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## Saanen and Alpine goats experience neophobia when offered novel feeds

Angélique Favreau-Peigné <sup>a,b,c,\*</sup>, Ophélie Dhumez <sup>a</sup>, Cécile Ginane <sup>d</sup>, Hans W. Erhard <sup>a</sup>

- <sup>a</sup> Université Paris-Saclay, INRAE, AgroParisTech, UMR Modélisation Systémique Appliquée aux Ruminants, 91120 Palaiseau, France
- <sup>b</sup> Université Paris-Saclay, UVSQ, INRAE, BREED, 78350 Jouy-en-Josas, France
- <sup>c</sup> Ecole Nationale Vétérinaire d'Alfort, BREED, 94700 Maisons-Alfort, France
- <sup>d</sup> Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France

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#### ABSTRACT

Feed neophobia is a reluctance to eat a novel feed that can last from minutes to days. While it can prevent animals from ingesting toxins, it may also have negative effects on growth and animal welfare. In ruminants, feed neophobia has mainly been studied in sheep and cattle, and only a few studies have focused on goats. This study assessed feed neophobia in goats and aimed to determine whether this is breed-dependent. Six-month-old Saanen (n = 18) and Alpine (n = 13) goats were repeatedly placed in an experimental environment, with a familiar feed, for 5 min per day (days 1-7). The familiar feed was then replaced by two novel feeds on consecutive days (d8d9), and the second novel feed was offered a second time (d10). Saanen goats habituated better to the situation as, on the last day with the familiar feed, they consumed more, with a greater intake rate, and spent less time near conspecifics than Alpine goats. They also showed a higher approach index than Alpine goats, meaning that they spent more time head in the feed trough while being close to it. Breed however did not influence the behavioural responses of goats when they were exposed to novel feeds. When first exposed to the novel feeds and regardless of their respective palatability, the goats reduced their intake compared to when they received the familiar feed, though they sampled the novel feeds by eating small amounts. They also approached the novel feeds less and spent more time near conspecifics. At the 2nd exposure to the second novel feed, the main behavioural responses were reversed, as intake increased and the time spent near conspecifics decreased, to reach the levels recorded with the familiar feed. Only the approach index remained lower. Thus, our results highlight that goats of both breeds displayed feed neophobia when first exposed to different novel feeds, but the situation was reversed after only one short-term exposure. Further research using more novel feeds, different feed-presentation schemes and longer tests will help to improve the understanding of feed neophobia in goats.

#### 1. Introduction

Ruminants may be confronted with abrupt feed changes, either during transition periods (e.g. from fresh to conserved forage or due to change in concentrate type), when they move from one farm to another or when climatic constraints impact feed availability. In response to these changes, ruminants can express feed neophobia, defined as a reluctance to eat an unknown feed (Demattè et al., 2013). They almost invariably reject a novel feed when offered for the first time (Chapple and Lynch, 1986) and this rejection can last from minutes to days (Provenza et al., 1995; Lecuelle et al., 2011; Egea et al., 2014). When it is long-lasting, it can have negative consequences on livestock performances such as a decreased body condition or wool production, and an extended period till slaughter (Nolan et al., 1975; Ortega-Reyes et al.,

1992). Welfare may also be affected because of the negative emotional state (Villalba et al., 2010) as well as the physical discomfort experienced by the animal refusing to eat an unfamiliar feed for several days (Chapple and Lynch, 1986). In most cases, ruminants sample the feed by ingesting small amounts (Chapple et al., 1987; Launchbaugh, 1995). This sampling behaviour can be a sign of a conflict between two motivations, approaching/eating the novel feed due to curiosity and hunger and avoiding it due to neophobia (Herskin et al., 2003). Sampling allows the animals to progressively experience the post-ingestive consequences of the feed, thus preventing them from ingesting potentially toxic and harmful plants in dangerous amounts. Sampling has been proposed as a survival mechanism for herbivores at pasture (Launchbaugh, 1995).

In ruminants, feed neophobia has mainly been studied in sheep (Chapple et al., 1987; Provenza et al., 1995; Launchbaugh et al., 1997;

<sup>\*</sup> Correspondence to: INRAE-UMR1198 BREED, Domaine de Vilvert, 78350 Jouy-en-Josas, France. *E-mail address:* angelique.favreau@inrae.fr (A. Favreau-Peigné).

Pedernera et al., 2022) with a focus on the role of the mother (Thorhallsdottir et al., 1990) and early experience (Catanese et al., 2012). Only a few studies have focused on goats. For instance, it was demonstrated that food learning can occur in utero and consequently improved the acceptability of a novel feed (Hai et al., 2013). Neophobia was not modified by pregnancy in goats (Knubel et al., 2004). Another study demonstrated that after a short-term exposure on one day, goats increased their intake of a novel palatable feed to an amount remaining stable during the following five days of exposure (Manuelian et al., 2010). Furthermore, differences in behaviours between breeds are rarely studied, even though it is of importance when comparing results from scientific studies carried out on different breeds, generalising results from one breed to the whole species, and also when managing a flock of different breeds on farm. To our knowledge, no studies have assessed a potential breed effect on feed neophobia in goats while such a breed effect was found in sheep (Simitzis et al., 2005). The authors attributed the lower susceptibility to feed changes of Karagouniki sheep, compared to Chios sheep, to the fact that the Karagouniki breed has evolved in harsh conditions and has become more adaptable to them. In goats, breed differences were found in young goats considering their exploratory behaviour in a non-feed novel environment (Erhard et al., 2012). In that study, the Saanen goats showed more exploratory behaviour compared to Alpine ones. Alpine and Saanen goats were the two breeds chosen in this study as they are commonly used in France, representing 52 % and 41 % of the French national herds, respectively (Races de France, 2014). Breeds were progressively selected to meet the requirements of different breeding objectives. These traits are mostly linked to qualitative and quantitative aspects of production (meat, milk, fertility, ...; D'Eath et al., 2010). As behavioural traits are rarely included in selection programmes (D'Eath et al., 2010), but they are sometimes co-selected unintentionally with production traits, there might be differences between breeds, and it is thus of importance for generalisation purpose to involve different breeds in our study.

Thus, our study focused on feed neophobia in goats and on the potential influence of breed on its display. The first hypothesis was that goats would show feed neophobia when a familiar feed was replaced by a novel feed, in our case two different novel feeds presented on subsequent days. The use of two different novel feeds was important to demonstrate the existence of the phenomenon whatever the nature of the novel feeds and their respective palatability. The second hypothesis was that a short-term exposure to a novel feed would increase its acceptance by the goats when subsequently being exposed for a second time to the same feed, in our case the second novel feed. This was previously observed in sheep (Chapple et al., 1987; Augner et al., 1998; Manuelian et al., 2010) and it needed to be confirmed in goats (observed with Olive tree leaves; Manuelian et al., 2010). The third hypothesis was that since Saanen goats had shown a lower neophobia in a non-feed related environment than Alpine goats (Erhard et al., 2012), they would also show a lower level of feed neophobia than Alpine goats.

#### 2. Material and methods

The experiment was performed at the experimental installation of INRAE unit MoSAR, Thiverval-Grignon (France) in 2011. At that time, in France, behavioural experiments were not subjected to ethical approval. However, the animals were handled by trained staff and all the experimental procedures were performed in accordance with the European Directive 86/609/EEC on the protection of animals used for scientific purposes and in respect of the 3Rs (Russell and Burch, 1959). At the end of the experiment, all the goats were managed as *per* before the experiment.

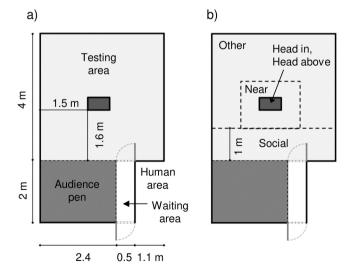
#### 2.1. Animals, housing environment and basal feeding

Thirty-one female goats from two different breeds (18 Saanen and 13 Alpine) were involved in this study. All goats were born at the

experimental installation of INRAE unit MoSAR, Thiverval-Grignon, in January, separated from their dams at birth and managed similarly from birth to the experiment. They were six months old at the beginning of the experiment. They were housed in the same rearing building, in two home pens separated by a wooden hurdle. The two breeds were mixed in each home pen (pen 1: 9 Saanen + 8 Alpine; pen 2: 9 Saanen + 6 Alpine). They were housed on wheat straw with water and mineral fortified salt licks available ad libitum. The following basal feeding procedure was applied throughout the experiment. A complete mixed diet for growing goats was daily offered. This feed also constituted the Familiar feed within tests as it was offered to the goats from 10 days before the first testing day. It was well accepted and consumed by the animals. It was composed of standard ingredients, either natural or industrial: Fluviajunior® concentrate (Agralis, France; 27 %), beat pulp (25 %), Rumi-Luz® (Désialis, France; 19 %), Fluvialac® concentrate (Agralis, France; 17 %) and oat straw (12 %) on a dry matter basis. It was offered ad libitum, at 16:30 h, on the floor, in front of each home pen. Then, on the following day, the feed refusals were pushed with a brush to be closer to the goats at 07:00 h, the refusals were removed at 15:00 h, and the goats were offered again the familiar feed ad libitum at 16:30 h.

#### 2.2. Experimental environment

The experimental area was located outside the main building, under a shed that was open to one side. It was situated at approximately 40 m from the building with the home pens and had a packed earth floor. The experimental environment consisted of four different areas: the audience pen, the waiting area, the human area and the testing area (Fig. 1a). The audience pen was adjacent to the waiting and testing areas and separated from them by barred hurdles (1 m high). The outside walls were solid, plain wood panels (1.6 m high). Each day, from day 1 to day 10, the same two adult goats were placed in the audience pen 30 min before the beginning of the tests and stayed there during the whole duration of the tests (4 h at the maximum). These two goats were not tested for feed neophobia but were used to avoid testing gregarious animals in social isolation. They were unfamiliar to the tested goats at the beginning of the experiment, to avoid any impact of their social relationship. They became familiar to the tested goats as the repeated



**Fig. 1.** The experimental environment and the virtual zones. a) The experimental environment consisted of an audience pen with two unfamiliar goats, a waiting area, a human area and a testing area. In the testing area, there was a plastic storage box containing the feed (familiar or novel - rectangle in dark grey). Solid lines represent plain panels and dashes represent barred hurdles. b) To record the location of the goat, the testing area was divided into 5 mutually exclusive virtual zones: social zone, head in the feed box, head above the feed box, near the feed box, and the other part of the testing area.

exposures to them and the experimental procedure went by. No agonistic behaviours were observed during the test trials. These audience goats were on wheat straw bedding with ad libitum access to feed and water, which were placed at the farthest point from the tested goats, close to the outside wall, to avoid any impact on the tested goats. The waiting area was a small corridor where the goat was placed for 1 min maximum before being tested. The human area was joined to the waiting and testing areas, and corresponded to the place where the two experimenters stayed hidden beyond plain panels during the tests. A plastic storage box (60  $\times$  40 x 22.5 cm) containing the feed (familiar or novel) was placed within the testing area, at a fixed position throughout the experiment.

#### 2.3. Experimental procedure

Considering the number of animals, the duration of the daily test and the time available each day to perform the tests, it was impossible to test all the goats in a unique experimental period. Thus, two experimental groups were created to be tested consecutively, on two 10-day long consecutive periods (one period per experimental group; Fig. 2). These two experimental groups were constituted of goats from the two breeds (group 1: 9 Saanen + 7 Alpine; group 2: 9 Saanen + 6 Alpine), coming from both home pens, and were balanced for body weight. The average weight of Alpine goats was  $37.4 \pm 4.5 \, \text{kg}$  (mean  $\pm$  SD) and  $38.1 \pm 4.3 \, \text{kg}$  for Saanen goats without any breed effect (ANOVA: F=0.17, P = 0.68), group effect (ANOVA: F=0.64, P = 0.43) or interaction between these two factors (ANOVA: F=0.26, P = 0.61).

A gradual exposure of the goats to the different steps of the experimental procedure was performed from day 1 (d1) to d7 with the familiar feed, before they were exposed to the novel feeds (d8 to d10, Fig. 2).

On d1 and d2, the goats were walked on a leash from their home pen to the experimental building. The transfer started in pairs to avoid social isolation and then, was performed individually. The number of transfers in pairs and individually was adapted to the goat so that each one walked on a leash without difficulties at the end of these two days.

On d3, between 10:00 h and 12:00 h, goats were exposed by groups of four to the novel experimental environment. Each group stayed for 10 min in the testing area, with 200 g of fresh matter (FM) of the familiar feed placed in the plastic box, before being walked back to their home pen.

From d4 to the end of the experiment (d10), the daily procedure was the same. Each day, each goat was tested individually and all the goats were tested successively in the same order, between 10:00 h and 13:30 h. Each goat was walked on a leash from its home pen to the experimental environment. The test started when the goat left the waiting area, with its two front legs in the testing area, and it lasted for 5 min (as performed by Van Tien et al., 1999; and similarly to Manuelian et al., 2010) before the goat was walked back to its home pen. The nature of the feed placed in the box changed depending on days but the amount of feed offered was still 200 g of FM (Fig. 2). From d4 to d7, each day, goats were exposed individually to the familiar feed. The aim was to habituate the goats to stay alone in the testing area and to consume this familiar feed. The last of these four days (d7) was used as the reference

for this period, and named thereafter Familiar. On d8, a novel feed was placed in the box (barley grains; named thereafter Novel 1; Table 1). On d9 and d10, another novel feed was used (rapeseed meal from a 00 variety, *i.e.* low-erucic/low-glucosinolate variety, Saipol, France; named thereafter Novel 2a and Novel 2b, respectively for d9 and d10; Table 1). We used two different novel feeds to dissociate the effect of feed neophobia from the one of initial palatability. Barley grain is an energetic concentrate commonly used for feeding ruminant (Humer and Zebeli, 2017), and it is considered palatable in cattle (Miller-Cushon et al., 2014) and sheep (Hutson and Van Mourik, 1981). Conversely, rapeseed meal is a feed of low palatability for ruminants (Stedman and Hill, 1987; Miller-Cushon et al., 2014).

The nutritional values of the novel feeds are extracted from INRA tables, national references of the nutritional values of feed under French conditions (Baumont et al., 2007).

#### 2.4. Measures

The amount of feed consumed during the test was determined by weighing the feed before and after each test and by calculating the difference.

The tests were recorded (Sony Handycam HDR-XR155 fitted with wide-angle lens VCL-HA07A, placed above the testing area), and the videos were analysed using a software for behavioural video analysis (The Observer XT, Noldus). The testing area was divided into 5 mutually exclusive virtual zones (Fig. 1b): "social" zone (the goat had at least its two front legs in a zone up to 1 m wide - approximately the length of a young goat - along the audience pen, the waiting area and the human area), "near" the feed box (close to the feed, i.e. 60 cm around the feed box; at least the two front legs in this feed zone), "head above" the feed box (when the animal had its head above the feed box; the goat could be looking at the feed), "head in" the feed box (when the animal had its head in the feed box; the goat could be eating, sniffing the feed, or looking closely at the feed), and the "other" part of the testing area. The percentage of time spent in the different zones of the testing area was calculated. Exposure to a novel stimulus induces both positive and negative emotional and behavioural responses (Mills et al., 2010), and

Table 1
Chemical characteristics of the novel feeds.

	Novel 1	Novel 2 a-b
	Barley grain	Rapeseed meal
Dry matter <sup>a</sup>	86.7	88.7
Organic matter <sup>b</sup>	974	921
Starch <sup>b</sup>	602	0
$CP^{b}$	116	380
NDF <sup>b</sup>	216	319
$ADF^{b}$	63	221
ADL <sup>b</sup>	11	108

CP= Crude Protein; NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre; ADL= Acid Detergent Lignin.

✓ Habituation to walk on a lead
 ☐ Habituation in group of 4
 ☐ Individual test with the familiar feed
 ☐ Individual test with Novel 1
 ☐ Individual test with Novel 2

**Fig. 2.** Timeline of the experiment. Goats were habituated to the transfer from their home pen to the experimental environment (days 1–2), then to stay in groups (d3) and alone (d4–7) in the experimental environment with their familiar feed. Day 7 was considered as the reference for the familiar feed, and named Familiar. On d8, a first novel feed was offered (Novel 1 - Barley grain) and then, a second one on d9–10 (Novel 2a-2b, respectively - Rapeseed meal).

a of Fresh Matter.

 $<sup>^{\</sup>rm b}\,$  per kg of dry matter.

can thus be the cause of an approach/avoidance conflict (Gray, 1987). As a consequence, an index of approach/avoidance related to the feeds was calculated as the ratio between "close investigation and/or ingestion of feed" (the percentage of time spent with the head in the feed trough) over "interest in the feed" (the sum of the percentages of time spent with the head in/above and the goat near the feed trough), i.e. "head in" / ("head in" + "head above" + "near"). The approach/avoidance index is the highest when the animal spent all the time "head in" the feed trough while being close to the feed trough; it is thereafter called "approach index". The intake rate was estimated by calculating the ratio between the amount of consumed feed and the time spent with the head in the feed box. The number of vocalisations was recorded, since novelty can induce negative emotions as fear or anxiety (Boissy and Bouissou, 1995), and vocalisations are considered as an indicator of negative arousal in goats (Briefer et al., 2015).

#### 2.5. Statistical analysis

All analyses were performed using XLSTAT Statistical Software (Addinsoft, Paris, France), with significance accepted at  $P \leq 0.05$  and tendencies (0.05 < P < 0.1) not discussed. Anderson-Darling tests and QQ-plots were used to assess the normality of the data and their residuals. Bartlett tests were performed to test variance homogeneity. The two groups were balanced for breed, home pen, and body weight at the beginning of the experiment so the data were pooled for statistical analyses.

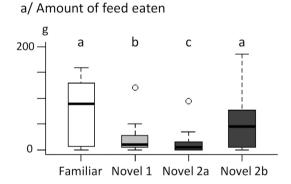
Four days (Familiar, Novel 1, Novel 2a and Novel 2b) were considered for the statistical analyses of feed intake and behavioural data (time spent in the social zone, approach index, intake rate and number of vocalisations). There was one data point per animal and per day. As the data and their residuals were not normally distributed and variances were unequal, non-parametric statistics were performed. The outliers

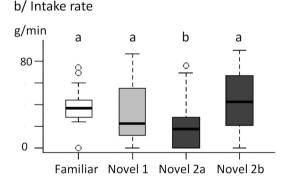
were not removed from the analyses as the individuals representing these outliers varied between days. The breed effect was tested with Mann-Whitney tests considering each feed type independently. For each variable, when there was a significant difference between the breeds with regard to the familiar feed, the difference between the measurement for the first day with each novel feed and the measurement for the familiar feed was calculated per animal and then, breeds were compared on this difference with Mann-Whitney tests. Regarding the results of the breed effect (which was mainly found during the habituation period), it was decided to pool the data from both breeds in order to focus on the analysis of the behavioural responses of all goats to feed novelty. Friedman tests were used to analyse the effect of feed type (Familiar, Novel 1, Novel 2a and Novel 2b) on goats' behaviours. When a significant feed effect was found, pairwise Wilcoxon tests were performed, with the consideration of the Benjamini-Hochberg correction for multiple comparisons, to identify which feed differed from one another. Thus, for pairwise Wilcoxon tests, adjusted p-values are reported thereafter.

#### 3. Results

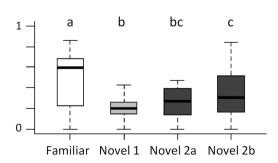
#### 3.1. Feed types and goat behaviour

One goat of each breed never ate any of the novel feeds while these two goats ate the Familiar one and put their head in/above the feed box during the Novel 1 and Novel 2a test. The feed intake, intake rate, approach index as well as the percentage of time spent in the social zone varied depending on feed types (Friedman: Q = 28.6, P < 0.001; Q = 21.2, P < 0.0001; Q = 18.88, P < 0.001; Q = 17.4, P < 0.001, respectively). Goats decreased their intake from Familiar to Novel 1 (Wilcoxon with adjusted p-value: V = 421, P < 0.0001), from Novel 1 to Novel 2a (V = 190, P  $\leq$  0.05), then increased their intake from Novel 2a to Novel





#### c/ Approach index



#### d/ Time spent in the social zone

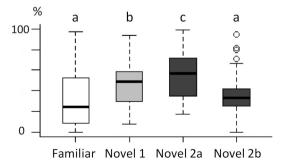


Fig. 3. Influence of feed type on a/ the amount of consumed feed (g of fresh weight), b/ the intake rate (g/min), c/ the approach index and d/ the time spent in the social zone (%), during 5 min tests, in goats (n = 31). Familiar was the last day with the familiar feed (d7). Box plots show medians (horizontal lines), interquartile ranges (boxes) and the minimum and maximum values (excluding the outliers; whiskers) and the outliers (circles). Pairwise comparisons were performed using Wilcoxon tests. Feeds with different letters differ at  $P \le 0.05$ .

2b (V = 5, P < 0.0001), to the level recorded on Familiar (V = 325, P = 0.06; Fig. 3a). The intake rate was the lowest for Novel 2a, and the ones of the three other feeds did not differ from each other (Fig. 3b). The approach index decreased when offered the novel feeds (Novel 1, Novel 2a and 2b) compared with Familiar (V = 429, P < 0.0001 - V = 412, P < 0.01 - V = 357,  $P \le 0.05$ , respectively), and neither differ between Novel 1 and Novel 2a nor between Novel 2a and Novel 2b (Fig. 3c). Goats spent more time in the social zone when offered novel feeds for the first time (Novel 1 and Novel 2a) than when Familiar (V = 131,  $P \le 0.05$ - V = 110, P  $\leq$  0.05, respectively) or Novel 2b (V = 353, P  $\leq$  0.05 - V = 454, P < 0.0001, respectively) were offered (Fig. 3d). This time increased from Novel 1 to Novel 2a (V = 143, P  $\leq$  0.05), but was similar between Familiar and Novel 2b (V = 195, P = 0.31; Fig. 3d). The number of vocalisations during the test did not differ depending on feed types when both breeds were included in the analysis (median,  $Q_1$ ,  $Q_3$ : 18, 5, 32; Friedman: Q = 2.9, P = 0.41).

#### 3.2. Breed and goat behaviour

The influence of the breed on feed intake was present all along the habituation period, with Saanen goats consuming the familiar feed in a greater amount than Alpine goats (Table 2).

Focusing on d7 - Familiar, the data showed that Saanen goats ate a greater amount of feed (Mann Whitney:  $U=43.5,\ P<0.01;\ Fig.\ 4a)$  with a greater intake rate (Mann Whitney:  $U=23,\ P<0.0001;\ Fig.\ 4b)$  than Alpine goats. They also showed a greater approach index (Mann Whitney:  $U=61,\ P\leq0.05;\ Fig.\ 4c)$  and spent less time in the social zone compared to Alpine goats (Mann Whitney:  $U=169,\ P\leq0.05;\ Fig.\ 4d)$ . Both breeds, however, vocalised the same amount (14, 4, 32; Mann Whitney:  $U=162,\ P=0.07)$ .

When offered novel feeds, the feeding behaviour and the time spent in the social zone did not differ between breeds (Fig. 4a, b, and d). The number of vocalisations on Novel 1 (16, 4, 28; Mann Whitney: U = 150, p = 0.20), Novel 2a (23, 10, 34; Mann Whitney: U = 131, P = 0.59) and Novel 2b (11, 5, 30; Mann Whitney: U = 148, p = 0.23) were also unaffected by the breed. Saanen goats only showed a higher approach index than Alpine goats on Novel 2a (Mann Whitney: U = 50, P < 0.01; Fig. 4c) while both breeds showed similar approach index on Novel 1 and Novel 2b (Mann Whitney: U = 100, P = 0.80 and U = 113, p = 0.89 respectively; Fig. 4c).

Due to the breed differences on Familiar and the absence of differences hereafter, the evolution between Familiar and the 1st day with the novel feeds was consistently different. In more details, the difference between Familiar and Novel 1 was higher in Saanen than Alpine goats for the amount of feed consumed (U = 179, P  $\leq$  0.05; Fig. 4a) and the approach index (U = 180, P  $\leq$  0.05; Fig. 4c). This was not the case considering the intake rate (U = 154, P = 0.15; Fig. 4b) and the time spent in the social zone (U = 71, P = 0.07; Fig4d).

The difference between Familiar and Novel 2a was higher in Saanen

**Table 2**Amount of familiar feed consumed (g), for each breed, during individual tests performed from day 4–7, *i.e.* a habituation period to the experimental procedure.

	Alpine goats	Saanen goats	p-value
Day 4	0	40	0.003
	(0, 21)	(15, 119)	
Day 5	0	55	0.023
	(0, 40)	(16, 113)	
Day 6	5	70	0.028
	(0, 15)	(11, 99)	
Day 7 - Familiar	5	108	0.003
	(0, 70)	(90, 150)	

Medians are presented with 1st and 3rd quartiles into brackets. Mann-Whitney tests were performed to compare the two breeds. The last day with the familiar feed, day 7, is thereafter considered as the reference for this habituation period, and named "Familiar".

than Alpines goats for the amount of feed consumed (U = 178, P < 0.01) but not for all the other measures (intake rate: U = 164, P = 0.06 for the intake rate; approach index: U = 147, P = 0.24 for the approach index; time spent in the social zone: U = 92, P = 0.33 for the time spent in the social zone).

#### 4. Discussion

The aim of this study was to find out if goats show feed neophobia when faced with novel feeds. Therefore, we compared the ingestion of a novel feed with that of the familiar one, then repeated this test with a 2nd novel feed to demonstrate the existence of neophobic responses whatever the palatability and nutritive value of the feeds. As a second control for 'novelty', we then offered the 2nd feed a second time. If the refusal to eat was due to the type of feed, the goats would maintain their reluctance to ingest it. If, however, novelty was the issue, then they would now be prepared to eat it. This study also aimed to determine whether the neophobic response is breed-dependent. Goats showed feed neophobia, by reducing their intake and their approach to the feed while increasing the time spent near conspecifics, when a familiar feed was replaced by a novel feed (two different novel feeds presented on subsequent days). A short-term exposure to a novel feed increased its acceptance by the goats when subsequently being exposed for a second time to the same feed. Interestingly, breeds influenced the behaviour of the goats on the last day with the familiar feed (Saanen goats better habituated than Alpine ones to the situation) whereas differences between breeds disappeared when exposed to novel feeds.

The study comes with some limitations such as the use of a single scheme of presentation of novel feeds and the consideration of only two novel feeds offered on very short-term tests performed over few days. The limited number of available animals did not allow to work with more than two types of feed and to test all orders of presentation, balanced among individuals. To decrease the possible negative impact (residual effect) of the first novel feed on the second one, two measures were taken: firstly, the more palatable novel feed was presented first (enhancing the likelihood of positive sensory experience) and secondly, the 1st novel feed was provided only once and for a brief period of time (reducing the likelihood of negative post-ingestive consequences). This study aims to provide original first results about feed neophobia in goats, and further research is needed to generalise them to other feed types, irrespective of their presentation order.

#### 4.1. Expression of feed neophobia in goats

The experimental procedure was designed to minimise the emotional reactivity of the goats during the tests. They were tested in presence of conspecifics, and they were gradually and repeatedly exposed to the experimental environment and procedure. This included the feed box, which remained familiar throughout the experiment, as this can impact novel feed acceptability (Chapple et al., 1987). This procedure aimed to limit the novelty in the tests to feedstuff only, so as to be able to assess feed neophobia. Feed neophobia is firstly displayed as and measured by the low intake of novel feeds. However, a low and reduced intake of novel feeds compared to that of a familiar one can be due to two non-exclusive phenomena: feed neophobia and/or a low initial palatability of the novel feeds. The palatability of a novel feed is influenced by the hedonic value of its sensory characteristics (odour, taste and texture), which can be modified through learning about the post-ingestive consequences of that feed or of similar feed (Favreau-Peigné et al., 2013). This plays a crucial role in the animals' feeding behaviour, including its acceptance (Herskin et al., 2003; Lecuelle et al., 2011). In an attempt to dissociate the effect of feed neophobia from the one of initial palatability, we used two different novel feeds. On one side, barley grain (Novel 1) is an energetic concentrate, considered as palatable in ruminants (Hutson and Van Mourik, 1981; Miller-Cushon et al., 2014). On the other hand, rapeseed

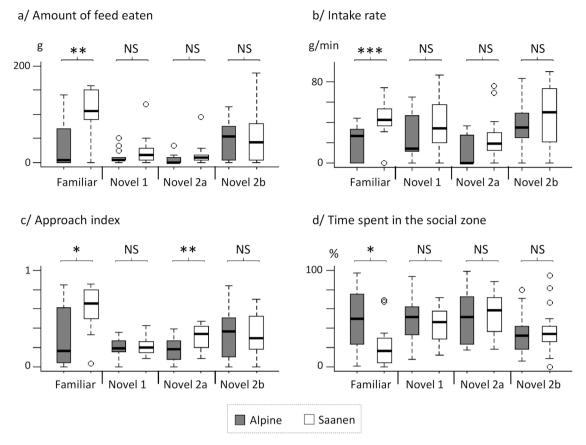


Fig. 4. Influence of breed, for each feed type, on a/ the amount of consumed feed (g of fresh weight), b/ the intake rate (g/min), c/ the approach index and d/ the time spent in the social zone (%), during 5 min tests, in goats (13 Alpine vs. 18 Saanen). Familiar was the last day with the familiar feed (d7). Box plots show medians (horizontal lines), interquartile ranges (boxes), the minimum and maximum values (excluding the outliers; whiskers) and the outliers (circles). The breed effect for each feed type was tested with pairwise comparisons for independent variables using Mann Whitney tests: \*\* for  $P \le 0.01$ ; \* for  $P \le 0.05$ ; NS for P > 0.05.

meal (Novel 2) is a feed of low palatability for ruminants (Stedman and Hill, 1987; Miller-Cushon et al., 2014). Even if it was a rapeseed meal from a low-erucic/low-glucosinolate variety, this low palatability may be due to the presence of sinapine, a bitter tasting phenolic compound (Mejicanos et al., 2016; Heuzé et al., 2020). Specific chemical analyses should be included in future studies to assess the presence of such compounds. The initial palatability of the novel feeds used in our study can be assessed by the intake rate measured on the short term (Baumont, 1996). These measurements indicate that for goats, as for other ruminants, rapeseed meal (Novel 2) appears to be less palatable than barley grains (Novel 1) on their first offer. Regardless of any difference in the palatability of the novel feeds, goats (both breeds combined) strongly decreased their intake of the two novel feeds on first offer compared to the last day with their familiar feed. This important decrease in intake observed for both novel feeds (-89 % with Novel 1 and -94 % with Novel 2a) suggests the expression of feed neophobia.

Our results did not demonstrate any improved acceptability of the 2nd novel feed contrary to a previous study in which lambs were repeatedly offered novel feeds for 3 consecutive days (Launchbaugh et al., 1997). In our study, any residual effect of Novel 1 on the behavioural responses of goats subsequently offered Novel 2 has been minimised working on short-term test and with only one exposure to Novel 1, thus minimising the onset of post-ingestive consequences and food learning on Novel 1. Despite this, we cannot exclude that some aspects of the behavioural responses of the goats to Novel 2 may be due to the fixed order of the feed presentation (Novel 1 has always been offered before Novel 2). Nevertheless, presenting the more palatable feed first increased the likelihood of a good experience with novelty (on a sensory basis) and thus the likelihood of reduced neophobia with the second

feed. The observation of effective neophobia with Novel 2 suggests that the residual effect was minimal or limited.

In addition to the decreased intake, goats showed a lower approach index and a longer time in the social zone with the novel feeds (at first offer) than with the familiar one. The lower approach index indicates that goats came near the novel feeds without approaching them too closely, thus suggesting that the novel feeds were challenging for them. The social zone was the place where they were near the audience goats and the humans (hidden behind a wall). As gregarious animals (Miranda-de la Lama and Mattiello, 2010), goats have been shown to express lower distress due to social isolation when they were allowed to maintain contact with their pen mates (Siebert et al., 2011) or with humans (Price and Thos, 1980). Furthermore, sheep in social isolation decreased their behavioural, autonomic and endocrine indices of stress when seeing a picture of the face of an unfamiliar conspecific (da Costa et al., 2004). It was thus assumed that the presence of real conspecifics, even if unfamiliar, would reduce the negative perception of social isolation and the distress of the tested goats, but this needs to be adequately tested. The social zone was also the place near the door to leave the testing area. So, the increased time in the social zone may have expressed the need to find reassurance near conspecifics/humans and/or the motivation to search something else to do than interacting with the feeds, due to their negative impact on goats' emotional state (disturbing and challenging new and unpredictable stimuli).

Lastly, the number of vocalisations was unaffected by the presence of the novel feeds. This can be explained by the fact that contact calls are the most common goat vocalisation (Briefer et al., 2015) and that the goats, in our study, might not have needed to express contact calls, as conspecifics were in the audience pen and may have provided them

reassurance during the tests.

#### 4.2. A short-lasting feed neophobia in goats

The second hypothesis of this study was that a short-term exposure to a novel feed could increase its further acceptance. This increased acceptance has been reported previously in sheep (Chapple et al., 1987; Launchbaugh et al., 1997; Manuelian et al., 2010). Our results are consistent with these previous observations as after only one exposure, goats of both breeds increased their feed intake and intake rate between Novel 2a and 2b. Such a rapid increase in acceptance can be explained by sampling, learning and habituation processes.

In our study, only 2 out of the 31 goats never ate any of the novel feeds while they consumed the familiar one and collected information on the novel feeds (they put their head in/above the feed box on Novel 1 and Novel 2a). Instead, the majority of the goats consumed small amounts of the novel feeds on their 1st offer. This is consistent with a sampling strategy (Provenza et al., 1995), which allows animals to learn about novel feeds' characteristics (including their post-ingestive consequences) without taking too much risk (Provenza et al., 1995; Egea et al., 2014). In a changing environment, feeds' diversity and quality vary and regular sampling allows animals to develop and update their knowledge about these feeds, via learning (Provenza, 1995; Forbes and Provenza, 2000). It may also provide animals the time needed to learn how to ingest a feed with different/unknown sensory properties such as texture (Chapple et al., 1987). The goats consumed Novel 2 in greater amounts and at a greater rate on the second exposure, which suggests that Novel 2 did not induce negative post-ingestive consequences and that goats had subsequently recognised it as safe (Burritt and Provenza, 1989).

At the first exposure, ten goats did not consume Novel 2 but 6 of them consumed it at the second exposure. For these goats, there was no sampling behaviour (no consumption) on Novel 2 at first exposure. Thus, a simple exposure to a novel feed may allow goats to perceive it as potentially edible, and then to reduce feed neophobia at the 2nd offer (Klein, 2013). After one exposure to the novel feed, goats increased the amount of feed consumed but also decreased the time spent in the social zone. To determine whether goats fully habituated to the novel feed, the comparison between Familiar and Novel 2b is useful. It showed that the approach index is lower on Novel 2b than on Familiar while the feeding behaviour (intake and intake rate) and the time spent in the social zone did not differ between these two days. Thus, goats habituated partially to the novel feeds. It would have been useful to extend our study for a few days to determine how many days would have been necessary for the goats to fully habituate to the novel feed, to adapt future scientific protocols.

#### 4.3. Goat behaviour and the influence of the breed

The third objective of this study was to assess a potential breed effect on goats' behaviour in a context of feed novelty. All the goats, in this study, were subjected to the same rearing conditions from birth to the experiment, and were mixed in the home pens. Consequently, breed can be considered as a major distinction between them. In our study, a breed effect was found for the amount of familiar feed consumed all along the habituation process (including Familiar) and, it was also the case for additional measures on the last day with the familiar feed. On Familiar, Saanen consumed more than Alpine goats, with a higher intake rate. Saanen goats furthermore showed a higher approach index, spent less time in the social zone and vocalised less than Alpine goats. Altogether, these results suggest that Saanen goats were less challenged and maybe better habituated to the experimental procedure than Alpine goats. Breed differences on behavioural responses were found in goats exposed to other different situations, such as social isolation (Alpine and Nubian goats - Carbonaro et al., 1992; Alpine and Saanen goats - Erhard et al., 2012) and a novel environment (Erhard et al., 2012). Other aspects of

feeding behaviour have been compared between adult Saanen and Alpine goats in previous studies, sometimes showing differences (Giger-Reverdin et al., 2020) and sometimes not (Cellier et al., 2021). As many parameters can influence the feeding behaviour of goats and explain these apparent inconsistencies (physiological stage, type of feed offered, duration of measures (long-term vs short term), etc.), further investigation is warranted to comprehend the breeds' differences (in various behavioural traits) and the conditions under which they are expressed. Hence, differences in emotionality (Savage and Eysenck, 1966), feeding motivation, or human handling (including the walk on a leash) may play a role in explaining the different levels of habituation between Alpine and Saanen goats to our experimental procedure. In future tests, precautions should be taken to base habituation on animal-based measures, such as a certain level of feed intake in the test pen, rather than only on a number of repetitions of the habituation procedure.

In our study, the aforementioned differences between breeds in their habituation (Familiar) have consistently resulted in differences in the evolution of some behavioural responses between Familiar and the 1st day with each novel feed (amount of feed consumed and approach index). Nonetheless, the key finding resides in the fact that the differences between breeds disappeared when the goats were offered novel feeds both at first and second exposures (except for the approach index on Novel 2a). If Alpine goats were only responding to the experimental procedure, without considering the novel feed as negative stimuli, we could have expected a progression of their habituation and thus an increase in their intake. Instead of that, both breeds showed low intakes. The novel feeds seemed to be perceived as challenging and important situations so that no differences between the two breeds did exist anymore. In rats, the behavioural responses (including feed intake) to a first exposure to a novel feed was also similar in all strains tested (Modlinska et al., 2015). Since feed neophobia has a biological significance, it is reassuring to observe that both breeds showed this behaviour, and are, therefore, still adapted to use environments with varying feed sources even after many generations spent in more and more intensive farming systems.

#### 5. Conclusion

Offering novel feeds for the first time in a familiar environment and according to a familiar procedure challenged the goats and decreased their feed intake, increased the time spent near conspecifics and decreased the approach index. All these elements reflect the expression of feed neophobia. Nevertheless, most individuals sampled the novel feeds by consuming small amounts from the first exposure, which has favoured habituation and learning about their sensory and postingestive properties. Then, from the second exposure, most behavioural responses of caution towards the novel feed decreased, making feed neophobia a short-lived process in our study. Breed influenced the behavioural responses of goats, but essentially during the exposure with their familiar feed, with Saanen goats appearing more habituated or less emotional in the test situation than Alpine goats. However, when the goats were exposed to the novel feeds, both breeds behaved in a similar way. This study presents interesting initial findings on feed neophobia in goats, although it has some limitations (limited number of novel feed types, presented in a single order) which would be addressed in future complementary studies.

#### CRediT authorship contribution statement

Hans W. Erhard: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. Ophélie Dhumez: Investigation, Methodology, Writing – review & editing. Cécile Ginane: Conceptualization, Formal analysis, Writing – review & editing. Angélique Favreau-Peigné: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration,

Supervision, Validation, Visualization, Writing – original draft, Writing review & editing.

#### **Declaration of Competing Interest**

We have no conflicts of interest to disclose concerning the manuscript entitled " Saanen and Alpine goats experience neophobia when offered novel feeds" by Favreau-Peigné, A. et al.

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