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Recognising the facial expression of frustration in the horse during feeding period

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ABSTRACT

Horses often present negative emotional states which are frequently poorly recognised, with much of our understanding of horse expressions based on anecdotes, rather than scientific evidence. The aim of this project was to identify potential facial markers of emotional states. 31 horses, aged between 2 and 23 years old (mean ± SD: 11.5 years, ± 6.6) and various genders (1 male, 10 geldings and 20 females) took part in the study. They were tested in three different scenarios involving the potential availability of food. Horses were trained to anticipate a reward after 10 s and then tested across the following three situations. Anticipation of a reward, considered a positive emotional state; frustration at waiting for a reward and disappointment at the loss of the reward - both considered negative emotional states. Tests were conducted in a stable with a feeding device fixed outside the stable within reach of the horse. Analysis of video recordings of facial expressions of the horses was undertaken using the Horse Facial Action Coding System (EquiFACS), an objective system for coding facial movements on the basis of the contraction of underlying muscles, as well as their behaviours. Specific facial markers associated with anticipation could not be characterised, however, we found that the occurrence of 9 actions and behaviours differed significantly between the two situations predicted to induce frustration and disappointment during the feeding period. The frustration phase was characterised by a higher likelihood of 'eye white increase' (AD1), 'ear rotator' (EAD104), and 'biting feeder' compared to the 'disappointment' situations. By contrast, 'blink' (AU145), 'nostril lift' (AUH13), 'tongue show' (AD19), 'chewing' (AD81) and 'licking feeder' were more likely in the 'disappointment' phase than in the 'frustration' situation. There was also a general gender effect with females more likely to blink than males. The findings of this research may help differentiate frustration and disappointment at least during the feeding period.

1. Introduction

In recent years, welfare researchers have increasingly focused on the mental health of horses (Mills et al., 2020) especially their emotions (Hall et al., 2018). An emotional reaction is an intense but short-lived affective response to a stimulus or event (Désiré et al., 2002) which is perceived as rewarding or punishing (Mendl et al., 2010). Emotions can be characterised along two main dimensions, their valence (such as positive/pleasant/attractive/rewarding versus negative/unpleasant/aversive/ punishing) and the level of arousal or activation (such as high or low intensity) which together make up core affect (Russell, 2003; Paul et al., 2005; Mendl et al., 2010). However there is increasing interest in the qualitative classification of animal emotions based on their ultimate biological function (Panksepp, 1998; Mills, 2017). It has been argued (Mills, 2017) that different types (qualities) of emotional state can be inferred using component process theory (Scherer, 1988) by triangulating evidence from four of the five components. Specific context can be used to identify potential emotionally competent stimuli relating to the appraisal processes associated with the cognitive component; signs of arousal may be detected relating to the neurophysiological component; behavioural tendencies described in relation to the motivational component and communicate signals described in relation to the motor expression component. Only the ability to generate evidence to support the subjective feelings component remain elusive. Any or all of these four lines of evidence can be used to inductively argue for and against the presence of a given emotional state at a given time. Consistent associations between specific metrics within or between any of these components can provide convergent evidence to reduce the uncertainty of inferring an emotional state (See Mills, 2017 for further details of this psychobiological approach). Thus identifying specific measures associated with a particular

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context of potential emotional significance, such as the communicative signals, behavioural tendencies or arousal shown at this time, is essential if we wish to build an evidence base for how to infer a specific emotion in a given species.

There is good reason to suppose that horses live in an emotionally complex world. They are generally gregarious animals, living within a complex social system (Feh, 2005; Cozzi et al., 2010), able to communicate with others with subtle visual signals (e.g. eyes direction, ears position and facial expressions) (Wathan and McComb, 2014; Wathan et al., 2015; reviewed by Fureix et al., 2012). For example, they can discriminate between different facial expressions of horse pictures such as agonistic, positive attention and relaxed (Wathan et al., 2016). Facial expressions have also been investigated as a communicative signal in order to detect pain in horses (Dalla Costa et al., 2014; Gleerup et al., 2015; Van Loon and Van Dierendonck, 2015). In these research studies, pain was typically acute and established in a variety of ways, from castration (Dalla Costa et al., 2014; Lencioni et al., 2021), application of noxious stimuli (a tourniquet and a capsaicin crème) (Gleerup et al., 2015) or association with acute colic (Van Loon and Van Dierendonck, 2015). The results show an alteration in facial expressions induced by the pain, especially in relation to the ears (e.g. ears pointed backward), eyes (e.g. activation of inner brow raiser) and lower face area (e.g. use of nostril dilator, tension above the mouth) (Dalla Costa et al., 2014; Gleerup et al., 2015; Van Loon and Van Dierendonck, 2015; Lencioni et al., 2021). These studies have thus enabled the development of a range of scales for assessing pain in horses, such as the Horse Grimace Scale (HGS) (Dalla Costa et al., 2014), Equine Pain Face (Gleerup et al., 2015) and The Equine Utrecht University Scale for Facial Assessment of Pain (EQUUS-FAP) (Van Loon and Van Dierendonck, 2015) with the potential for automated detection beginning to be realized (Lencioni et al., 2021). More recently, a pain scale instrument based on facial expressions has also been developed more specifically for ridden horses (FEReq) (Mullard et al., 2017). These studies highlight the importance of recognising the communicative component of emotion in the horse.

Indeed, the face is a major source of emotional information in many species (cat: Finka et al., 2019; cow: Sandem et al., 2002; pig: Camerlink et al., 2018; rat: Finlayson et al., 2016; sheep: Reefmann et al., 2009), but there is a danger that facial expressions are evaluated holistically (e.g. as a grimace) and the inference drawn subject to bias as a result. To address this problem in humans, the Facial Action Coding System (FACS) has been developed (Ekman et al., 2002) to facilitate the recording of facial expressions based on the movement of underlying facial musculature (i.e. description of facial changes based on the contraction of defined muscle groups). This system has been developed in several species: cats (*Felis catus*) (Caeiro et al., 2017a), dogs (*Canis familiaris*) (Waller et al., 2013), and several primates (chimpanzees (*Pan troglodytes*) (Vick et al., 2007), common marmosets (*Callithrix jacchus*) (Caeiro et al., 2022), gibbon (*Hylobatides*) (Waller et al., 2012), orang outangs (*Pongo pygmaeus*) (Caeiro et al., 2013), rhesus macaques (*Macaca mulatta*) (Parr et al., 2010) and horse (*Equus caballus)* (Wathan et al., 2015). Of these only the horse has laterally placed eyes and an elongated face. The positioning and size of their eyes make it easier to observe feature changes in this area (Merkies et al., 2019). Hintze et al. (2016) have previously focused on eye wrinkles, related to one of the action units from EquiFACS (AU101), as a potential tool to evaluate emotional valence in horses, testing horses in four situations, with two of them related to the feeding period: food anticipation (positive emotional valence) and food competition inducing frustration (negative emotional valence) (Hintze et al., 2016). They found that the angle between the highest wrinkle and the line through the eyeball increase during food competition compared to a control (period of one minute before the test), whereas during food anticipation there is no change (Hintze et al., 2016). However, it is clear that emotional expression in horses goes beyond this region, with structures like the ears often referred to in relation to states such as fear (Leiner and Fendt, 2011), vigilance (Hausberger and Muller, 2002) and pain (Dalla Costa et al., 2014).

Anticipation can be defined as the time between a cue signalling the availability of an expected reward and the arrival of this reward (Spruijt et al., 2001; Van Den Bos et al., 2003; Boissy et al., 2007; Anderson et al., 2020). This period of time is typically associated with a positive emotional state (Van Den Bos et al., 2003; Boissy et al., 2007; Mendl et al., 2010; Anderson et al., 2020). However, if the expectation is not met due to its absence (Amsel, 1992; Papini and Dudley, 1997), reduction (Amsel, 1992; Papini and Dudley, 1997), delay (Amsel, 1992) or inaccessibility (Panksepp, 2005), a negative emotional state result, such as frustration and/or disappointment (Amsel, 1962; McNaughton, 1989; Flaherty, 1996; Papini and Dudley, 1997). The difference between these two latter negative states is that frustration can be considered a high arousal state whereas disappointment is of lower arousal (Mendl et al., 2010). Differences in the intensity of arousal can be inferred from the assessment of physiological measures such as heart rate (Wascher, 2021). Thus the assessment of components of emotion help in the evaluation of both qualitative (which emotion) and quantitative (intensity) aspects of emotion.

The current report uses an adaptation of an experimental paradigm developed in a previous study with dogs by Bremhorst and colleagues (2019) to infer facial expressions of anticipation, frustration and disappointment in horses. Bremhorst et al. (2019) examined dogs' facial expressions using DogFACS during positive (anticipation of a food reward) and negative (denial access to the food reward) contexts. They found that during the positive context, 'Ears adductor' (EAD102) was more frequent whereas during the negative context 'Blink' (AU145), 'Lips part' (AU25), 'Jaw drop' (AU26), 'Nose lick' (AD137) and 'Ears flattener' (EAD103) were more common. However, facial emotional expressions should not be generalised across species (Caeiro et al., 2017b). Species might also differ in their behavioural tendencies too. For example, anticipatory behaviours differ between species (Van Den Bos et al., 2003; Krebs et al., 2017). In rats and cats, subjected to the same Pavlovian conditioning paradigm (with a tone as conditioned stimulus and food reward as unconditioned stimulus), it was found that cats have a decrease in behavioural transitions (i.e. the number of times that an animal changes behaviours) during the anticipatory phase, whereas the opposite occurs in rats (Van Den Bos et al., 2003). In the current study, we trained 30 horses to expect a reward after 10 s; our aim being to identify any signs that might be useful for distinguishing between the positive emotional expression of reward anticipation in the horse and negative emotional states when the food was not delivered as anticipated: specifically disappointment (a low arousal state) and frustration (a high arousal state). Critically, all three emotions can occur in relation to a delayed reward. We hypothesised, firstly, that horses' facial expressions would differ between the situations predicted to induce positive (anticipation) and negative (frustration and disappointment) valence and secondly, that they would also differ between the two potentially negative emotional states that might reflect: 'disappointment' and 'frustration'.

2. Methods

The delegated authority of the University of Lincoln Research Ethics Committee approved this research (UoL2021_6910) and all methods were carried out in accordance with the University Research Ethics Policy and the ethical guidelines of ISAE (Sherwin et al., 2003). Written informed consent was obtained from the owner of all horses used in the research.

2.1. Study animals

This study took place between January and February 2022 in the Haras d'Emeraude (St Lunaire, France). 31 horses of various breeds (Cob Normand, French saddle, Haflinger, Hungarian, Pinto cross Trotter, unknown) aged 2–23 years old (mean ± SD: 11.5 years, ± 6.6) representing 1 entire male, 10 geldings and 20 females were used in this experiment (Supplementary material Table S1).

2.2. Experimental set-up

The horses were tested in a stable measuring 4 m x 3.50 m with free access to water. A feeder with two panels (Fig. 1a) was attached to the outside of the stable, along with a food delivery system. The dimensions of the feeder were 40 centimetres long, 32 centimetres wide and 20 centimetres high. The opaque panel was located 7.5 centimetres from the bottom of the feeder. A transparent Perspex panel closed the feeder. On the outside of the stable, a device was installed with two screens allowing the experimenters to remain hidden during the tests (Fig. 1b). From behind the screen on the left of the picture, the experimenter could manipulate the different panels and behind the right screen the other experimenter could fill the feeder. Two cameras were installed in front of the stable to record during the tests.

2.3. Baseline

The horses were filmed when they were at rest with their head outside their stable in order to record their facial expressions in a neutral relaxed situation. Three seconds of each film were selected to provide baseline data as per Bremhorst et al. (2019).

2.4. Experimental procedure

2.4.1. Step 1: Habituation to the feeder

The horse was brought to the stall and the head collar was removed. The experimenter encouraged the animal to investigate the device and then manipulated the different panels to get the horses used to the mechanism. The habituation phase lasted 15 min and was repeated on multiple occasions until the horses were accustomed to the mechanism. During this habituation phase the cameras were already present.

2.4.2. Step 2: Reward anticipation

The transparent Perspex panel was initially closed. With the horse less than 1 m from the feeder, the experimenter poured food (500 g, the usual pellet of the horses) into the feeder. After 10 s the other experimenter slid the Perspex panel to give the subject access to the food (Fig. 2). This context was repeated on multiple occasions so that the horse learned to anticipate the arrival of food after 10 s. The learning performance criterion was that the front feet of the horse stayed within 1 m of the feeder for 10 s

Step 2 began the day after Step 1. For each horse, Steps 1 and 2 were to be completed within the same week. They then started Step 3 the following week.

2.4.3. Step 3: Consolidation of food anticipation

Once the horse had learned to anticipate the arrival of food after 10 s, the horse needed to repeat five consecutive trials during which he/she stayed within 1 m of the feeder. When the horse reached this criterion, he/she was considered ready to start testing (Step 4). This began two days later.

2.4.4. Step 4: Testing

On the test day, one of the experimenters brought the horse into his/her stable and removed the head collar. The two cameras then began to record and each experimenter stood behind one of the screens. When the horse was 1 m from the feeder the food was released, which signalled the beginning of one of the three tests: anticipation (Fig. 2) / frustration (Fig. 3) / disappointment (Fig. 4).

The frustration test was similar to the anticipation one, but after the 10 s, the Perspex panel was not removed. This marked the beginning of the food frustration test, which finished when the subject was given access to the food 1 min later (Fig. 3). Frustration was not assessed in the preceding 10 s, during which anticipation could be expected to occur, given the prior learning.

For the disappointment test, the procedure was initially as per the food anticipation test: the horse had no access to food for the first 10 s. During this period, the transparent Perspex panel was closed and the opaque panel was opened. After this period, the opaque panel was closed and then the transparent Perspex panel was opened, allowing the horse access to an empty bucket for 1 min (Fig. 4).

The experimental order of testing subjects was as per Tables 1 and 2, with horses randomly but evenly allocated to one of two groups. There were 10–15 min between subsequent tests during which the feeder was cleaned.

2.5. Video sampling

For each horse, ten 3-second samples of video were extracted using Shotcut software (version 21.03.21) to be used for EquiFACS coding (Bremhorst et al., 2019). These 10 samples related to the baseline sample, three anticipation phases, three frustration phases and three disappointment phases. The starting point of these samples was randomly selected using the *'sample'* function in R software (version 4.1.1). The criterion for considering a sample valid was that the horse was within one metre of the feeder and that it was visible for at least two seconds. If this criterion was not met then another random starting point was generated. For the anticipation phase, the samples were extracted

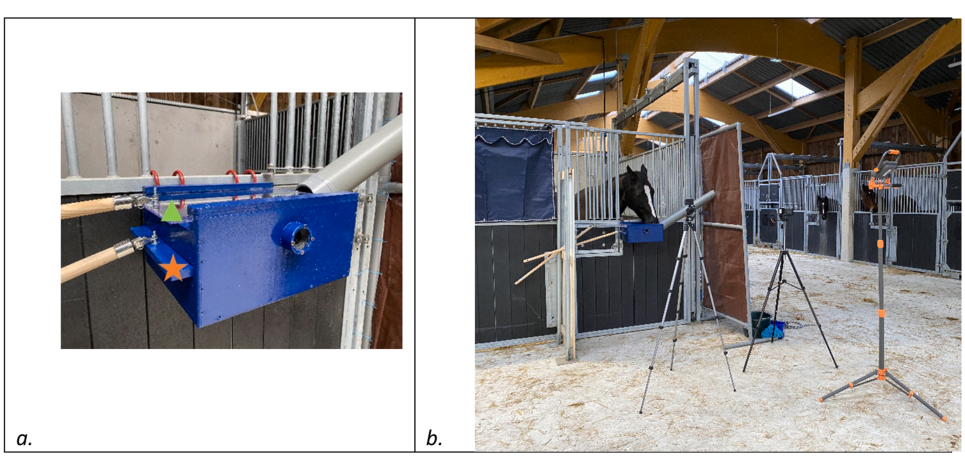

Fig. 1. a. Feeder with the two different panels (orange star = opaque panel, green triangle = transparent Perspex panel), along with a device to put the feed in (grey pipe), b. experimental set-up with the feeder attached outside the stable, two screens allowing the experimenters to remain hidden during the tests and two cameras to record during the tests.

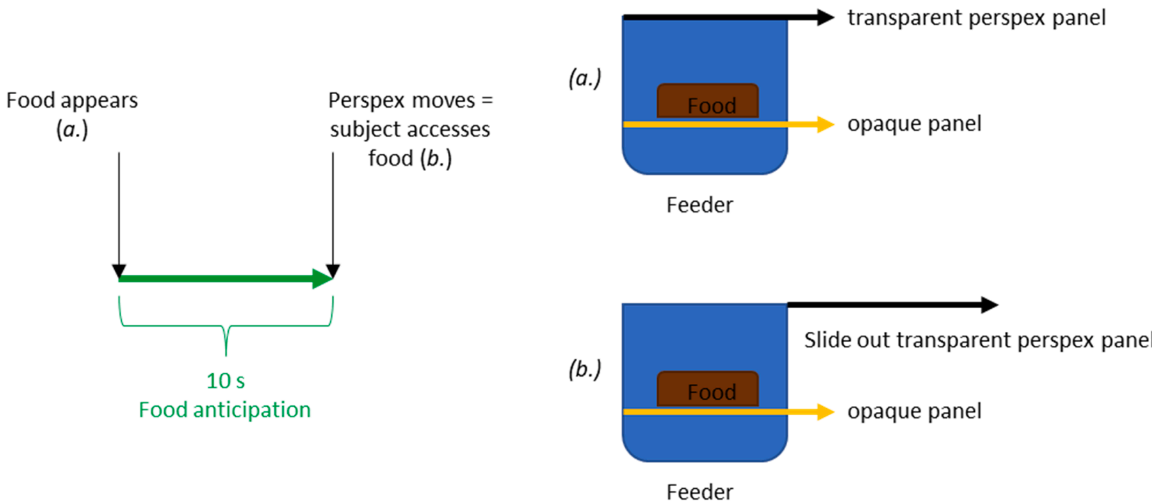

Fig. 2. *Food anticipation test showing the procedure with the movement of the different panels.*

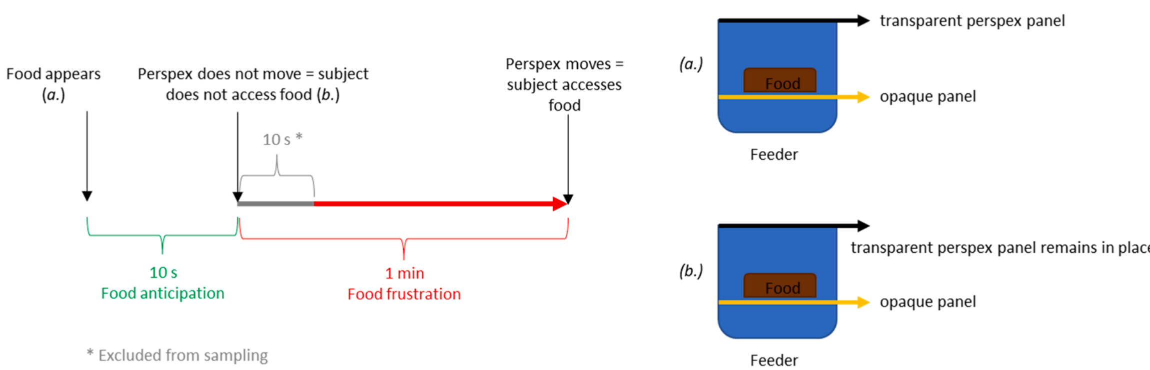

Fig. 3. Food frustration test showing the procedure with the movement of the different panels.

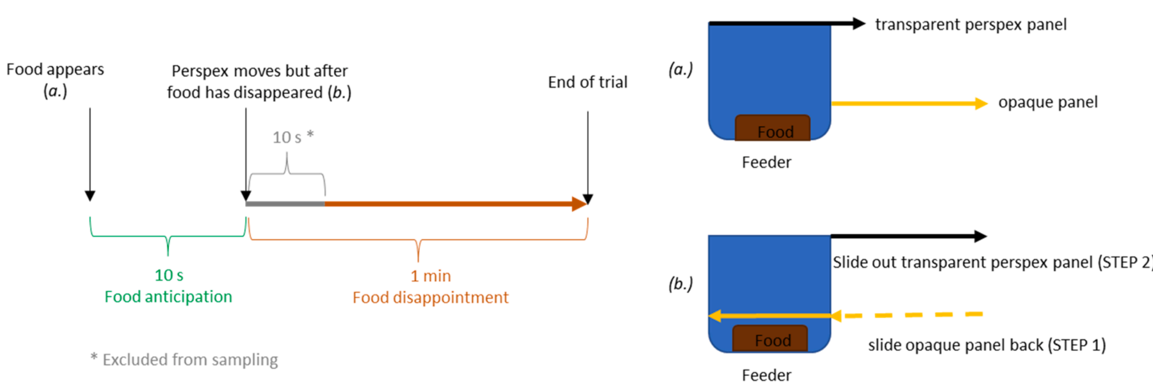

Fig. 4. Food disappointment test showing the procedure with the movement of the different panels.

Table 1
Order of tests for a horse starting with the frustration test, followed by the disappointment test (Note: the number corresponds to the order of tests, bold = period when the measurements were taken).

Part 1	1- **Anticipation**	2- **Anticipation** and **frustration**	3- **Anticipation**	4- Anticipation and **frustration**	5- Anticipation and **frustration**
Part 2	6- Anticipation	7- Anticipation and **disappointment**	8- Anticipation	9- Anticipation and **disappointment**	10- Anticipation and **disappointment**

from the first ten seconds of the first three tests (Table 1). The sampling for the frustration and disappointment phases did not take place within the first 10 s of the start of these phases in order to minimise the effect of the panels' movements on the horses' behaviour (Figs. 3 and 4).

296 videos samples were selected, with 4 samples not used as the horses were not visible for at least two seconds within them. Among these video samples, for the disappointment phase, it was not possible to have the entire horse's face visible during 3 s on 74 videos out 90 (43 videos with the lower face not visible at all and 31 videos where the lower face was partially visible). For these 43 videos, it was not possible

Table 2
Order of tests for a horse starting with the disappointment test, followed by the frustration test (Note: the number corresponds to the order of tests, bold = period when the measurements were taken).

Part 1	1- **Anticipation**	2- **Anticipation** and **disappointment**	3- **Anticipation**	4- Anticipation and **disappointment**	5- Anticipation and **disappointment**
Part 2	6- Anticipation	7- Anticipation and **frustration**	8- Anticipation	9- Anticipation and **frustration**	10- Anticipation and **frustration**

to find a sample where the horse's face was outside the feeder for at least three seconds.

2.6. Measurements

All the video samples were coded by a certified EquiFACS coder (C.R. B.) based on the EquiFACS manual (https://animalfacs.com/equifacs_new). All Action Units, Action Descriptors and Visibility Codes were coded as present or absent using one-zero sampling method (Bateson and Martin, 2021) with these 3-second video samples being the coding interval (Fig. 5 and Table 3). For the Action Descriptors, two variables which were not present in the EquiFACS manual were added: licking and biting the feeder. For the analysis, only EquiFACS variables occurring in more than 10% of one of the four situations (baseline, anticipation, frustration and disappointment) were considered. Fig. 6.

2.7. Analyses

2.7.1. Intercoder reliability

In order to ensure the reliability of the coding, a second certified EquiFACS coder (N.J.) coded 30 videos samples, i.e. more than 10% of the videos samples created. These video samples were randomly selected (by using the '*sample*' function in R software) and renamed so that the second coder was blind to the condition. Pretraining between the two coders was undertaken to minimise the risk of coding problems. The level of agreement between the two coders was calculated for the 28 variables with a prevalence above 10% using Cohen's Kappa coefficient of concordance (with the R-package '*irr*'). Only the variables with a strength of agreement of at least "substantial" (>0.60) were kept in the following analyses (Landis and Koch, 1977).

2.7.2. Potential effect of repeated measurements and group order

For each situation (anticipation, frustration and disappointment) multiple samples (i.e. three for each) and the group to which the horses belong were included in a binomial mixed effect model (with the R-packages: '*lme4*', '*car*', '*MASS*' and '*glmer*' function). The behavioural variables (coded as 0 for absent and 1 for present) were used as responses, with sample order (1,2,3) and the group included as fixed factors and the subject's identity as a random factor. The Holm-Bonferroni method was used to correct for multiple hypothesis testing in order to control for type 1 errors.

For the disappointment phase, the following variables were not analysed as they were not visible (the lower face of the horse was in the feeder box): upper lip raiser, lip corner puller, lower lip depressor, lip pucker, lips part and jaw drop.

2.7.3. Investigation of situations and gender on horse's facial expressions

As no effect of repeated measurements and group order were found in the initial analyses, the data from the different situations were pooled.

In order to investigate which variables differed as a function of the situation, a binomial mixed effect model was run with the occurrence of the behavioural variables (coded as 0 for absent and 1 for present) used as responses, the situation (anticipation/frustration/disappointment) and the gender (male and female) as fixed factors, and the subject's identity as a random factor. The baseline was excluded from the analysis as changes in facial expression and behaviour during this time were extremely rare so analysis could not be undertaken with their inclusion due to zero inflation.

As the subjects were of very different ages (2–23 years old) and breeds (with 11 horses of unknown breed), these factors were not taken into account in the analysis.

3. Results

The horses needed 2–7 trials to learn to anticipate the arrival of a reward after 10 s (mean ± SD: 3 trials, ± 1.39). Only one horse failed the training phase for food anticipation, i.e. the horse did not stay within 1 m of the feeder for 10 s for five consecutive trials. Consequently, this

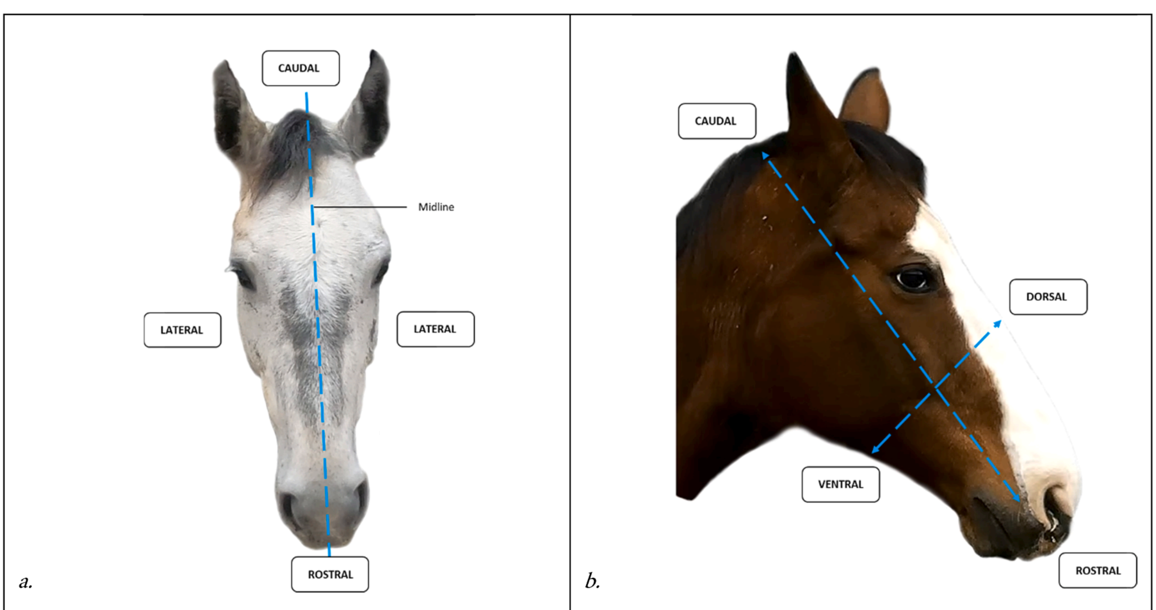

Fig. 5. Anatomical directions used for the definition of the EquiFACS (Horse Facial Action Coding System) variables in frontal (a.) and profile (b.) view of the horse.

Table 3
EquiFACS (Horse Facial Action Coding System) variables used for video samples coding. Note: AU = Action Unit, AD = Action Descriptor, EAD = Ear Action Descriptor; italic: variables added and not present in the EquiFACS manual; bold: EquiFACS variables with a prevalence of more than 10% in one of the four situations (baseline, anticipation, frustration and disappointment); bracket: variables with a strength of agreement between coders below 'substantial'; definition of gross behaviours are adapted from McDonnell (2003).

Category	Code	Variable name	Definition (adapted from EquiFACS manual)
Action Unit			
Upper Face Actions Units	AU101	**[Inner brow raiser]**	Skin above the inner corner of the eye is pulled dorsally and obliquely towards the medial frontal region.
	AU143	Eye closure	Both eyelids move towards each other to close the eye for at least 0.5 s
	AU145	**Blink**	Both eyelids move towards each other to close the eye for less than 0.5 s
	AU47	**Half blink**	Both eyelids move towards each other but the eye is not closed completely.
	AU5	**[Upper lid raiser]**	Upper eyelid raises by pulling it caudally and dorsally, leading to an increase of the eye opening.
Lower Face Actions Units	AU10	**Upper lip raiser**	Central part of the upper lip raises straight up.
	AU12	**Lip corner puller**	Lip corners are pulled back caudally.
	AU113	Sharp lip puller	Upper lip's corner pulls up towards the bridge of the nose.
	AUH13	**Nostril lift**	Nostril elongates.
	AU16	**Lower lip depressor**	Lower lip pulls down ventrally.
	AU17	Chin raiser	Lower lip and skin covering the mental region are pushed upwards.
	AU18	**Lip pucker**	Upper lip protrude, being pushed forward.
	AU122	Upper lip curl	Upper lip everts and curl up.
	AU24	Lip presser	Upper lip lowers and lower lip raises to press the lips together.
	AU25	**Lips part**	Lips separation.
	AU26	**Jaw drop**	Lower jaw is lowered in a relaxed movement; teeth separation can be clearly seen or at least inferred.
	AU27	Mouth stretch	Lower jaw is pulled down and mouth stretched open; teeth separation is visible.
Action Descriptor			
Upper Face Action Descriptor	AD1	**Eye white increase**	White sclera becomes visible in any part of the eye.
Lower Face Action Descriptor	AD160	Lower lip relax	Lower lip is visibly relaxed and hangs loose with no tension.
Ear Actions Descriptors	EAD101	**Ears forward**	One or both ears are turned or swivelled forward (rostrally).
	EAD102	**[Ear adductor]**	One or both ears are pulled towards the midline.
	EAD103	**Ear flattener**	One or both ears are flattened and abducted.
	EAD104	**Ear rotator**	Ears are rotated laterally and caudally.
Miscellaneous Actions and Supplementary Codes	AD19	**Tongue show**	Tongue is shown and it reaches beyond the teeth; the jaw must be lowered and the lips separated.
	AD29	Jaw thrust	Lower jaw is pushed forward and lower teeth extends in front of the upper teeth.
	AD30	Jaw sideways	Lower jaw is moved sideways; chin and lower lip displaced from the midline to one side or the other.
	AD103	Blow	Nostril is dilated briefly, with a rounder shape, when air is blown out sharply.
	AD38	**[Nostril dilator]**	Nostril wings are flared and increase of the nostril aperture.
Gross Behaviour Codes	AD50	Vocalization	Sound produced for communication.
	AD76	Yawning	Deep and long inhalation with mouth widely opened. The jaws are either directly opposed or moved from side to side.
	AD80	Swallow	Saliva going from the mouth to the stomach.
	AD81	**Chewing**	Upper and lower jaws side-to-side grinding movement.
	AD84	Head shake side to side	Head moves rapidly and rhythmically side-to-side along a vertical axis.
	AD85	Head nod up and down	Head moves rapidly and rhythmically up-and-down.
	AD86	Grooming	Horse scratches a part of his/her body.
	AD87	Ear shake	Ears move rapidly and rhythmically.
	-	***Licking feeder***	Tongue extends through the teeth and the lips, and makes contact with the feeder.
	-	***Biting feeder***	Horse takes a part of the feeder in his/her mouth by grabbing it between his/her teeth.
Head Movements Codes	AD51	**Head turn left**	Head moves left along a vertical axis.
	AD52	**Head turn right**	Head moves right along a vertical axis.
	AD53	**Head up**	Head moves upwards.
	AD54	**Head down**	Head moves downwards.
	AD55	**[Head tilt left]**	Head is tilted to the left side.
	AD56	**Head tilt right**	Head is tilted to the right side.
	AD57	**[Nose forward]**	Nose is pushed forward, and this is not due to a head toss.
	AD58	Nose back	Nose is brought in towards the chest.

horse was excluded from the experiment.

3.1. Intercoder reliability

Of the 28 variables with a prevalence above 10% in one of the four phases, 22 of them had a strength of agreement between coders 'almost perfect' or 'substantial'. The following 6 variables had agreement between coders below 'substantial': 'inner brow raiser' (AU101), 'upper lid raiser' (AU5), 'ear adductor' (EAD102), 'nostril dilator' (AD38), 'head tilt left' (AD55) and 'nose forward' (AD57) (Table 3 and Supplementary material Table S2). Consequently, these variables were not analysed further.

3.2. Potential effect of repeated measurements and group order

The repetition of measurements (3 samples) for each situation and the group (Table 1 & 2) to which the subject belonged had no significant effect on the variables of interest (Table 4).

This analysis of sample number measures and group order could not be done for the following variables as these behaviours were rare (zero inflation): chewing (anticipation and frustration phases), licking feeder (anticipation phase) and biting feeder (anticipation and disappointment phases).

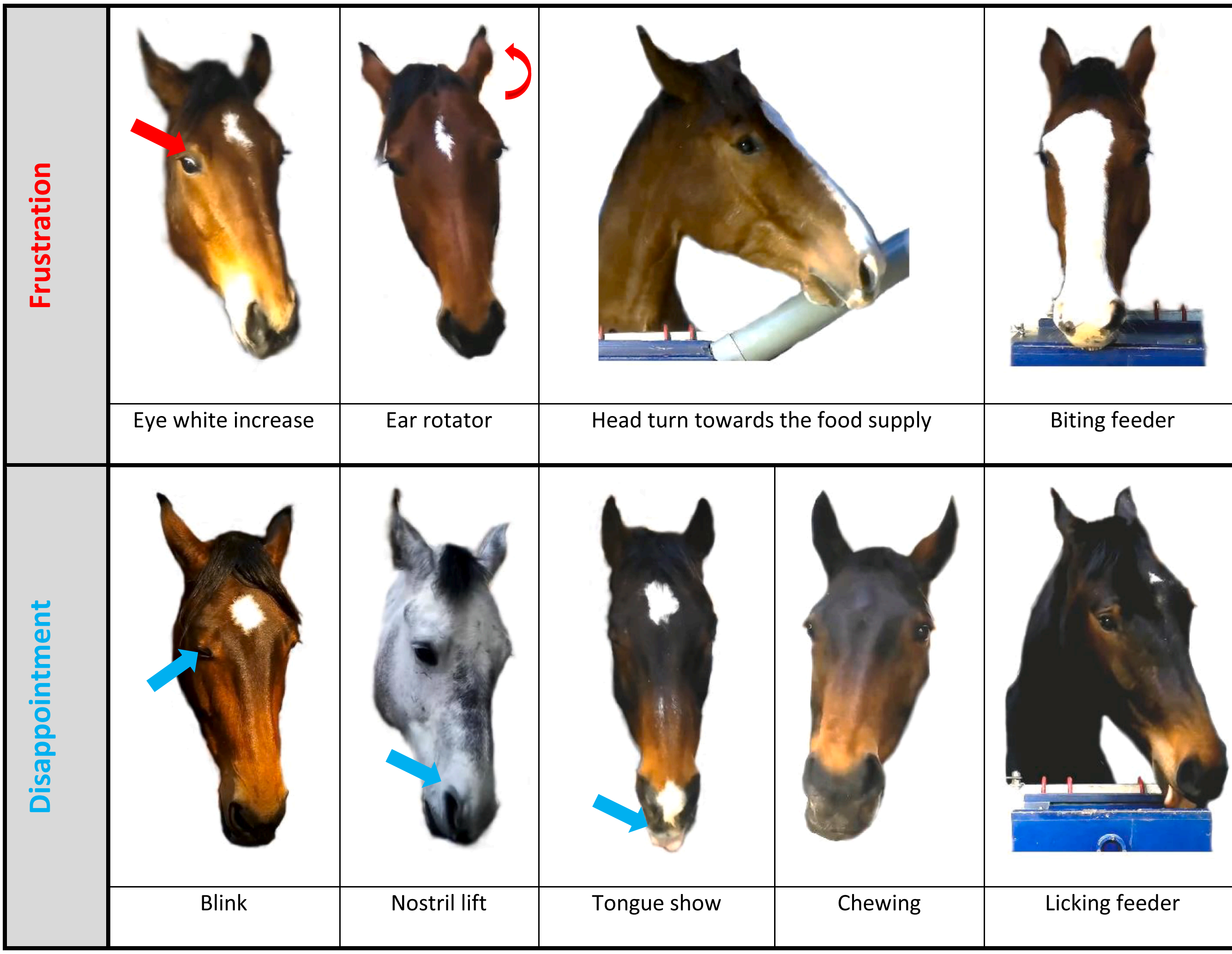

Fig. 6. Horse's facial expressions and behaviours typical of inferred frustration and disappointment.

3.3. Investigation of situations and gender on horse's facial expressions

9 out 22 variables varied significantly between the situations (Table 5 and Supplementary material Table S3). During the disappointment phase, the following variables were more likely to occur than during the anticipation and frustration phases: 'nostril lift' (AUH13), 'tongue show' (AD19), 'chewing' (AD81) and 'licking feeder', whereas 'eye white increase' (AD1), 'ear rotator' (EAD104), and 'head turn left' (AD51) were more likely during the anticipation and frustration phases than the disappointment phase. 'Biting box' was more likely in the frustration phase than the disappointment phase whereas the variable 'blink' (AU145) was more likely in the disappointment phase than the frustration phase. Moreover, this is the only variable which was affected by the subject's gender. Females were more likely to blink than males.

4. Discussion

The aim of this study was to identify significant facial differences potentially associated with positive emotional facial expression (related to the anticipation of a food reward) versus negative ones (frustration from the denial of access to a visible food reward and disappointment characterised by the loss of food before it can be accessed). The method of one-zero sampling allowed us to identify differences in the likelihood of a response occurring during equivalent-length, randomly chosen samples of the horse's state, rather than the frequency with which it occurs. This was chosen as a method because from a pragmatic perspective it is easier for observers to appreciate differences in the likelihood of a behaviour occurring at all than relative frequency. Perhaps surprisingly, we did not find evidence to allow the differentiation of phases involving the potential positive anticipation of food and the extended denial of access to it (frustration phase). However, 9 out of 22 actions and behaviours were found to vary significantly between the frustration and disappointment situations. The disappointment phase was characterised by a higher likelihood of occurrence of 'blink' (AU145), 'nostril lift' (AUH13), 'tongue show' (AD19), 'chewing' (AD81) and 'licking feeder' compared to the frustration situation. By contrast, 'eye white increase' (AD1), 'ear rotator' (EAD104), 'head turn left' (AD51) and 'biting feeder' were more likely in the frustration phase than the disappointment situation. Fewer (7) and generally weaker (see Table 5) significant contrasts between positive anticipation and disappointment contexts were found. In addition, there was a gender effect in the present study with females more likely to blink than males. It is important to note that the horses have only been tested in a feeding context and therefore we cannot conclude that our findings are directly related to underlying emotion as they might be specific to feeding motivation. In order to make a stronger claim that specific facial expressions are related to an emotional state, horses would need to be tested in additional contexts, potentially associated with equivalent emotional responses but not related with food. The ability to reliably infer emotional states would also be strengthened by contingent evidence from other components of the given emotion, such as physiological measurements of arousal.

Table 4
Results of the comparison between samples and groups variables within each situation (Note: statistical test: binomial mixed effect models; -: variables not analysed are not visible in this situation; #: variables rare so impossibility to analysed due to zero inflation).

		Anticipation		Frustration		Disappointment	
Variable name (Code)	df	χ^2	P-value (Holm-Bonferroni corrected)	χ^2	P-value (Holm-Bonferroni corrected)	χ^2	P-value (Holm-Bonferroni corrected)
Blink (AU145)							
Group	1	0.85	0.356 (1)	1.13	0.288 (1)	0.67	0.412 (1)
Sample	2	0.37	0.831 (1)	1.28	0.527 (1)	1.75	0.417 (1)
Half blink (AU47)							
Group	1	1.48	0.224 (1)	0.24	0.624 (1)	0.15	0.703 (1)
Sample	2	1.80	0.407 (1)	3.13	0.209 (1)	0.10	0.950 (1)
Upper lip raiser (AU10)							
Group	1	0.06	0.807 (1)	0.23	0.633 (1)	-	-
Sample	2	2.18	0.336 (1)	1.85	0.397 (1)	-	-
Lip corner puller (AU12)							
Group	1	0.04	0.847 (1)	3.31	0.069 (1)	-	-
Sample	2	3.24	0.197 (1)	0.79	0.672 (1)	-	-
Nostril lift (AUH13)							
Group	1	2.15	0.143 (1)	0.006	0.938 (1)	2.22	0.136 (1)
Sample	2	0.37	0.830 (1)	1.02	0.600 (1)	0.62	0.735 (1)
Lower lip depressor (AU16)							
Group	1	0.42	0.517 (1)	0.51	0.475 (1)	-	-
Sample	2	0.15	0.926 (1)	0.61	0.737 (1)	-	-
Lip pucker (AU18)							
Group	1	0.20	0.656 (1)	1.49	0.223 (1)	-	-
Sample	2	1.21	0.545 (1)	0.81	0.669 (1)	-	-
Lips part (AU25)							
Group	1	1.26	0.261 (1)	0.12	0.732 (1)	-	-
Sample	2	0.56	0.756 (1)	1.67	0.433 (1)	-	-
Jaw drop (AU26)							
Group	1	1.71	0.191 (1)	1.38	0.240 (1)	-	-
Sample	2	0.03	0.986 (1)	3.38	0.184 (1)	-	-
Eye white increase (AD1)							
Group	1	1.25	0.263 (1)	0.31	0.580 (1)	5.21	0.022 (0.33)
Sample	2	0.52	0.771 (1)	0.39	0.822 (1)	0.91	0.634 (1)
Ears forward (EAD101)							
Group	1	1.82	0.177 (1)	0.70	0.403 (1)	0.00	1.000 (1)
Sample	2	1.41	0.495 (1)	1.91	0.386 (1)	3.83	0.147 (1)
Ear flattener (EAD103)							
Group	1	0.001	0.977 (1)	0.002	0.965 (1)	1.40	0.237 (1)
Sample	2	0.73	0.693 (1)	1.07	0.586 (1)	3.42	0.181 (1)
Ear rotator (EAD104)							
Group	1	0.09	0.767 (1)	1.28	0.257 (1)	0.05	0.827 (1)
Sample	2	0.54	0.764 (1)	1.29	0.526 (1)	0.00	1.000 (1)
Tongue show (AD19)							
Group	1	0.12	0.732 (1)	0.61	0.434 (1)	0.16	0.686 (1)
Sample	2	1.02	0.601 (1)	3.41	0.182 (1)	1.44	0.488 (1)
Chewing (AD81)							
Group	1	#	#	#	#	3.22	0.073 (1)
Sample	2	#	#	#	#	3.56	0.168 (1)
Licking feeder							
Group	1	#	#	0.19	0.667 (1)	0.15	0.698 (1)
Sample	2	#	#	1.60	0.450 (1)	5.77	0.056 (0.84)
Biting feeder							
Group	1	#	#	0.06	0.813 (1)	#	#
Sample	2	#	#	0.38	0.829 (1)	#	#
Head turn left (AD51)							
Group	1	2.15	0.142 (1)	0.96	0.328 (1)	0.05	0.828 (1)
Sample	2	0.81	0.667 (1)	2.22	0.329 (1)	1.21	0.547 (1)
Head turn right (AD52)							
Group	1	0.24	0.628 (1)	0.09	0.759 (1)	1.67	0.196 (1)
Sample	2	2.77	0.250 (1)	0.15	0.928 (1)	1.12	0.570 (1)
Head up (AD53)							
Group	1	1.03	0.311 (1)	2.19	0.139 (1)	0.04	0.838 (1)
Sample	2	0.50	0.778 (1)	5.55	0.062 (1)	0.87	0.647 (1)
Head down (AD54)							
Group	1	0.01	0.921 (1)	3.82	0.051 (1)	0.00	0.997 (1)
Sample	2	1.32	0.516 (1)	1.90	0.386 (1)	3.37	0.185 (1)
Head tilt right (AD56)							
Group	1	0.16	0.689 (1)	0.03	0.854 (1)	0.21	0.643 (1)
Sample	2	2.33	0.313 (1)	0.76	0.683 (1)	3.15	0.207 (1)

Table 5
Results of the comparison between tests (anticipation/frustration/disappointment) and gender on each variable for the 30 horses in order to investigate which behavioural variables differed as a function of the situations (Note: statistical test: binomial mixed effect models; Ant. = Anticipation, Fru. = Frustration, Dis. = Disappointment, S.E. = Standard error; bold: significant result with $p < 0.05$).

Variable name	Estimate	S.E.	Z	P-value
Blink (AU145)				
Ant. vs Fru.	-0.30	0.32	-0.92	0.357
Ant. vs Dis.	0.39	0.32	1.21	0.226
Fru. vs Dis.	0.68	0.32	2.13	**0.033**
Gender	-0.83	0.36	-2.31	**0.021**
Nostril lift (AUH13)				
Ant. vs Fru.	-0.54	0.36	-1.51	0.130
Ant. vs. Dis.	1.77	0.44	4.03	**< 0.001**
Fru. vs. Dis.	2.31	0.45	5.14	**< 0.001**
Gender	0.10	0.59	0.17	0.861
Eye white increase (AD1)				
Ant. vs Fru.	-0.23	0.35	-0.65	0.513
Ant. vs. Dis.	-1.66	0.39	-4.25	**< 0.001**
Fru. vs. Dis.	-1.43	0.38	-3.72	**< 0.001**
Gender	0.43	0.62	0.70	0.485
Ear rotator (EAD104)				
Ant. vs Fru.	0.20	0.34	0.59	0.555
Ant. vs. Dis.	-1.40	0.34	-4.16	**< 0.001**
Fru. vs. Dis.	-1.60	0.34	-4.69	**< 0.001**
Gender	0.14	0.35	0.39	0.698
Tongue show (AD19)				
Ant. vs Fru.	1.04	0.59	1.77	0.077
Ant. vs. Dis.	3.37	0.77	4.39	**< 0.001**
Fru. vs. Dis.	2.33	0.65	3.61	**< 0.001**
Gender	0.22	0.65	0.34	0.733
Chewing (AD81)				
Ant. vs Fru.	-0.76	0.89	-0.85	0.393
Ant. vs. Dis.	1.64	0.59	2.77	**0.006**
Fru. vs. Dis.	2.40	0.78	3.09	**0.002**
Gender	0.19	0.56	0.33	0.740
Licking feeder				
Ant. vs Fru.	0.72	0.63	1.14	0.253
Ant. vs. Dis.	4.02	0.65	6.20	**< 0.001**
Fru. vs. Dis.	3.30	0.54	6.14	**< 0.001**
Gender	-0.26	0.64	-0.40	0.688
Biting feeder				
Ant. vs Fru.	0.89	0.62	1.43	0.152
Ant. vs. Dis.	-0.90	0.80	-1.13	0.261
Fru. vs. Dis.	-1.79	0.76	-2.35	**0.019**
Gender	-0.66	1.24	-0.53	0.594
Head turn left (AD51)				
Ant. vs Fru.	0.20	0.32	0.61	0.539
Ant. vs. Dis.	-0.72	0.32	-2.23	**0.026**
Fru. vs. Dis.	-0.91	0.32	-2.84	**0.005**
Gender	-0.04	0.36	-0.11	0.914

By contrast, Bremhorst and colleagues (2019) who were working with dogs, found evidence of a difference in facial expressions between the anticipation and frustration situations. There are several possible explanations for why we might not have seen the equivalent difference in our study.

Firstly, the lack of significant differences in facial movements and behaviours could potentially reflect a genuine lack of facial differences between these two emotions (Anderson et al., 2020). For example, in horses, pawing has been reported to be associated with anticipation (Peters et al., 2012) as well as frustration (Ninomiya et al., 2013; Briefer Freymond et al., 2020; Rørvang et al., 2021); individual behaviours might therefore be uninformative. It is possible that anticipation and frustration differ in terms of the intensity rather than likelihood of behaviours shown (Anderson et al., 2020). This suggestion is potentially supported by our finding of a stronger differentiation of disappointment-phase response from the frustration-phase response than from the positive anticipation-phase response. Given our method of sampling we did not measure rates. Correia-Caeiro et al. (2020), found the rate of production of different action units particularly useful for distinguishing several emotional states in dogs, while Yayou et al. (2009), found that ewes had a more general increase in frequency of behaviours during frustration, which is generally associated with higher arousal and an intensification of behavioural response.

The focus of our behavioural assessment might also be a contributing factor to the lack of statistically significant differences between the positive anticipation and frustration phases. We focused on facial movements and behaviours related to the head area (e.g. licking, head turn, etc.). These regions are perhaps not relevant for differentiating differences between these two emotions and measurement from other regions may be more informative. Gygax et al. (2013) found that during frustration situations (no access to the food), goats had an increase in their locomotor and prefrontal cortical activity whereas when they had access to food, they had a decrease in their locomotor activity and had a greater sympathetic reaction. More recently, Rørvang et al. (2021) found a higher heart rate in horses who showed behavioural tendencies indicative of frustration when they could not solve visible and invisible displacement tasks than successful horses. Anecdotally, during our experiments, we noticed some horses pawing or turning in circles in the stable. Thus these more overt behavioural expressions (often referred to as displacement and redirected behaviours) might be more relevant for recognising frustration (Broom, 2019) than facial expressions.

Another possibility is that the anticipation and frustration situations did not elicit responses with the differential emotional valences expected, i.e. a positive valence for the anticipation and a negative valence for the frustration (Mendl et al., 2010). It is possible that during the anticipation period the positive emotion of waiting for access to the food can turn into frustration (Moe et al., 2009). However, in order to try to prevent this type of emotional shift from happening in our study, we trained the horses to reliably wait for the reward for 10 s. Nonetheless, it is possible that in both our experimental situation and in general that the pre-feeding period is not perceived as a positive event, since in horses it is often prolonged and they have no control over the situation, i.e. they cannot control when they will have access to the food as they are fed when the human decides to do so. Indeed the feeding period is recognised by experts as a potential cause of frustration in horses (Pannewitz and Loftus, 2023). The fact that in our experiment the horses could detect that the food was in the feeder but could not access it because of the transparent Perspex panels may have simply induced frustration from the outset. For example, delaying or changing the timing of the feeding period in horses can induce frustration behaviours (Ninomiya et al., 2004; Cooper et al., 2005) and increase the occurrence of stereotypic behaviours before the food arrives.

Finally, despite the training of all the horses to anticipate the food after 10 s, individual differences in terms of emotional response might be present. It has been reported that there appears to be variation in a horse's specific susceptibility to frustration (Fureix et al., 2011), as is the case in humans (Caprara et al., 1985; Seymour et al., 2016) and dogs (McPeake et al., 2019). Another possibility is that other aspects of the personality of the horses affected their appraisal of the situation and consequently, their emotional response (Boissy, 1995) and behavioural reactions (Roberts et al., 2016; Mott et al., 2020).

Our results did however distinguish the context designed to elicit disappointment. 'Head turn left' (AD51) was more likely to occur in the anticipation and frustration situations than the disappointment one. This difference in the incidence of head movement during these two situations may be explained by a change in focus and/or an increase in activity (Ninomiya et al., 2004; Peters et al., 2012) resulting from a high arousal state. For example, Peters and colleagues (2012) found a significant increase in the frequency and duration of arousal and investigation and locomotion, with standing also accompanied by an increase in heart rate. There is also growing scientific interest in the use of lateralised responses as a measure of emotional valence in animals (Simon et al., 2022). A bias in head movement toward the left side has been observed in dogs when presented images of differing emotional valence during feeding (Siniscalchi et al., 2010), with them turning their head towards the left when the image was of a negative valence (black shape of a snake or of a cat in defensive threat posture). In the literature on horses, a preferential leftward bias in either head or eye movement (due to increased right hemispherical activity) has been associated with an object of negative valence of an object (De Boyer des Roches et al., 2008), a conspecific (Austin and Rogers, 2012, 2014), a human (Smith et al., 2016; d'Ingeo et al., 2019), attention and vigilance behaviours (Austin and Rogers, 2012, 2014; Rochais et al., 2018). The left side bias in the present experiment may suggest that the horses perceived the experimental condition as a negative situation. However, another and arguably more likely reason for this difference is that the pipe bringing the food into the wooden box was on the left side of the horse. So horses may be turning their head in this direction in anticipation of the arrival of food during the anticipation and frustration situations. This behaviour is less common in the disappointment situation, and this might be because the food had already fallen into the feeder before that the feeder was open for one minute without food. This highlights the importance of considering potential incidental environmental confounds in studies of lateralised responses.

The position of the horses' ears is an important source of information concerning their appraisal of a situation (Waring, 2003). In the present study, 'Ear rotator' (EAD104) was more likely to occur during anticipation and frustration situations than during the disappointment one. Moreover, no difference in terms of absolute likelihood of occurrence has been found across the three situations for 'Ears forward' (EAD101) and 'Ear flattener' (EAD103). In horses, 'ears forward' is often associated with positive interactions (Hausberger and Muller, 2002; McDonnell and Poulin, 2002; Fureix et al., 2009) and attention/vigilance (McDonnell and Haviland, 1995; Hausberger and Muller, 2002) whereas the position 'ears backward' is often present during negative interactions (McDonnell and Haviland, 1995; Hausberger and Muller, 2002; Fureix et al., 2009) and pain (Dalla Costa et al., 2014; reviewed by Hausberger et al., 2016). There was no difference in the likelihood of occurrence of 'Ears forward' between the three situations probably due to the fact that the horses pay attention towards the feeder, waiting or looking for food.

'Ear rotator' was more commonly recorded during anticipation and frustration than during disappointment situations. This result is in accordance with the finding of Rochais et al. (2018) that horses spend more time with the ears backwards when they express frustration behaviours toward a food device (e.g. teeth pulling, head pushing). However, it seems that sometimes in the literature the term 'ears backward' is used for both 'ear flattener' (EAD103) and 'ear rotator' (EAD104) (Rashid et al., 2020). Thus these movements do not use the same muscles (Wathan et al., 2015) and emphasise the value of a FACS coding type of approach. The ears flattened to the head/neck seem to correspond to a negative facial expression (Lansade et al., 2022) whereas the ears backward can be associated with a positive facial expression especially during grooming (Lansade et al., 2018; Trösch et al., 2020). It would therefore be useful to investigate further whether the position of the ears is associated with a general emotional or more contextually specific situation.

There was also a difference in the eye regions in relation to the experimental contexts for the actions 'Eye white increase' (AD1) and 'Blink' (AU145). In our study, we found that more horses showed 'Eye white increase' during anticipation and frustration than disappointment situations. This result initially seems contradictory to what is present in the literature on ungulates, i.e. that an increased visibility of the white sclera is associated with negative situations such as fear and/or stress (cows: Sandem et al., 2004; horses: Hintze et al., 2016; Lundblad et al., 2021). However, two separate studies in cows have shown an increase in the white area during food anticipation (Sandem et al., 2006) but also when food is inaccessible for 6 min (the cow can see and smell the food in the wooden box through a Plexiglas cover panel with holes) (Sandem et al., 2002) compared to when they have access to it. It is therefore possible that the increased visibility of sclera is associated more with increased arousal rather than emotional valence or quality. Anticipation of a reward and frustration due to blocked access (e.g. delay, inaccessibility) are generally recognised as leading to high arousal whereas the consumption of this reward or disappointment are more typically related to lower arousal (Mendl et al., 2010).

Contrary to the results of Bremhorst et al. (2019), who found that the action unit 'Blink' (AU145) was more likely in the frustration phase than the anticipation one in dogs, we did not find any difference between these two situations. However, in our research, 'Blink' was more likely to occur in the disappointment phase than in the frustration phase sampled. In horses, eye blink rate has been used as an indicator of stress (Merkies et al., 2019; Mott et al., 2020; Lundblad et al., 2021) but the results of these studies are inconsistent. For example, Merkies et al. (2019) tested horses in four situations of three minutes each (three treatments tested: feed restriction, separation with conspecifics and startle test, and one control situation: horse in paddock with visual contact with others) and found that horses blinked significantly less often in the three treatments compared to the control situation. Moreover, they concluded that the feeding restriction was perceived as the most stressful situation by the horses, with an increase in heart rate noted. By contrast, Mott and colleagues (2020) found an increase in eye blink rate correlated with an increase in salivary cortisol and a decrease in heart rate variability during the stressful situation of sham clipping for 10 min compared to before it started. However, the authors also found a significant reduction in eye blink rate during the first minute of the procedure. Consequently at the beginning of a stressful situation, the individual may be more focused (e.g. to gather information in this environment) which results in a decrease in eye blink rate (Giannakakis et al., 2017). In the present study, the frustration and disappointment situations were of short duration (one minute and 10 s) but the inaccessibility to the food may have been perceived as more stressful than the empty feeder.

Previous research has proposed 'Inner brow raiser' (AU101) as an indicator of emotional valence (Hintze et al., 2016). The authors found an increase in expression of eye wrinkles (angle) during food competition compared to a control (period of one minute before the test) but no change during food anticipation. This result could not be confirmed in the present study as this variable was not retained in the statistical analyses given the lack of agreement between the evaluators.

In the nose region, 'Nostril lift' (AUH13) was more likely to be observed during the disappointment situation than during anticipation and frustration situations. Flared nostrils are often used as an indicator of stress (Lundblad et al., 2021; Pearson et al., 2021) or pain in horses (Dalla Costa et al., 2014; Gleerup et al., 2015; Van Loon and Van Dierendonck, 2015). However, the flare of at least one nostril can also be seen when a horse uses his/her sense of smell to investigate a stimulus (Draaisma, 2017) such as an object (Masko et al., 2020) or a sample of horse body odour (Hothersall et al., 2010; Péron et al., 2014). Thus, when the horse has his/her head in the feeder, it might be that there was increased flaring associated with the food delivery system.

Other expressions also differed across contexts. 'Tongue show' (AD19), 'Chewing' (AD81) and 'Licking feeder' were more likely to be observed during the disappointment situation than the anticipation and frustration ones. Tongue movements (in and out the mouth) associated with feeding periods have been reported by Houpt et al. (1978). They found that horses expressed this behaviour when they cannot access the food. More recently, 'Tongue show' has been observed to occur during stressful situations such as isolation and transportation (Lundblad et al., 2021). This movement of the tongue is possibly a displacement behaviour related to conflicting or stressful situations (Goodwin, 1999). In the present study, it seems that 'Tongue show' was associated with 'Licking feeder'. These oral movements of licking and chewing are generally present after a stressful situation (e.g. horse chase away in a round pen) (Krueger, 2007; König von Borstel et al., 2010) and could be a sign of relaxation or to a lesser extent a release of tension (Krueger, 2007; Thorbergson et al., 2016; Broom, 2019). In fact, after a stressful situation, there is a shift in the sympathetic - parasympathetic tone, associated with relaxation restarting saliva production in the process. Licking and chewing might allow saliva to return to the mouth (König von Borstel et al., 2010). It is possible that in the present study, waiting for the food reward may have caused tension in the horse which decreased during the disappointment situation as the feeder box was opened. Another possibility, is that the horses were more likely to lick the box in the disappointment situation because there could be residues of pellet powder accessible as the box was opened in this situation.

'Biting feeder' was more commonly seen to occur during the frustration situation than the disappointment one. This result is in accordance with previous studies which found that horses expressed frustration behaviours towards a device, such as biting it, when the expectation of a reward was unmet (Goodwin et al., 2007; Rochais et al., 2018; Briefer Freymond et al., 2020).

The only gender effect was on the variable 'Blink' (AU145) which was more commonly recorded in females than males. This is in accordance with the human literature which has found that women blink more often than men (Dreisbach et al., 2005; Sforza et al., 2008; Pult et al., 2013). This difference in humans can be related to the menstrual cycle which has an effect on fluctuations in dopaminergic activity with the latter associated with spontaneous eye blink rate (see review Jongkees and Colzato, 2016). The reason why it occurred in mares in this study is less clear, unless there are more general differences in dopaminergic tone in this gender.

It was not possible to use EquiFACS on the baseline recordings, although we recorded this baseline when the horses were at rest. The aim was to obtain the horse's neutral facial expression but in this situation the horse had very little facial movement and FACS depends on an expressive change for coding. FACS enables the description of facial behaviour through facial muscle movements and more specifically their contraction (Ekman et al., 2002). In conclusion, a limitation of FACS is that it cannot be used in situations where the facial expression is static, which may be more common in many non-human species. As a result of this limitation, some have started to use a morphometric approach to the assessment of facial emotion (Finka et al., 2019; Feighelstein et al., 2022; Gris et al., 2022).

5. Conclusion

This research demonstrates how specific measures of facial expression in the horse can potentially be used to assist the differentiation of the emotional state of the animal. The results highlight how there may be different qualities of a given emotional valence (frustration and disappointment) which can be distinguished from the facial expressions shown at the time. However we must emphasise that our result might be limited to the feeding situation, and so further research is required. Frustration was potentially characterised by a higher likelihood of 'eye white increase', 'ear rotator', 'head turn left' and 'biting feeder' compared to the disappointment situations. By contrast, disappointment might be characterised by a greater likelihood of 'blink', 'nostril lift', 'tongue show', 'chewing' and 'licking feeder'. These changes of facial expressions and behaviours could also be associated with the horse's perception of the situation: using vision (seeing the food through the transparent Perspex panel), sound (hearing the food falling in the wooden box), or smell (smelling residues of pellet food). Facial expressions indicative of anticipation could however not be demonstrated, and so these results raise the important question as to whether the pre-feeding period is a positive event for horses.

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CRediT authorship contribution statement

Conceived and designed the experiments: C.R.B and D.M.; performed the experiments and analysed the data: C.R.B.; interpreted the data and wrote the paper: C.R.B and D.M.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2023.105966.

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