Facial expressions in cattle in different affective states

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Ansiktsuttryck hos nötkreatur vid olika affektiva tillstånd

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Abstract

In order to understand, manage, and help cattle, with the aim of ensuring the animals' welfare, care, and production; it is of utmost importance to gain an understanding of these animals' signals. Understanding facial expressions may help by being a cheap, easy, and non-invasive approach. This study aimed to investigate which facial muscles are present in cattle, which facial expressions can be performed, and whether cattle show a range of facial expressions during different affective states.

This was done in two parts, a dissection of a cow skull, and analyzing video sequences of cows in different affective states. Two dissection models were created, a skinned face and a face mask. The result showed that most of the muscles found were previously documented, however four previously documented muscles were not found in the present study. For example, there was no distinct small muscle, *M. levator anguli oculi medialis*, by the medial side of the eye as previously documented but instead it looked like M. frontalis continued down and connected to a larger portion of *M. orbicularis oculi*.

A total of 21 cattle were studied using 45 second video sequences in four different affective states: neutral state, stress, pain, and pleasure. The footage was collected at different times of the day in their loose housing system, regularly used chute, and pastures. The cows used in this study were of the breeds SRB (the Swedish red-and-white) and SLB (the Swedish Friesian) - with an age range of seven months to eight years according to farm personnel. There were 23 different facial movements observed and registered. The median of those facial movements was then calculated to gather more information about the data and see the value it has in practice. After that, figures were created of the observed facial movements in each individual affective state as well as the frequency of each movement. There were some correlations of higher frequencies of movements in different affective states, such as ear flick in pleasure and neutral state, and increased eye whites during stress and pleasure.

Facial expressions are complicated and have many different aspects and components. More research in this area is needed to get a better understanding of facial expressions in cattle and what they can indicate. Understanding cattle's facial expressions and behavior would benefit not only the animal's production but also their welfare.

Keywords: Affective states, cattle, cow, facial expressions, welfare, dissection

Sammanfattning

För att kunna förstå, hantera och hjälpa nötkreatur i syfte att upprätthålla djurens välfärd, skötsel och produktion är det av yttersta vikt att förstå dessa djurs signaler. Möjligheten att förstå och tolka djurens ansiktsuttryck kan vara ett billigt, enkelt och icke-invasivt tillvägagångssätt för att uppnå dessa mål. Denna studie undersökte vilka ansiktsmuskler som finns hos nötkreatur, vilka ansiktsuttryck dessa djur kan göra och om det fanns en skillnad mellan vilka ansiktsuttryck de visade under olika affektiva tillstånd.

Studien gjordes i två delar, först genom en dissektion av en ko-skalle och därefter genom att filma kor i olika affektiva tillstånd och registrera ansiktsuttrycken manuellt. Vid dissektionen skapades två dissektionsmodeller, ett där ansiktet flåddes (skinned face) och en ansiktsmask (face mask). Resultatet visade att de flesta av de musklerna som hittades är kända och dokumenterade sedan tidigare men också att det fanns fyra dokumenterade muskler som inte kunde hittas i denna studie. I denna dissektion påträffades till exempel ingen distinkt liten muskel, *M. levator anguli oculi medialis*, medialt om ögat, vilket har dokumenterats tidigare, utan i stället såg det ut som att *M. frontalis* fortsatte ned och förenades med en större del av *M. orbicularis oculi*.

Vid videoanalysen studerades totalt 21 nötkreatur i fyra olika affektiva tillstånd, neutralt tillstånd, stress, smärta, och njutning. Videosekvenser var 45 sekunder långa med ansiktsuttryck i fokus. Filmerna spelades in vid olika tidpunkter på dygnet i lösdriften, på betet, samt vid regelbundet användande av verkningsstol. Nötkreaturen som användes i denna studie var av raserna SRB (svensk röd-vit) och SLB (svensk låglandsboskap), med ett åldersintervall om sju månader till åtta år enligt personalen. Totalt observerades och registrerades 23 ansiktsrörelser. En tabell över vilka ansikts-rörelser som observerats i alla fyra affektiva tillstånd skapades och sedan beräknades medianen för varje rörelse för att få mer information om resultaten och dess värde i praktiken. Därefter upprättades diagram av de observerade ansiktsrörelserna över varje affektivt tillstånd samt frekvensen av varje rörelse. Det fanns viss korrelation mellan frekvensen av vissa rörelser och olika affektiva tillstånd. Exempelvis förekom en högre frekvens av öronrörelser vid njutning och neutralt tillstånd. Vidare kunde även ökad ögonvita observeras under både stress och njutning.

Ansiktsuttryck är ett komplicerat ämne med många olika aspekter och komponenter. Mer forskning inom detta område behövs för att ge en bättre förståelse för ansiktsuttryck hos nötkreatur och hur de bör tolkas. Att förstå nötkreaturens ansiktsuttryck och beteende skulle gynna inte bara produktion utan också djurens välfärd.

Nyckelord: Affektiva tillstånd, nötkreatur, ko, ansiktsuttryck, välfärd, dissektion

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1. Introduction

There are approximately 1,389,900 cattle in Sweden today (Jordbruksverket 2022), constituting a large part of Sweden's agriculture. In order to continuously improve the welfare for these animals, it is of utmost importance from a veterinary perspective to understand signals of wellbeing and disease to effectively support their care and production.

There are multiple ways to evaluate the welfare of cattle: through physiological parameters; daily performance; and among other things, behavior (Sørensen *et al.* 2001). Behavior is becoming an important aid in assessing the welfare and emotions of cattle, and has received attention in assessing pain in both cattle and other species (Holton *et al.* 2001; Pritchett *et al.* 2003; Gleerup *et al.* 2015). Facial expressions have been seen as an important variable when evaluating welfare and emotion in a variety of species due to the time efficiency afforded by animals having a higher frequency of facial expressions than body behaviors (Leach *et al.* 2011, 2012). Although assessing emotions, otherwise known as affective states in animals, is no easy task due to the lack of verbal confirmation of the affective state experienced (Ahloy-Dallaire *et al.* 2018); there have been extensive studies done on facial expressions in humans confirming that facial expressions are closely linked to several emotions: such as fear, anger, happiness, and pain (Dores *et al.* 2020). There is a growing state of knowledge in animals' facial expressions, especially in dogs, cats, and horses (Waller *et al.* 2013; Wathan *et al.* 2015; Caeiro *et al.* 2017). In the present, however, there is a lack of knowledge regarding cattle's facial muscles and their specific facial expressions in different affective states.

Gaining a deeper understanding of cattle's facial expressions in different affective states, could increase the ability for owners and animal healthcare professionals to understand the animal's emotions, thus, being able to help the animals in a quicker, easier, and more effective way, therewith increasing the welfare of cattle.

1.1 Aim

This study aimed to investigate which facial muscles were present in cattle and compare them to previously documented literature; as well as which facial expressions could be performed. That was done by dissection of a cow skull and evaluation of videos taken of cows in specific affective states. The affective states studied were neutral state, pain, stress, and pleasure. The hypotheses were that the muscles found would correspond with the muscles previously documented and that cattle show a range of facial expressions during different affective states.

2. Literature Review

Welfare in animals has become a popular subject, not only in pets that are often seen as family members, but also in production animals. The majority of assessments and goals to increase their welfare has had a focus on negative experiences (Yeates & Main 2008). The importance of positive experiences has been brought to light on numerous occasions by welfare scientists, but little has been done to put this knowledge into effect (Fredrickson 1998). Understanding and assessing affective states, both positive and negative, is an important component in animal welfare (Mechanic & Bradburn 1970; Boissy *et al.* 2007).

The cattle's natural behavior, their spontaneous reactions to different stimuli, as well as their facial expressions could be some of the components used to evaluate their different affective states – methods that have been seen to be effective in other species (Jirkof *et al.* 2019), as well as being a non-invasive approach to evaluate animals' welfare.

2.1 Natural behavior

Cattle are highly social gregarious animals that were domesticated approximately 10,000 years ago from their wild ancestor, aurochs (Jensen 2012). The aurochs went extinct in 1627, causing the knowledge of wild cattle's behavior to be limited (Clutton-Brock 1999). Most of the research done on cattle's natural behavior is therefore done with semi-wild herds or domestic cattle in captivity.

They are grazing animals, and when able to choose, spend up to nine hours per day grazing (Jensen 2012). In their most natural environment, freely out in a meadow or held in what is called ranching, which refers to the practice of raising herds of animals on large tracts of land (Jensen 2012; Boudreau *et al.* 2022), the cows form small groups with mutually respected rankings where they wander, graze, and rest together (Jensen 2012). Cows have a very high social need and always want to be in groups. Cows with calves usually live in the same herd their entire lives, while bulls move freely between different groups (Jensen 2012).

2.2 Affective states

To be able to make a statement about the welfare or well-being of an individual, a sustainable spectrum of methods is required (Jirkof *et al.* 2019). When studying emotions in human beings, the researcher can simply ask the patients what they are feeling, setting a gold standard for research; although even with that component, it is still a controversial subject (Chalmers 2010; Ede *et al.* 2019). Understanding and evaluating how animals feel is even harder, because unlike humans, we cannot receive a verbal confirmation on how the animal is feeling (Ahloy-Dallaire *et al*. 2018). That makes inferences the only way to determine an animal's affective state.

The well-being of animals is a large and complex area of study; with multiple different theories and approaches as far as affective states, feelings, or emotions. Therefore, in this study, the term affective state will be used, although resources used can refer to emotions, feelings, or other similar terms. The term "affective state" is referring to the animal's emotional state in relation to subjective perceptions of either internal or external stimuli (Jirkof *et al.* 2019). It may otherwise be defined as a "multifaceted phenomena with neuronal, physiological, behavioral, cognitive, and subjective aspects" (Désiré *et al.* 2002; Špinka 2012) and can have different levels of arousal that are either of positive or negative valence (Désiré *et al.* 2002).

2.2.1 Positive

Play has been seen as crucial in child development, both in an intellectual as well as a social aspect, for quite some time (Gray 2011). Generally, play is considered a sign of well-being and good health, as well as creating normal cognitive development in children. Play has long been suggested to be an indicator of welfare in animals too and relatively recently been proposed to be a sign of positive affective states in animals (Lawrence 1987; Spinka *et al.* 2001). In another social aspect, allogrooming (licking one another) (Sato & Tarumizu 1993), has been suggested as a display of a social bond between individuals (Sato *et al.* 1993). A connection between the mechanical brushes, that are becoming popular on loose housing dairy farms, and the release of the "feel good" hormone oxytocin has recently been made (Neumann 2007; Keeling *et al.* 2016).

2.2.2 Negative

Pain and distress have long been associated with a negative affective state and have therefore been of great focus in animal welfare. One of the most famous welfare measurements in animals, known as the "five freedoms", focuses on the animals being free from pain or injury, as well as fear and distress, which refers to the kind of stress that causes mental suffering (FAWC 1993). Lameness in cattle has a large impact on their welfare (Alban *et al.* 1996), due to the association between lameness and pain and discomfort, often of a longer duration period. Not only can pain in the hooves cause a negative affective state, but the stress of routine hoof care and trimming can cause substantial stress as well (Shearer & van Amstel 2001; Pesenhofer *et al.* 2006). Although there is discussion about which trimming chutes are more stressful, there is an agreement that the general process, either way, is considered stressful. Not only can pain and stress be seen as the cause of negative affective state but also not being able to express natural behavior (FAWC 1993).

2.3 Facial expressions

Facial expressions are seen as central in communication of emotion. It is a highly studied and advanced science in human beings, whereas there has not been quite as extensive research explored in other species. In humans, there has been a focus on a set of six basic categories of facial expressions; happiness, surprise, anger, sadness, fear and disgust (Ekman *et al.* 2013). With that knowledge of those six main categories, compound emotion categories were created (Du *et al.* 2014). Compound categories are those emotions that can be constructed by combining the basic categories to create new ones, such as happily surprised and angrily surprised. In the work of Du *et al.* 2014, 21 distinct emotion categories were described in facial expressions in humans, showing the complexity of this area of science and communication model, but also the potential it has in assessing subjective feelings (Müller *et al.* 2019).

Although the knowledge of animal facial expressions is growing, the research that has been done has had a larger focus on dogs, cats and horses (Waller *et al.* 2013; Wathan *et al.* 2015; Caeiro *et al.* 2017). In those and other animals, animal-FACS has been created based on the human facial action coding system (FACS). However, there is currently no FACS for cattle. Also in dogs, cats and horses, the facial muscle anatomy is well known, and it is important to determine the relationship between the involuntary activation of specific facial muscles and the emotions felt connected to those muscle activations (Ekman & Friesen 1976; Müller *et al.* 2019).

2.3.1 Facial muscles

The facial anatomy and muscles have been used to determine the correlation between the activation of specific facial muscles and specific emotions. FACS has been created to objectively assess the movements of the face (Ekman *et al.* 1971). FACS has established 44 “fundamental anatomical components of facial movements” and is used to observe independent units of movement in the face, referred to as action units (AUs), through terms of underlying muscle movements that cause the appearance of facial markers to change. For example, an eyebrow movement is an action unit. Using an anatomical baseline and comprehension approach when evaluating facial movements causes the practice to be relatively straightforward when modifying for the use in other species (Ekman & Friesen 1978). There has however been some inconsistencies in the terminology used while describing the anatomy of domestic mammals, which has caused the comparisons to be difficult (Wathan *et al.* 2015).

When looking at the musculature of animals in the purpose of examining facial movement and expressions, two main techniques have been presented, the more traditional “skinned face” and a newer technique referred to as a “face mask” (Burrows *et al.* 2009). The “skinned face” refers to the removal of the skin and superficial fasciae from the skull, leaving all muscles behind and attached to the skull in their natural state. The “face mask” refers to the removal of skin, superficial fasciae plus all the superficial musculature from the skull, leaving only the deepest facial muscles that have a direct attachment to the skull. Some studies have used a combination of the two techniques to allow a better understanding of the muscles, their attachments, and the three-dimensional relationships between one another (Burrows *et al.* 2009). For that reason, both the “skinned face” and the “face mask” were performed in the present study.

The details of the bovine’s facial anatomy documented today is limited. Some anatomy books focus mostly on the muzzle and cheeks of the bovine (Ashdown 2010), while others go further into details of the entire facial anatomy including the eyes and ears (Nickel *et al.* 1954; Budras & Habel 2011). Books have also used other species that are close in anatomy to describe the different muscles, adding information regarding the differences the species might have (Schaller & Constantinescu 2007). In table 1, the already documented muscles in cattle have been listed. However, there are some inconsistences in the anatomy depending on what litera-ture is being used. An example of inconsistencies is that *M. parietoauricularis*, according to Nickel *et al.* is one muscle that goes from the parietal bone to concha auriculae, whereas Schaller believes that from the parietoauricularis muscle, another muscle is presented, known as *M. parietoscu-tularis*. N/A, not applicable is written where there is no information given.

*Table 1. List of previously documented facial muscles in cattle. Muscles marked with one * is from the author Nickel* et al. *(1954) and when marked with ** is the author Schaller & Constantinescu (2007). The colours represent different anatomical areas: green: muzzle region, yellow: cheek, blue: eyes, orange: ears*

Muscle	Connection	Function
M. incisivus maxillaris * / M. incisivus superior**	Fibers in the upper lip	Raises upper lip
M. incisivus inferior **	Fibers in the lower lip	Depresses lower lip
M. orbicularis oris */** -pars marginalis ** -pars labialis **	- Part near rima oris - Main Part of the lips	Sphincter of mouth
Lip part of M. cutaneous faciei* / M. depressor anguli oris **	Part of Platysma (M. cutaneous faciei). Connects to M. orbicularis oris	Depresses the angle of the mouth
M. depressor labii maxillaris * / M. depressor labii superioris **	From rostrally of tuber faciale to M. orbicularis oris, M. lateralis nasi and, in numerous thin tendons, the upper lip and muzzle.	N/A
M. depressor labii mandibularis * / M. depressor labii inferioris **	Lies along corpus mandibulae and inserts into the lower lip	N/A
M. mylohyoideus **	Transverse fibers throughout the intermandibular space.	Raises floor of the oral cavity
M. mentalis **	From the lateral part of the corpus mandibulae to the lower lip	Stiffens lower lip
M. zygomaticus */**	Area of crista facialis or arcus zygomaticus to angle of the mouth deep to platysma	Retracts angle of the mouth
M. buccinator pars buccalis */ **	Superficial portion of M. buccinator. Along molar area on the maxilla and mandible	Muscle of the cheek
M. buccinator pars molaris */**	Deeper portion of M. buccinator. Longitudinal fibres extending caudally to Ramus mandibulae	Muscle of the cheek
M. malaris*/**	Ventrally directed cutaneous fibrers from area of lacrimal bone.	N/A

M. masseter */** -pars superficialis */** -pars profunda */**	Lateral muscle of mastication - From the zygomatic arch to the caudal and ventral side of the mandible - The deeper portion of the muscle	Mastication
M. levator nasolabialis */**	Flat muscle arising rostral to orbita, inserting in upper lip and nostril	Raises upper lip and dilates nostril
M. levator labii maxillaris propr. * / M. levator labii superioris **	Arises in front of and on tuber faciale and goes towards the bridge of the nose, between the two portions of m. levator nasolabialis to the dorsolateral angle of the nostril and upper edge of the muzzle	Raises upper lip
M. caninus */**	Perforates m. levator nasolabialis. Inserts to upper lip and nostril	Dilates nostril and raises upper lip
M. dilatator naris apicalis */**	Transverse muscle fibers from medial line to the medial side of each nostril	Dilates the nostril
M. lateralis nasi ** / M. Dilatator naris lateralis *	Ventral and dorsal along the incisors and the caudal portion of the nostril	Assists in dilating the nostril
M. dilatator naris medialis*	Arises from the dorsomedial side of the nose cartilage wall by the nostril and continues of the nose bridge.	Moves dorsal angle of the nostril and the dorsal edge of the medial nasal wing, assisting with the dilation of the nostril
M. orbicularis oculi */** -pars palpebralis** -pars orbitalis **	Positioned within and around the orbita, -Part within the eyelid -Part surrounding the orbita	Sphincter of the eyelids
M. cutaneus frontalis*/ M. Frontalis **	Lies over the frontal bone	N/A
M. levator anguli oculi medialis **	From frontal bone, rostro-laterally pointed, to medial portion of the upper eyelid. According to Nickel et al. (1954) M. frontalis takes over the role of M. levator anguli occuli medialis.	Lifts medial portion of the upper eyelid

M. levator palpebrae superioris	From dorsal to canalis opticus to the upper eyelid	N/A
M. frontoscutalaris */**	Linea temporalis in caudal direction to the scutiformis cartilage	Pulls scutiformis cartilage, that sits cranial medial to the ear, rostrally
-pars temporalis* / M. zygomaticoscutularis ** (part of auricularis rostrales)	Processus zygomaticus ossis frontalis to cartilago scutiformis	Pulls the cartilago scutiformis rostrally
-pars frontalis* / M. frontoscutularis ** (part of auricularis rostrales)	Linea temporalis to cartilago scutiformis	Pulls the cartilago scutiformis rostrally
M. retractor anguli oculi lateralis **	Continuation of m. frontoscutularis to the lateral ocular angle	N/A
M. interscutularis */**	Transverse fibers from linea temporalis to cartilage scutiformis	N/A
M. cervicoscutularis */**	Caudal to m. interscutularis from linea temporalis to the caudal portion of the cartilago scutiformis	N/A
M. scutuloauriculares superficiales dorsalis et accessorius* / Mm. scutuloauriculares superficiales ** (part of auricularis rostrales)	From dorsal surface of the cartilago scutiformis to concha auriculae	Turns the incisura intertragica (a lateral part of the ear) rostrally
M. scutuloauriculares profundi major* / Mm. scutuloauriculares profundi **	From ventral surface of the cartilago scutiformis to eminentia conchae	Turns the incisura intertragica caudally
M. zygomaticoauricularis */M. zygomaticoauricularis (part of auricularis rostrales)**	From arcus zygomaticus to concha auriculae	Turns the incisura intertragica rostrally
M. cervicoauricularis superficialis */**	From funiculus nuchae to concha auriculae	Raises concha
M. cervicoauricularis medius*/**	Caudal and deep to M. cervicoauricularis superficialis	Turns incisura intertragica laterally

M. cervicoauricularis profundus major et minor */ M. cervicoauricularis profundus **	Deep to M. cervicoauricularis medius	Turns incisura intertragica laterally
M. styloauricularis ** (part of Mm. auriculares ventrales)	From mandibula or pars tympanica ossis temporalis to concha auriculae	Depress concha auriculae
M. auricularis ventralis * / M. parotidoauricularis (part of Mm. auriculares ventrales)**	Lies over the dorsal side of Gl. Parotis to concha auriculae	Depress concha auriculae
M. parietoscutularis **	Separates from M. parietoauricularis to attach to cartilage scutiformis	N/A
M. parietoauricularis */**	From the parietal bone to concha auriculae	Raises concha

* Lehrbuch der Anatomie der Haustiere (Nickel *et al.* 1954)

** Illustrated Veterinary Anatomical Nomenclature (Schaller & Constantinescu 2007)

2.3.2 Facial expressions in cattle

There are a few previous studies on facial expressions in cattle, some focusing on an overall change in the facial expressions, while others focusing on specific components in the face proven to be useful, such as eye and ear movement.

In one study, 35 cattle were filmed with a digital camera with the focus on their face before, during and after the branding of a hot branding iron to study changes in the cattle's facial expressions when experiencing pain (Müller *et al.* 2019). Heifers and bulls were examined as well as two different breeds, Nellore, and a crossbreed. Each video being one (1) minute long, to capture the branding. A software system was then used to capture each animal's facial expressions and create a "pain" and "no-pain" picture. Afterwards, a table of action units related to facial expressions of pain in different species and in previously collected data on beef cattle were created in the study. A clear activation of muscles and movements of the face was seen, where pulled back ears, dilated nostrils, open mouth, and raised forehead was significantly associated with the presence of an acute painful stimulus. Only five of the 15 action units were observed in all 35 animals, those being orbital tightening, tension above eye, brow lowering, eye closure, and raised inner brow. Although there was no shown difference between heifers and bulls, a higher frequency of mouth opening was shown in the crossbreeds. Müller *et al.* discussed that these results should be considered when developing additional pain assessment methods based on facial expressions in cattle (Müller *et al.* 2019).

In another study, it was investigated whether ear position could be used to evaluate the positive emotional status of cattle (Proctor & Carder 2014). In the study of Proctor & Carder 2014, a total of 381 video sequences were recorded and observed on 13 different cows. Each video sequence was 15 minutes long. The positive stimulus used in this study was stroking of their head, neck, and withers. The 15-minute sequences consisted of five minutes pre-stroke, which was used as a baseline, stroking, as the stimulus, and post-stroking, which was the post-stimulus. Four ear postures were predefined for this study; upright, forward, backward and hanging (ears fell loosely to the side). Although the results need to be further developed through the use of different stimuli, the results suggest that the relaxed (loosely hanging) ear postures could be an indicator of positive, low arousal emotional state in cattle. The authors concluded that ear position could be useful as a quick, simple, and inexpensive way to evaluate emotional status in cattle and might be incorporated in on-farm assessments. These results were strengthened when a similar study was done evaluating whether eye white and ear posture are reliable indicators. The study suggests that half-closed eyes and ears hanging down or backwards are indicators of low arousal and positive emotion, also having the highest percentage while in a pasture compared to their housing facilities (Battini *et al.* 2019).

A closer look at the eyes of cattle has been taken to see if they can be used as a measurement of the cattle's emotional status (Lambert (Proctor) & Carder 2017). Excitement and frustration are the emotional states this study chose to focus on, using both positive and negative stimuli to elicit these emotions. A total of 22 Holstein dairy cows were part of the experiment and underwent ten 15-minute observations each. The observations were grouped into neutral stimulus, positive stimulus, and negative stimulus. The stimuli used for this study were different types of feed, with the neutral stimulus being a standard feed that the cows continuously have access to, making it not novel nor highly desirable, but edible. The positive stimulus was a feed that is highly desired and which they have limited access to, making it even more desirable. The negative stimulus was inedible woodchips. Both heart rate and percentage of visible eye whites were measured. The results showed that when the cows emotions changed from neutral to either positive or negative, the percentage of visible whites of the eyes also changed. With the understanding of this information and more comparable research done, the eye whites of a cow could be a useful measurement of emotional state according to Lambert *et al.* (Lambert (Proctor) & Carder 2017).

3. Method and material

This study consisted of two parts of data collection and analysis. The first part consisted of a dissection of a heifer skull to evaluate which muscles cattle have in their face. The second data collection took place at the Swedish Livestock Research Center (Lövsta, Uppsala) where 15 cows were filmed in different affective states. Afterwards, the film material as well as previously collected film material of six cows was studied in shorter video sequences with a focus on facial expressions. No animals were euthanized or harmed for the purpose of this study.

3.1 Dissection

A heifer skull was dissected to study which facial muscles are found in cattle. The skull that was used was from an earlier euthanized heifer that was acutely euthanized due to an injury. After euthanasia, the head was severed from the neck at the C3-C4 region and frozen in -21 degrees Celsius. Four days prior to dissection, the skull was transferred to a refrigerator to slowly thaw in a temperature of 5 degrees Celsius. The heifer used in the study was of the breed SRB (the Swedish red-and-white) and of the age 8 months old.

The dissection began with dividing the face into two sides, a right and a left side by a midline incision from the dorsal side of the occipital region, moving in a rostral direction down the face, over the nose and mouth and over the ventral side of the neck, therefore, creating the possibility for two different models on the same skull: the skinned face and the face mask.

By using these two models on the same skull, a better understanding of the muscle's placement on the skull as well as their relationship to each other may occur. There are some muscles on the face that have different names depending on what literature is being used.

3.1.1 Skinned face

The first model made was created by only separating the skin and superficial fasciae from the skull, leaving all muscles in their place on the skull. The skin, sub-cutaneous tissue, and fat was carefully removed with a size 24 blade scalpel starting at the neck, dorsal to the mandibula, where the flap was created when the head was severed from the body, and working rostral towards the nose and mouth, up over the ear. After the skinning, a study of each muscle was done that included photographic and video documentation. The procedure took approximately eleven hours to complete. Afterwards, the face was wrapped in plastic and again frozen at -21 degrees Celsius.

3.1.2 Face mask

The second model was created by separating the skin, superficial fasciae, and all detachable muscles from the skull, leaving only the muscles that had full bone attachment on the skull (m. masseter and m. temporalis). A size 24 blade scalpel was used, beginning at the neck caudal to the mandibula where the flap was created when the head was severed from the body. The ear was cut away with the skin and muscles. After the face mask removal, a study of each muscle was done that included photographic and video documentation. The procedure took approximately seven hours to complete. Afterwards, the face was vacuum sealed and frozen at -21 degrees Celsius.

3.2 Video sequences

A total of twenty-one (21) cattle were studied in shorter video sequences in different affective states with their facial expressions in focus. All cows were filmed at the Swedish Livestock Research Centre (Lövsta, Uppsala), fifteen (15) of the cattle being filmed for this specific study, and six cattle were used from previously collected footage. The footage was contained at different times of the day in their loose housing system, regularly used chute, and pastures. The cattle used in this study were of the breeds SRB (the Swedish red-and-white) and SLB (the Swedish Friesian), with an age range of seven months to eight years according to the personnel at the research centre. In table 1, the affective states, number of cows and different situations used are listed.

Table 2. Filming data collection

Affective states	Number of cows in each situation	Situation 1	Situation 2	Situation 3
Neutral state	3	Ruminating	Sleeping	
Pain	3	Lameness		
Pleasure	3	Grooming, mechanical brush	Social interactions	Grazing
Stress	3	Trimming chute		

The different situations for each affective state were chosen from the information contained in the literature study about a cow's natural behavior and what is seen to cause stress, pain, and pleasure in cattle.

After the video sequences were obtained with a digital camera (Canon HD Legria HF R78), they were loaded onto a computer where each video was viewed in 45-second sequences, registering each facial movement manually. Afterwards the videos were observed another time focusing on the frequency of each movement. With that data, the median of each facial movement was calculated, and figures were created.

4. Results

4.1 Dissection

The dissection gave a clearer picture of each muscle's connection to the skull as well as to each other. However, not all muscles were visible in each of the models. It was therefore necessary to cut through some muscles to gain visual access to others. Most of the muscles that were found during this dissection were expected and previously documented. There were, however, four muscles previously documented that could not be found in the present study: *M. styloauricularis, M. parietoscutularis, M. retractor anguli oculi lateralis*, and *M. levator anguli oculi medialis*. There was also some indication that *M. parietoauricularis* and *M. cervicoauricularis superficialis* could be the same muscle.

4.1.1 Skinned face

This model gave a well-formed picture of the muscles and their correlation to the skull. It was clear where they connected and what structures were close by. Some skin/surface muscles were damaged while removing the skin. The deepest set muscles around the ears were only accessible by cutting through the more superficial muscles.

Figure 1 is taken laterally to give an overview of all the muscles.

Figure 2. The skinned face with all muscles in place, lateral view. Photograph by Cain, B. (2023).

In figure 2, *M. orbicularis oculi* (black arrow), a circular muscle around the eye, can be seen. Over the frontal bone is *M. frontalis* (green arrow), which continues down and connects into a larger portion of *M. orbicularis oculi*.

Figure 2. The skinned face with all muscles in place. Black arrow pointing at M. orbicularis, green arrow pointing at M. frontalis. Photograph by Cain, B. (2023).

Figure 3 is taken from the side with the ear pulled caudally to show the rostral side of the ear and its corresponding muscles.

Figure 3. Cranial side of the ear. Photograph by Cain, B. (2023).

Figure 4 is taken from the back to show the caudal and dorsal side of the ear and its corresponding muscles. All muscles are intact.

Figure 4. Caudal side of the ear. Photograph by Cain, B. (2023).

4.1.2 Face mask

The face mask gave a well-formed visual of the deepest set muscles that are otherwise hard to see. In addition, it was possible see how the deepest muscles relate to the more superficial muscles, while also seeing how they correlate with the skin.

Figure 5 gives an overview look of the entire face mask.

Figure 5. Face mask of a cow. Photograph by Cain, B. (2023).

Figure 6 shows a close-up of the muzzle region (left) and the ear (right).

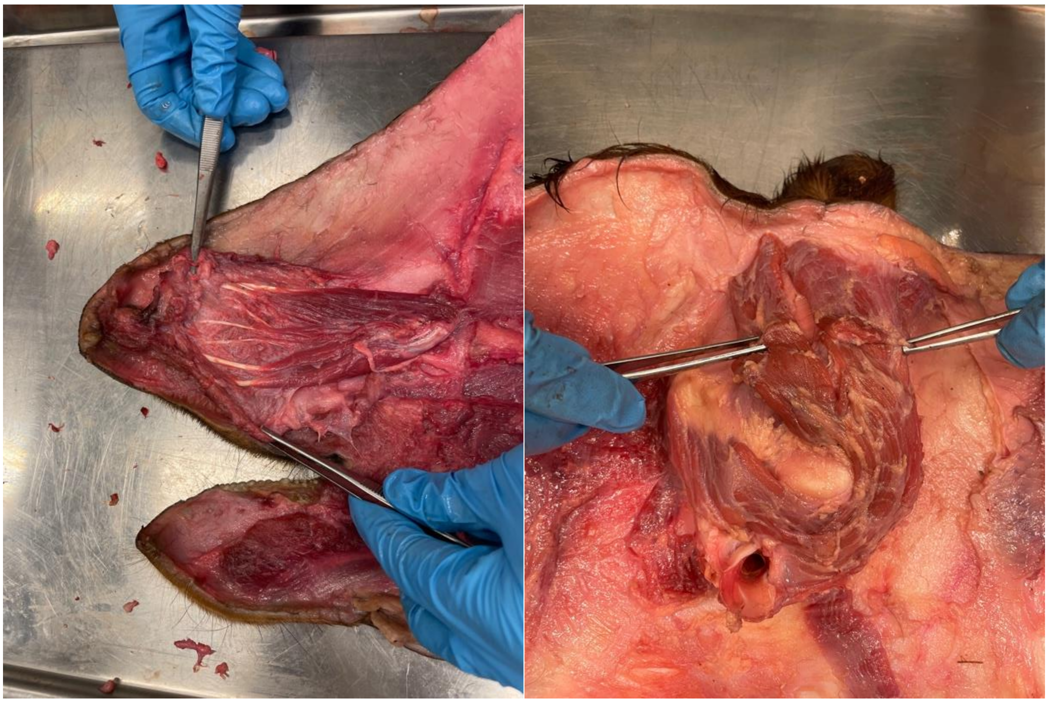

Figure 6. Face mask of a cow. Photograph by Cain, B. (2023).

4.2 Video sequences

In the 21 videos of the 21 cows observed, there was a total of 23 different facial movements observed and registered. Each individual video sequence was 45 seconds long and the total length of all video sequences was approximately 16 minutes.

Some of the registered movements are not directly correlated with the face but were kept in the study because they are considered to be important while evaluating the affective state of cattle.

Table 3 shows the collected observed facial movements in all video sequences as well as their median of occurrence per video sequence. Many of the movements had a median of 1 or 2. There were only a few movements with a median over 3; blinking having the highest median at 9.

Table 3. Facial movements

Facial movements observed	**Definition**	**Median**
Ears forward	Ears facing forward	3
Ears back	Ears pulled slightly back, turned in a slight ventral direction	2
Ear lift	The ears lifting upwards	2
Pinned ears	Ears pinned back against the head	2
Ear flick	Flicking motion, ears move quickly back and then forwards again, to return to the position they were in	4
One ear forward, one ear back		3
Eyebrow movement	The raising or tensing of the area above the eyes. Pulling in an upward and medial motion causing a crease in the skin around the frontalis area	2
Slightly closed eyes	Eyes being approximately 50% closed	2
Closed eyes		1,5
Blinking		9
Increased eye white	The portion of the sclera visible in the eye	3
Nose lift	The dorsal side of the nose, rostral of the bridge, moves upwards and in a caudal direction	1
Nose cleaning	Curling the tongue upward into the nostril to clear it	2
Nostril dilation	The widening of the nostrils dimension	3
Lower lip moving down	Lower lip moving in a downward motion	3

Upper lip moving up	Upper lip moving in an upward motion	3,5
Maxilla muscles contraction	What could be the contraction of masseter muscle along the cheekbone	3
Clenched jaw	The muscles around the jaw being contracted	2
Eating	The normal movements of the mouth and tongue with the intake of food	1
Ruminate	The chewing of cud to then be swallowed again	1
Swallow		1
Salivating	Saliva hanging or dripping from the mouth	1
Allogrooming	Licking face, ears, neck and back of another cow	2,5

Figure 7 shows before (left) and during (right) the eyebrow movement that was observed. The entire eyebrow raises, which causes a crease above the eye.

Figure 7. Eyebrow movement before and during contact with the mechanical brush. Photographs by Cain, B. (2023).

4.2.1 Frequency of facial movements in each affective state

The number of each facial movement in the different states is shown in figures 8-11. There are some irregularities in the number of registrations due to the variation of the duration of each movement in each video, resulting in fewer registrations.

In figure 8, the frequency of each movement for their neutral state (sleeping and ruminating) shows that ear flick was dominant, followed by nose lift and nostril dilation. Even ears back and upper lip movement was a recurring movement.
The following facial movements continued throughout the duration of the video for some cows, resulting in fewer registrations: ruminate, one ear forward, one back, ears forward, ears back, closed eyes.

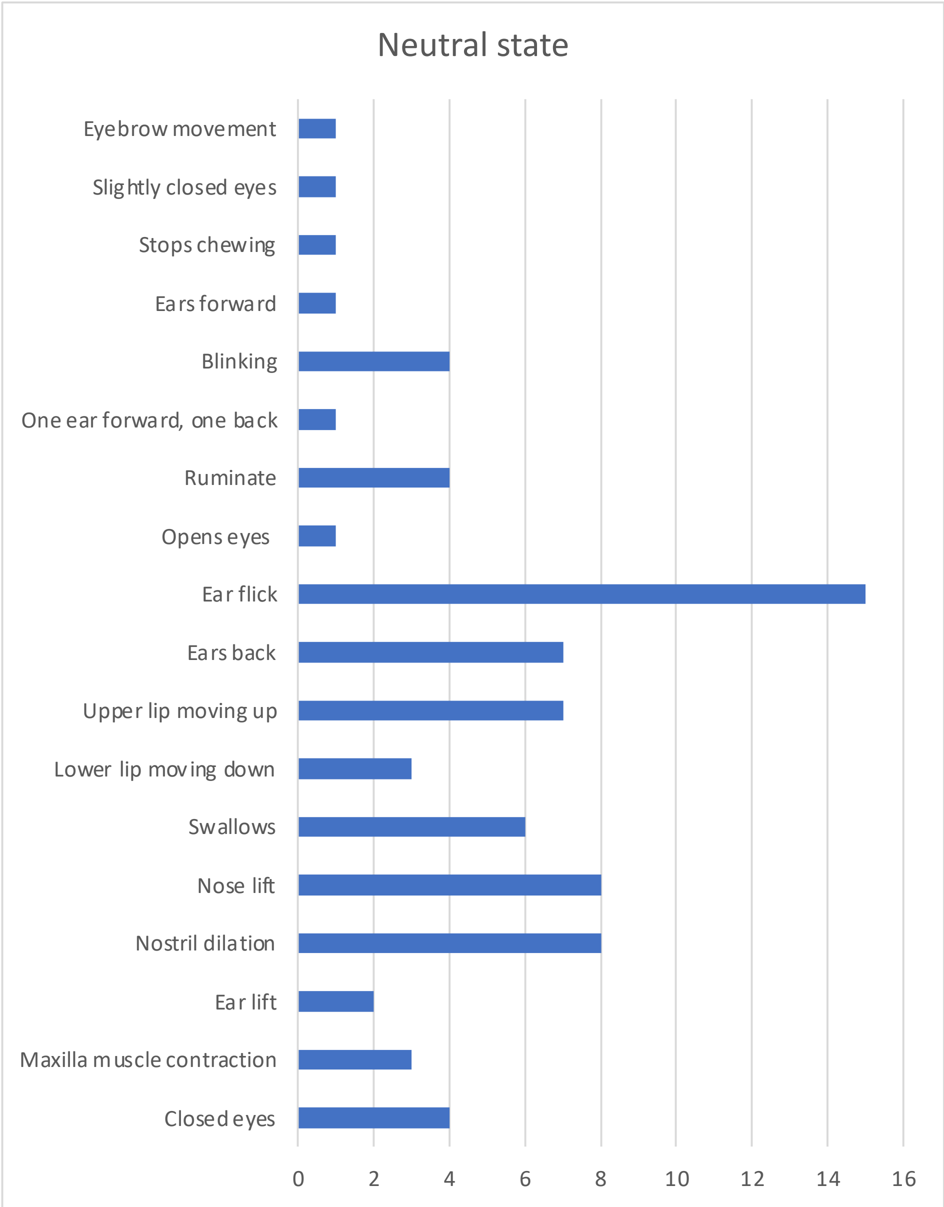

Figure 8. Neutral state.

In figure 9, the frequency of each movement for pleasure (social interaction, grazing and brushing with the mechanical brush) shows that blinking and ear flick was dominant, but even ears back had a significant number. The following facial movements continued throughout the duration of the video for some cows, resulting in fewer registrations: eating, ears back, slightly closed eyes, allogrooming.

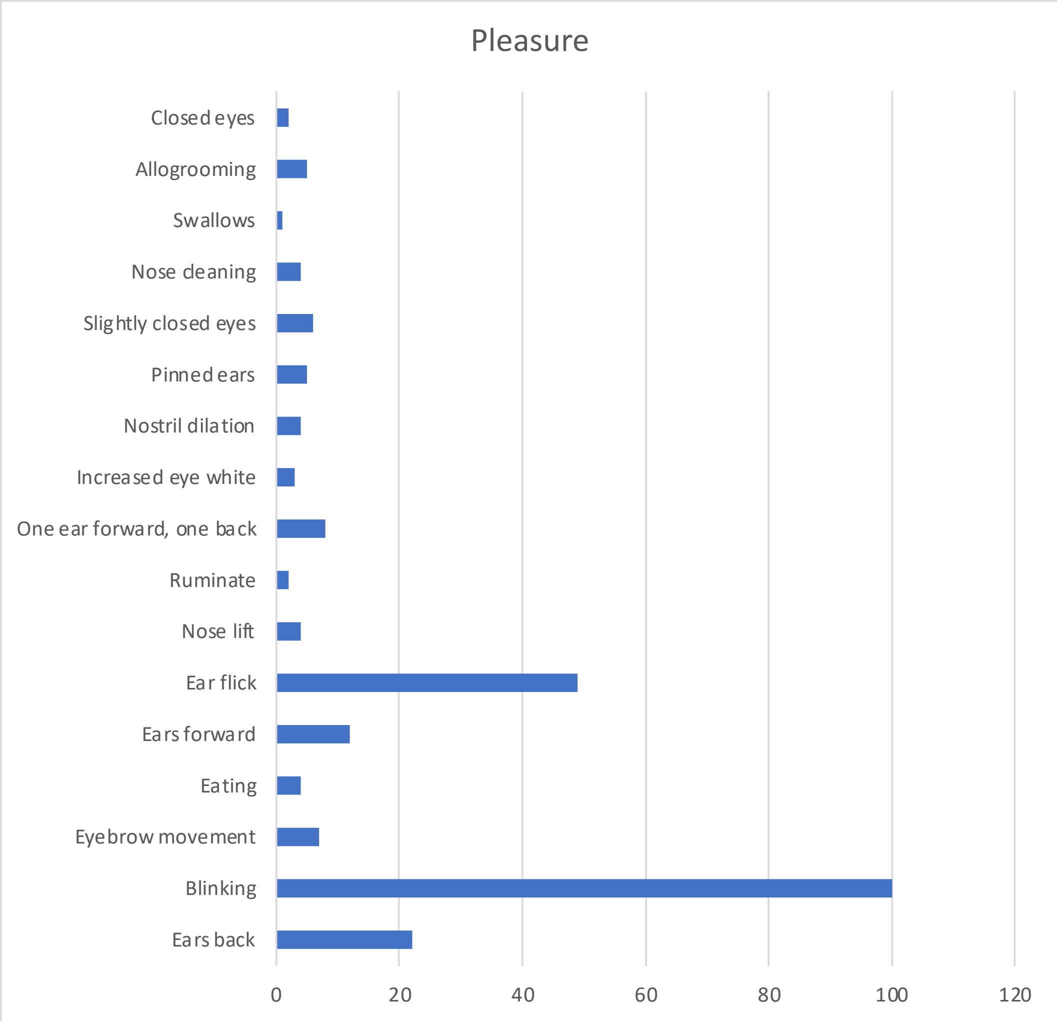

Figure 9. Pleasure.

In figure 10, the frequency of each movement in stress (trimming chute) shows that blinking and ears forward were highest, but even ears back, eyebrow movement, and increased eye whites were dominant. The following facial movements continued throughout the duration of the video for some cows, resulting in fewer registrations: salivating, increased eye white.

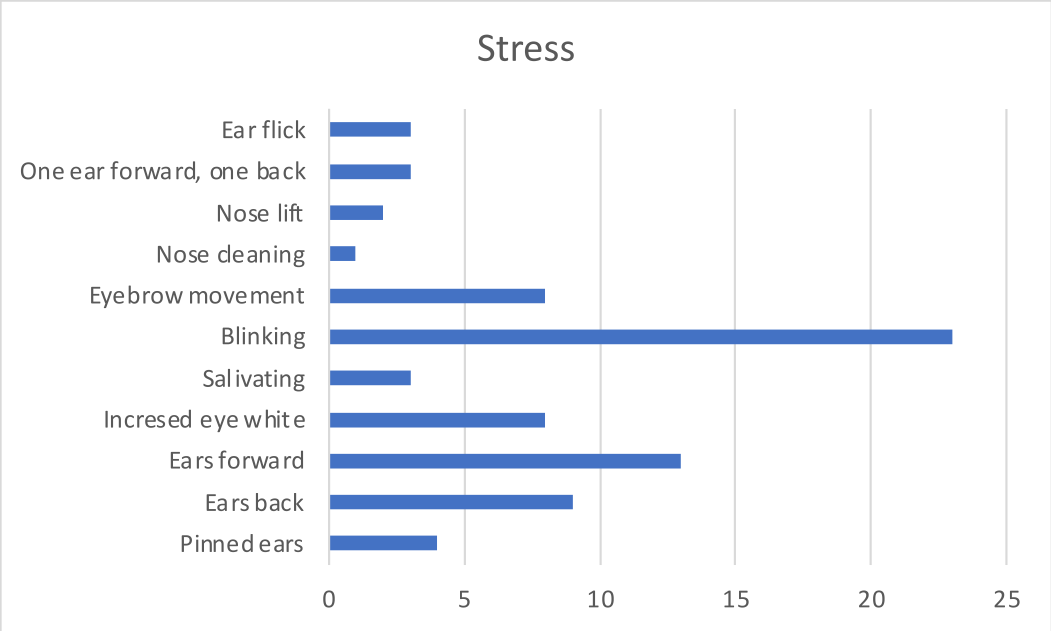

Figure 10. Stress.

In figure 11, the frequency of each movement in pain (lameness) shows that blinking and nostril dilation were dominant, followed by ears forward and eyebrow movement.

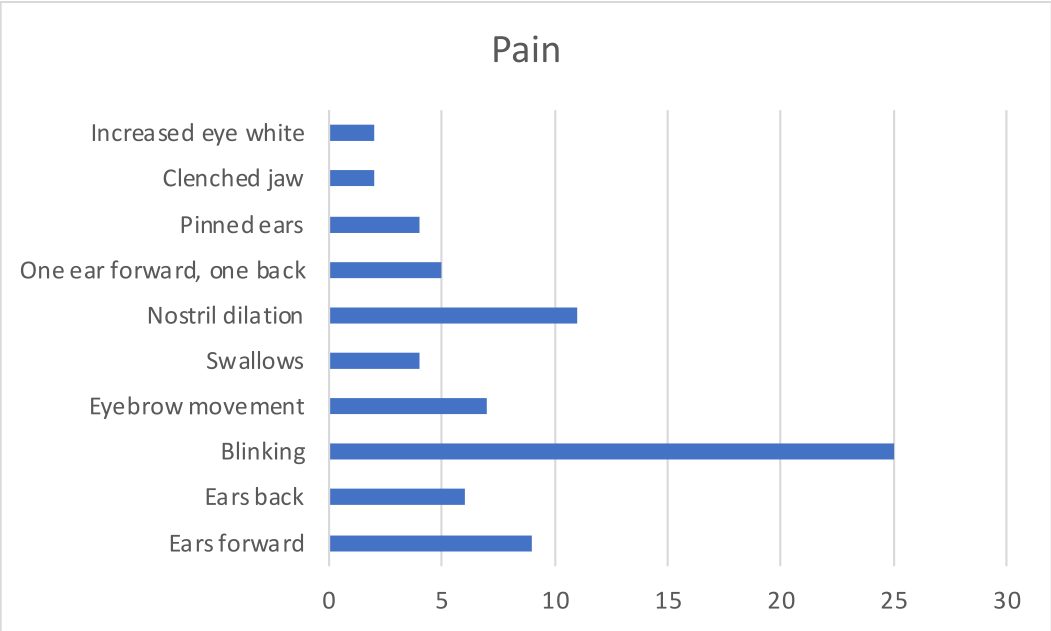

Figure 11. Pain.

5. Discussion

This study aimed to investigate which facial muscles were present in cattle and compare them to previously documented literature, as well as which facial expressions could be performed. This was done by dissection of a cow skull and evaluation of videos taken of cows in different affective states. The hypotheses being that the muscles found are similar to the muscles previously documented and that cattle show a range of facial expressions during different affective states. These questions are explored and elaborated on in the following discussion.

5.1 Dissection

In this study, the dissection consisted of two different models, the skinned face, and the face mask. The skinned face showed to be useful for seeing all the natural connections and correlations the muscles had. Not only was it an appropriate model to see each muscle and where it sat on the skull, but it was also useful and considered necessary when creating the face mask. The reason being that when the face mask was removed from the skull, it was challenging to imagine where each muscle sat on the skull and therefore which muscle was which. Hence, having the skinned face skull to compare to, made the process more attainable.

In other species there is clear and extensive research on the anatomy of the face. Therefore, studies that created FACS for species such as cat, skipped the dissection all together since it was determined to be unnecessary (Caeiro *et al.* 2017). In the study creating animal FACS for horses, it was considered necessary to only create a face mask (Wathan *et al.* 2015) to confirm which facial muscles were present. It was the equine face mask (Wathan *et al.* 2015) that the present face mask study was based on, using a similar technique. Although it was deemed necessary to include the skinned face since previously documented facial muscles in cattle was so limited.

This was a pilot dissection. Because of that, some muscles were damaged during the process. *M. frontalis* is a thin skin muscles that lies over the frontal bone. This muscle was mistakenly partially removed during the skinning of the skinned face which damaged the muscle slightly. The positioning and connections of the muscle were still possible to determine. It is therefore important to be careful when skinning that region.

While creating the face mask it was difficult to remove the deepest set of muscles, e.g. *M. buccalis* from the cheek's mucous membrane causing some damage to the deeper portion of the muscle. In future dissections it is suggested to keep the mucous membrane with the whole face mask, and then afterwards carefully remove the mucous membrane allowing the *M. buccalis* to stay intact.

There were multiple muscles deviating from one another depending on the source (table 1). Some of the names differed, as well as whether some muscles were even present or not. The muzzle and cheek regions were consistent with that previously documented, whereas the eye and ear region differed a bit more. The results in this study indicates that *M. parietoauricularis* and *M. cervicoauricularis superficialis* could be the same muscle. The fibers of those muscles were intertwined and difficult to separate clearly, despite previously been considered to be two separate muscles. If the muscles are separate, then they cross over one another to connect onto the ear. Whether this would make a difference in ear movement is hard to say without doing a separate test where a contraction test is performed on each muscle. *M. styloauricularis* is an ear muscle that Schaller & Constantinescu (2007) mentions, which Nickel *et al.* (1954) do not. This muscle was concluded to not be found during this study's dissection. *M. parietoauricularis,* according to Nickel *et al.* is one muscle that goes from the parietal bone to concha auriculae, whereas Schaller & Constantinescu (2007) believes that from that muscle, another muscle is presented, known as *M. parietoscutularis.* The present study's dissection did not show any clear indication that *M. parietoscutularis* was present.

For the muscles in the eye region, they were mostly in agreement with previous literature, however, according to Schaller & Constantinescu (2007), cattle have the muscle *M. retractor anguli oculi lateralis*, (which is present in horses and give that clear angle shape to the lateral side of the eye), while Nickel *et al.* (2007) does not mention this muscle at all. In this study's dissection, muscle fibers were found protruding from the lateral ocular angle, although they did not seem significant enough to call them an individual muscle. Therefore, it was unclear whether or not these were muscle fibers from *M. retractor anguli oculi lateralis*. Another eye muscle Schaller & Constantinescu (2007) claims to be present in cattle is *M. levator anguli oculi medialis*, which is the medial eye muscle in horses that cause the classic "point" of the inner eyebrow to make them look concerned/uncomfortable. Nickel *et al.* (1954) has categorized that muscle area to be an extension from *M. frontalis*. In the present dissection, there was no distinct small muscle as described in Schaller & Constantinescu (2007), but instead it looked like *M. frontalis* continued down and connected to a larger portion of *M. orbicularis oculi* (figure 2), which seems to instead cause the entire eyebrow to raise, thereby causing a crease above the eye (figure 7). It would appear that Schaller has a good amount of knowledge of the horses' facial anatomy and correlated it directly with cattle, while Nickel *et al.*

(1954) focuses only on cattle but does not go into as much detail in some areas of the face, such as the lips.

Based on the results of this study in comparison to previously published material, there are some inconsistencies, and therefore a need for more research. An updated anatomy book would also be beneficial and could be seen as necessary. The horse's facial anatomy is, in many ways, similar but is not exactly the same as cattle. Therefore, it is important to not take what we know about horses and assume it is the same for cattle. The classic "point" in the eye being a good example.

5.2 Video sequences

5.2.1 On-farm filming

The data gathered for this part of the study was done at the Swedish Livestock Research Centre. The milking cows there are used to people coming and going and visitors observing them. They are social and for the most part unafraid of people. Moreover, the filming for this study was done on an average day where they were following their routine as usual, this causing the least amount of disturbance as possible. However, there was someone standing there with a camera for a long period of time watching them. Some of the movement registrations could therefore be affected by the camera presence, but also by employees walking by, machinery, loud noises etc. Moreover, facial movements could be missed when the cows turned away from the camera or another cow walked past, and therefore blocking the camera's view of the observed cow. The environment otherwise was an adequate representation of an average milk farm.

The situations chosen for each affective state was based on previous research that explored cattle's affective states and emotions. There are multiple other situations that could be interesting to consider while studying positive and negative affective states. For positive, play would be fascinating to look at, both in younger and older cattle. Although older cattle may not play in the same way that calves do, they do bounce around when released outside to the pastures. For negative, the milking line would be interesting to look at, being a confined space with multiple cows of different rankings. Another stressful situation could be transport, especially if there are different groups combined.

5.2.2 Observed facial movements

In the observations of the 21 cows, there was a total of 23 different facial movements registered. Of those 23, only 18 were actual facial muscle contractions that cause a movement of the face. The remaining five, eating, ruminating, swallow-wing, salivating, allogrooming, were included because they were deemed important while evaluating facial expressions and affective states. Salivating is not a facial muscle contraction but was present for each and every cow during the affective state stress. That could be an indication of a stress signal in cattle. Rumina-ting is another that was kept in this study because there has been connections between decreased rumination and stress and disease (Rizk *et al.* 2012; Braun *et al.* 2015).

An interesting finding in this study is that during stress (i.e. in the trimming chute), increased eye whites were one of the facial movements with the highest frequency. At the same time, it was also seen during pleasure, especially for the cows using the mechanical brush. This can indicate that eye whites can be a sign for stress, but also a sign of pleasure, which is similar to the results in Lambert and Carders study about eye whites. As known earlier, a connection has been made between the mechanical brush and raised oxytocin levels, why it would be interesting to further explore potential correlations between eye whites and oxytocin levels.

To compose the videos of social interaction, one video focused on the cow doing the allogrooming, one video focused on the cow receiving the allogrooming, and the last video on two cows interacting together, thus enabling the span of social interactions as broad as possible.

Another observation seen in this study was the eyebrow movement. As mentioned earlier, in horses, the classic “point” in the inner eyebrow is very distinct, but it appears to be more of a whole eyebrow lift in these cattle, causing a crease above the eye (figure 7). This correlates well to the muscles found in the dissection.

5.2.3 Movement frequency

The difficult part of this study is comparing the frequency of each movement in an affective state to another affective state. The cause being that there was a different number of cows for each affective state. For pain and stress there were only three cows each, whereas for pleasure there were nine cows. One reason being that the study of pleasure in cattle has not been explored to the extent that pain and stress have been, therefore it was of higher interest in this study. There is also a larger variation to what pleasure is, which is why the area of study is larger.

With the data gathered from this study and the facial movements’ median, an idea of how many times an “average” cow does each movement can be gathered. This being interesting to see if a movement with a high frequency was gathered by just one cow or if it is in fact a representative observed movement that most cows do. Blinking is a good example of that. Blinking had a frequency anywhere from 2 to 17, depending on the affective states, but had a median of nine. This suggests that most cows do blink multiple times in different affective states. Comparing then the frequency of blinking in each individual affective state could be a sign of how they are feeling. Although this has not been studied in cattle, it has been seen that horses have a lower spontaneous blinking rate while in stressful situations (Merkies et al. 2019). It could be interesting to do a study on blinking in cattle as well.

Another interesting median result is ear flick. Ear flick has a median of 4, which isn't that high of a number in all affective states, but when looking at the individual affective states, ear flick varies quite a bit. Ear flick isn't even present in pain, whereas in the neutral state and pleasure, ear flick is dominant. That could suggest that ear flick is seen in more positive or neutral states. Now, it is very difficult to know for sure if these cows were actually in a pleasure or in a neutral affective state. The bases of those suggestions are from what is known in cattle today and how they are affected by different situations and experiences in their lives. Another dominant movement in pleasure is ears back, which is interesting because studies done before have had similar results showing ears hanging down or backwards as indicators of low arousal and positive emotion (Proctor & Carder 2014; Battini *et al.* 2019).

Median can also depend on the chosen time for each video, and it can be discussed whether 45 seconds was long enough to gather the information needed or not. A 45 second timeline was chosen partly because of the practicality of filming in the middle of a loose housing facility, where other cows walked in front of the camera or took over the mechanical brush, as well as testing a distinct timeline to create a baseline. Future studies may want to try other timelines, such as 30 seconds, 1 minute or even longer. In previous studies, timelines from 1 minute to 15 minutes have been included.

The number of animals could also be an important factor in the results. It should be considered whether the size of this group has an effect on not only the frequency of each movement but on the median as well. It is not completely straight forward when choosing a sample size in a behavioral study and seems to vary depending on the behavioral scientist (Taborsky 2010). For the most part the choice seems based on a "best guess" strategy.

In this study the frequency of each facial movement was chosen as the way to register the facial movements seen. That caused some irregularities in the number of registrations, due to the variation of the duration of each movement in each video, resulting in fewer registrations. As mentioned earlier, increased eye white was dominant in stress, but also seen in pleasure. Although during pleasure, multiple cows had only one registered "increased eye white" because it stayed present during the whole duration of the video. It would therefore be very interesting to register the duration of each facial movement and testing the correlations between frequency and duration.

5.3 Limitations

It was planned to have at least two different situations for each affective state. Because of the timeline of this study the other situations for stress and pain did not come to pass. One of the reasons being that there were no ill cattle on that farm at the time of filming. If the timeline would have allowed it, other situations could have been used, or gaining filming access to other farms or animal clinics. Another limitation was the amount of time available to spend on the gathered film data. The higher number of situations or cattle in each situation increases the amount of material to go through. Because of this, 3 cattle per situation was chosen.

6. Conclusion

The present study aimed to investigate which facial muscles that are present in cattle and thereby, which facial expressions that can be present, and the range of facial expressions exhibited during different affective states. Most of the muscles found were previously documented. However, four muscles previously documented could not be found in the present study, *M. styloauricularis*, *M. parietoscutularis*, *M. retractor anguli oculi lateralis*, and *M. levator anguli oculi medialis*. There was also an indication that *M. parietoauricularis* and *M. cervicoauricularis superficialis* could be the same muscle. Both the skinned face and face mask were deemed necessary dissections due to the limited literature available for reference. There were 23 facial movements found. Correlations of higher frequencies of movements in different affective states were seen, such as ear flick having a higher frequency during pleasure and neutral state. It is important to not take what is known in other species and assume it is the same for cattle.

The study of facial expressions is a complicated subject that has many different aspects and components. More research is needed in this area to get a better understanding of facial expressions in cattle and what those expressions can indicate. Understanding cattle's facial expressions and behavior would benefit not only the animal's production but also their welfare.

References

Ahloy-Dallaire, J., Espinosa, J. & Mason, G. (2018). Play and optimal welfare: Does play indicate the presence of positive affective states? *Behavioural Processes*, 156, 3–15. https://doi.org/10.1016/j.beproc.2017.11.011

Alban, L., Agger, J.F. & Lawson, L.G. (1996). Lameness in tied Danish dairy cattle: the possible influence of housing systems, management, milk yield, and prior incidents of lameness. *Preventive Veterinary Medicine*, 29 (2), 135–149. https://doi.org/10.1016/S0167-5877(96)01066-5

Ashdown, R.R. (2010). *The Ruminants*. 2. ed. Mosby/Elsevier. (Color Atlas of Veterinary Anatomy; 1)

Battini, M., Agostini, A. & Mattiello, S. (2019). Understanding cows' emotions on farm: Are eye white and ear posture reliable indicators? *Animals*, 9 (8), 477. https://doi.org/10.3390/ani9080477

Boissy, A., Manteuffel, G., Jensen, M.B., Moe, R.O., Spruijt, B., Keeling, L.J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissier, I. & Aubert, A. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiology & Behavior*, 92 (3), 375–397. https://doi.org/10.1016/j.physbeh.2007.02.003

Braun, U., Zürcher, S. & Hässig, M. (2015). Evaluation of eating and rumination behaviour in 300 cows of three different breeds using a noseband pressure sensor. *BMC Veterinary Research*, 11 (1), 231. https://doi.org/10.1186/s12917-015-0549-8

Budras, K.-D. & Habel, R.E. (2011). *Bovine Anatomy*. Schlütersche.

Burrows, A.M., Waller, B.M. & Parr, L.A. (2009). Facial musculature in the rhesus macaque (Macaca mulatta): evolutionary and functional contexts with comparisons to chimpanzees and humans. *Journal of Anatomy*, 215 (3), 320–334. https://doi.org/10.1111/j.1469-7580.2009.01113.x

Caeiro, C.C., Burrows, A.M. & Waller, B.M. (2017). Development and application of CatFACS: Are human cat adopters influenced by cat facial expressions? *Applied Animal Behaviour Science*, 189, 66–78. https://doi.org/10.1016/j.applanim.2017.01.005

Chalmers, D.J. (2010). Facing Up to the Problem of Consciousness. In: Chalmers, D.J. (ed.) *The Character of Consciousness*. Oxford University Press. 0. https://doi.org/10.1093/acprof:oso/9780195311105.003.0001

Clutton-Brock, J. (1999). *A Natural History of Domesticated Mammals*. 2. ed. Cambridge University Press.

Désiré, L., Boissy, A. & Veissier, I. (2002). Emotions in farm animals:: a new approach to animal welfare in applied ethology. *Behavioural Processes*, 60 (2), 165–180. https://doi.org/10.1016/S0376-6357(02)00081-5

Dores, A.R., Barbosa, F., Queirós, C., Carvalho, I.P. & Griffiths, M.D. (2020). Recognizing emotions through facial expressions: a largescale experimental study. *International Journal of Environmental Research and Public Health*, 17 (20), 7420. https://doi.org/10.3390/ijerph17207420

Du, S., Tao, Y. & Martinez, A.M. (2014). Compound facial expressions of emotion. *Proceedings of the National Academy of Sciences*, 111 (15), E1454–E1462. https://doi.org/10.1073/pnas.1322355111

Ede, T., Lecorps, B., von Keyserlingk, M.A.G. & Weary, D.M. (2019). Symposium review: Scientific assessment of affective states in dairy cattle. *Journal of Dairy Science*, 102 (11), 10677–10694. https://doi.org/10.3168/jds.2019-16325

Ekman, P. & Friesen, W.V. (1976). Measuring facial movement. *Environmental Psychology and Nonverbal Behavior*, 1 (1), 56–75. https://doi.org/10.1007/BF01115465

Ekman, P., & Friesen, W. (1978). *Action coding system: a technique for the measurement of facial movement.* Consulting Psychologists Press, Palo Alto.

Ekman, P., Friesen, W.V. & Ellsworth, P. (2013). *Emotion in the human face: Guidelines for research and an integration of findings*. Elsevier. https://books.google.com/books?hl=en&lr=&id=dOFFBQAAQBAJ&oi=fnd&pg=PP1&dq=info:X5y6BUEx4TMJ:scholar.google.com&ots=zjyCuLYyZf&sig=uOdaZ6UYevSbmKOcztwvsPRnXL4 [2023-11-21]

Ekman, P., Friesen, W.V. & Tomkins, S.S. (1971). Facial affect scoring technique: A first validity study. *Semiotica*, 3 (1), 37–58. https://doi.org/10.1515/semi.1971.3.1.37

FAWC (1993). *Report on Priorities for Animal Welfare Research and Development*. Farm Animals Welfare Council, Surrey. https://edepot.wur.nl/134980

Fredrickson, B.L. (1998). What good are positive emotions? *Review of General Psychology*, 2 (3), 300–319. https://doi.org/10.1037/1089-2680.2.3.300

Gleerup, K.B., Andersen, P.H., Munksgaard, L. & Forkman, B. (2015). Pain evaluation in dairy cattle. *Applied Animal Behaviour Science*, 171, 25–32. https://doi.org/10.1016/j.applanim.2015.08.023

Gray, P. (2011). The decline of play and the rise of psychopathology in children and adolescents. *American Journal of Play*, 3 (4), 443–463

Holton, L., Pawson, P., Nolan, A., Reid, J. & Scott, E.M. (2001). Development of a behaviour-based scale to measure acute pain in dogs. *Veterinary Record*, 148 (17), 525–531. https://doi.org/10.1136/vr.148.17.525

Jensen, P. (2012). *Hur mår maten? [Elektronisk resurs]*. Natur & Kultur.

Jirkof, P., Rudeck, J. & Lewejohann, L. (2019). Assessing affective state in laboratory rodents to promote animal welfare—What is the progress in applied refinement research? *Animals*, 9 (12), 1026. https://doi.org/10.3390/ani9121026

Jordbruksverket (2022). *Antal nötkreatur i december 2021*. https://jordbruksverket.se/om-jordbruksverket/jordbruksverkets-officiella-statistik/jordbruksverkets-statistikrapporter/statistik/2022-02-15-antal-notkreatur-i-december-2021 [2023-09-14]

Keeling, L., De Oliveira, D. & Rustas, B.-O. (2016). Use of mechanical rotating brushes in dairy cows – a potential proxy for performance and welfare? In: Kamphuis, C. & Steeneveld, W. (eds.) *Precision Dairy Farming.* [Conference Proceedings, Leeuwarden, The Netherlands, June 21-23, 2016]. Wageningen Academic Publishers. 343-347.

Lambert (Proctor), H.S. & Carder, G. (2017). Looking into the eyes of a cow: Can eye whites be used as a measure of emotional state? *Applied Animal Behaviour Science*, 186, 1–6. https://doi.org/10.1016/j.applanim.2016.11.005

Lawrence, A. (1987). Consumer demand theory and the assessment of animal welfare. *Animal Behaviour*, 35 (1), 293–295. https://doi.org/10.1016/S0003-3472(87)80236-1

Leach, M.C., Coulter, C.A., Richardson, C.A. & Flecknell, P.A. (2011). Are we looking in the wrong place? Implications for behavioural-based pain assessment in rabbits (Oryctolagus cuniculi) and beyond? *PLOS ONE*, 6 (3), e13347. https://doi.org/10.1371/journal.pone.0013347

Leach, M.C., Klaus, K., Miller, A.L., Perrotolo, M.S. di, Sotocinal, S.G. & Flecknell, P.A. (2012). The assessment of post-vasectomy pain in mice using behaviour and the mouse grimace scale. *PLOS ONE*, 7 (4), e35656. https://doi.org/10.1371/journal.pone.0035656

Mechanic, D. & Bradburn, N.M. (1970). The structure of psychological well-being. *American Sociological Review*, 35 (5), 948. https://doi.org/10.2307/2093340

Merkies, K., Ready, C., Farkas, L. & Hodder, A. (2019). Eye blink rates and eyelid twitches as a non-invasive measure of stress in the domestic horse. *Animals*, 9 (8), 562. https://doi.org/10.3390/ani9080562

Müller, B.R., Soriano, V.S., Bellio, J.C.B. & Molento, C.F.M. (2019). Facial expression of pain in Nellore and crossbred beef cattle. *Journal of Veterinary Behavior*, 34, 60–65. https://doi.org/10.1016/j.jveb.2019.07.007

National Geographic (n.d.). *Ranching*. Education - Encyclopedic entry. https://education.nationalgeographic.org/resource/ranching [2023-10-08]

Neumann, I.D. (2007). Oxytocin: The neuropeptide of love reveals some of its secrets. *Cell Metabolism*, 5 (4), 231–233. https://doi.org/10.1016/j.cmet.2007.03.008

Nickel, R., Schummer, A. & Seiferle, E. (1954). *Lehrbuch der Anatomie der Haustiere, Band 1, Bewegungsapparat.* Paul Parey.

Pesenhofer, G., Palme, R., Pesenhofer, R.M. & Kofler, J. (2006). Comparison of two methods of fixation during functional claw trimming - Walk-in crush versus tilt table - In dairy cows using faecal cortisol metabolite concentrations and daily milk yield as parameters. *Wiener Tierarztliche Monatsschrift*, 93, 288–294

Pritchett, L.C., Ulibarri, C., Roberts, M.C., Schneider, R.K. & Sellon, D.C. (2003). Identification of potential physiological and behavioral indicators of postoperative pain in horses after exploratory celiotomy for colic. *Applied Animal Behaviour Science*, 80 (1), 31–43. https://doi.org/10.1016/S0168-1591(02)00205-8

Proctor, H.S. & Carder, G. (2014). Can ear postures reliably measure the positive emotional state of cows? *Applied Animal Behaviour Science*, 161, 20–27. https://doi.org/10.1016/j.applanim.2014.09.015

Rizk, A., Herdtweck, S., Meyer, H., Offinger, J., Zaghloul, A. & Rehage, J. (2012). Effects of xylazine hydrochloride on hormonal, metabolic, and cardiorespiratory stress responses to lateral recumbency and claw trimming in dairy cows. *Journal of the American Veterinary Medical Association*, 240 (10), 1223–1230. https://doi.org/10.2460/javma.240.10.1223

Sato, S. & Tarumizu, K. (1993). Heart rates before, during and after allo-grooming in cattle (Bos taurus). *Journal of Ethology*, 11 (2), 149–150. https://doi.org/10.1007/BF02350048

Sato, S., Tarumizu, K. & Hatae, K. (1993). The influence of social factors on allogrooming in cows. *Applied Animal Behaviour Science*, 38 (3), 235–244. https://doi.org/10.1016/0168-1591(93)90022-H

Schaller, O. & Constantinescu, G.M. (2007). *Illustrated Veterinary Anatomical Nomenclature*. Georg Thieme Verlag.

Shearer, J.K. & van Amstel, S.R. (2001). Functional and corrective claw trimming. *Veterinary Clinics of North America: Food Animal Practice*, 17 (1), 53–72. https://doi.org/10.1016/S0749-0720(15)30054-2

Sørensen, J.T., Sandøe, P. & Halberg, N. (2001). Animal welfare as one among several values to be considered at farm level: The idea of an ethical account for livestock farming. *Acta Agriculturae Scandinavica, Section A — Animal Science*, 51 (sup030), 11–16. https://doi.org/10.1080/090647001316922992

Špinka, M. (2012). Social dimension of emotions and its implication for animal welfare. *Applied Animal Behaviour Science*, 138 (3), 170–181. https://doi.org/10.1016/j.applanim.2012.02.005

Spinka, M., Newberry, R. & Bekoff, M. (2001). Mammalian play: Training for the unexpected. *The Quarterly Review of Biology*, 76, 141–68. https://doi.org/10.1086/393866

Taborsky, M. (2010). Sample size in the study of behaviour. *Ethology*, 116 (3), 185–202. https://doi.org/10.1111/j.1439-0310.2010.01751.x

Waller, B.M., Peirce, K., Caeiro, C.C., Scheider, L., Burrows, A.M., McCune, S. & Kaminski, J. (2013). Paedomorphic facial expressions give dogs a selective advantage. *PLOS ONE*, 8 (12), e82686. https://doi.org/10.1371/journal.pone.0082686

Wathan, J., Burrows, A.M., Waller, B.M. & McComb, K. (2015). EquiFACS: The equine facial action coding system. *PLOS ONE*, 10 (8), e0131738. https://doi.org/10.1371/journal.pone.0131738

Yeates, J.W. & Main, D.C.J. (2008). Assessment of positive welfare: A review. *The Veterinary Journal*, 175 (3), 293–300. https://doi.org/10.1016/j.tvjl.2007.05.009

Popular science summary

It is of utmost importance from a veterinary perspective to understand the welfare of cattle to uphold their care and production. Behavior is becoming a popular way to assess the welfare and emotions in cattle, and is already used for, among other things, assessing pain in cattle and other animals. Facial expressions can help when evaluating welfare and emotion in various species due to the time efficiency. This is a result of animals having a higher frequency of facial expressions than body behaviors. This study aimed to investigate which facial muscles were present in cattle and which facial expressions could be exhibited. In addition, facial expressions during certain affective states were studied: a neutral state, pain, stress, and pleasure.

The study consisted of two parts, a dissection of a cow skull, and assessment of video sequences of cows in different affective states. Two dissection models were created: a skinned face and a face mask. The results showed that most of the muscles found were previously documented but that there were multiple muscles previously documented that were not found in this study. Both models were deemed necessary to get the best possible understanding of the muscles.

For the video sequences, a total of twenty-one cattle were studied in 45 second videos during different affective states, with their facial expressions in focus. The videos were then observed twice, and each facial movement seen was registered manually. There were twenty-three facial movements observed and registered. There were some correlations of higher frequencies of movements in different affective states, such as ear flick in pleasure and neutral state. During stress, increased eye whites were one of the facial movements with the highest frequency, but at the same time, it was also seen during pleasure, especially for the cows using the mechanical brush. This illustrates that facial expressions are a complicated subject that has many different aspects and related components.

More research in this area would give a better understand of facial expressions in cattle and what it can indicate. Understanding cattle's facial expressions and behavior would benefit not only the animal's production but also their welfare.

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