THE GRIMACE SCALE
AS A METHOD OF ACUTE PAIN ASSESSMENT FOR NEONATAL LIVESTOCK

A THESIS
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

Maria Elizabeth Lou

IN PARTIAL FULFILLMENT OF THE REQUIRMENTS
FOR THE DEGREE OF MASTER OF SCIENCE

Yuzhi Li

August 2020

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr. Yuzhi Li for accepting me as your graduate student. Thank you for always having faith and believing in me. You gave me the incredible opportunity to choose the topic of and design my own project. You pushed me to think of new ideas and because of that to the best of our knowledge I may be the first to apply 3D technology towards the piglet grimace scale and the first to develop a goat kid grimace scale. I would like to thank my committee members, Dr. Beth Ventura and Dr. John Deen. Dr. Beth Ventura, you continuously provided me with beautiful words of encouragement and your guidance as mentor, professor, and coach remind me every day of my love and passion for animal welfare. Dr. John Deen, you encouraged me think critically and to always ask questions. I am blessed to have been trained by all three of you. I will forever be grateful for all of your support and guidance. This research would have not been possible without the help and support of various collaborators. I would like to extend my sincerest gratitude to Dr. Whitney Knauer for providing me the opportunity to be a part of her goat kid project. To Dr. Samantha Porter, at the UMN Advanced Imaging Services of Objects and Spaces, thank you for all of your instruction in building the photogrammetry pig rig and 3D models and always offering to help in any way. To Dr. Jason Massey, thank you for your instruction in geometric morphometrics and statistical analysis and reminding me to stay positive. It was an extraordinary experience applying 3D technology to my research and you are amazing at what you do. The opportunities presented by Drs. Knauer, Porter, and Massey truly exceeded my expectations of what I hoped to earn from a master's degree and the knowledge I gained from their expertise will be invaluable to my future career.

Furthermore, a big thank you to Tracy Rheingans for your help in data collection and Clara Richard for designing the animal sketches. It was an absolute pleasure working with both of you. Thank you to Hannah Phillips for your help and patience in statistical analysis, you are a wonderful teacher. Thank you to Dr. Nichole Anderson for her instruction on vocalization analysis. I would like to thank Dr. Lee Johnston for your ingenuity in designing and building the piglet cradle. To my office mates and friends, Edward, Jinlong, Tina, Yuan-Tai, and Kasey thank you for welcoming me with open arms and becoming my Minnesota family. I'm grateful for our love of boba tea and pho for bringing us closer together.

Lastly, I could not have done this (or survived the Minnesota winters) without the continuous love and support from my family. To my parents, you showed that with hard work and determination anything is possible. Mama, your unwavering faith in me gave me strength. Papa, my love for animals began with you. Carlota, thank you for being my biggest supporter and making sure I had everything I needed. To Ito, Ita, Erick, Sergio, Lily, Joshua, Paula, and Andrea, it is an absolute blessing from God to have such a beautiful and loving family like you, thank you for always being there for me. To Crystal, Estevan, and Alicia, thank you for always making sure happiness and laughter were part of the journey. To my fiancé, AJ, thank you for being my #1 fan. You cheered me on in all of my greatest movements, but most importantly you helped built my confidence when I needed it the most. You and Max remind me that every day is a new day and I can do anything. Last but not least, to my best friend, my dog Lucas, thank you for being by my side every step of the way. You made Minnesota feel like home.

DEDICATION

This thesis is dedicated to my parents, Vilma and Carlos Lou.

Thank you for always encouraging me to do whatever makes me happy.

ABSTRACT

Neonatal livestock animals commonly undergo management procedures that induce acute pain and have negative implications on their well-being. This research consists of two studies, one focused on piglets and one on goat kids. The first study was designed to assess behavior of male piglets as an indicator of pain before, during, and after surgical castration. The four objectives of this study were: 1) To assess acute pain during castration through behavioral indicators, 2) To evaluate the utility of the Piglet Grimace Scale (PGS) to detect acute pain, and 3) To refine the current method of data collection for the PGS using photogrammetry and 3D landmark-based geometric morphometrics. The third objective had two sub-objectives: a) To determine if photogrammetry and 3D landmark-based geometric morphometrics can obtain clear, analyzable images of piglet faces and b) To evaluate 3D landmark-based geometric morphometrics to quantify changes in piglet facial shape.

Eighty-eight male piglets were randomly allocated to one of two treatments: surgical castration (C; n=43) and sham-castration (S; n=45). Within 24 hours after birth, identical procedures were followed for both treatment groups, except sham-castrated piglets were not castrated. For objective 1, struggle behavior (curl ups, leg kicks, and body flailing) and vocalization parameters (duration and peak frequency) during the castration (or sham-castration) period were recorded and analyzed. For objectives 2-4, photographs of piglets were taken at four time points using 11 cameras mounted on a photogrammetry rig: baseline (T1 - immediately before castration), post-castration (T2 - immediately after castration), 4 h post-castration (T3), and 22 h post-castration (T4).

Four trained raters scored the piglet images using five facial action units (FAUs): orbital tightening, ear position, temporal tension, lip contraction, and nose bulge/cheek tension. 3D facial models were generated for each piglet at each time points using the Agisoft Metashape software. Landmarks corresponding to 3 facial action units (FAUs) (orbital tightening, lip contraction, and nose bulge/cheek tension) were placed onto the models using the Markers feature for geometric morphometric analysis. Data were analyzed using the Glimmix, Mixed, and Genmod procedures of SAS software, and Generalized Procrustes Analysis (GPA) procedure of Morpheus software.

During castration, castrated piglets kicked more frequently than sham-castrated piglets (28.8 vs. 21.3 kicks/min, SE=0.09; P=0.02). Additionally, 51.2% of castrated piglets displayed body flailing, whereas only 4.4% of sham piglets displayed the same behavior (P=0.03). Castrated piglets also responded with more high frequency (≥1,000 Hz) calls than sham-castrated piglets (23.6 vs. 18.6 calls/min, SE=0.26; P=0.04) and high frequency calls tended to be of longer duration for castrated piglets (0.45 vs. 0.27 sec/call, SE=0.04; P=0.08). These results indicate that surgical castration increased the frequency of leg kicks, body flailing, and high frequency calls compared to sham-castration, suggesting these may be useful behavioral indicators of acute pain in piglets.

The reliability of the PGS was tested by evaluating the agreement among the four raters. The intra-class correlation coefficient (ICC) for orbital tightening, ear position, nose bulge/cheek tension, temporal tension, and lip contraction were 0.68, 0.67, 0.54, 0.40, and 0.28, respectively. For all time points (T1-T4), the odds of castrated to sham-castrated piglets for all FAUs did not differ from 1, suggesting that castration did not change any FAU. Moreover, geometric morphometric analysis did not discriminate differences between the treatment groups or among time points (all $P > 0.10$). These results suggest orbital tightening and ear position are more reliable than all other FAUs for a PGS, as indicated by the ICCs. However, neither the PGS nor 3D landmark-based geometric morphometrics were sensitive enough to detect pain in piglets post-castration in the current study.

The objective of the second study was to develop a Goat Kid Grimace Scale (GKGS) as a novel method of pain assessment following disbudding. Goat kids (n=42) of mixed sex and breed between the age of 2-15 days were randomly assigned to one of seven treatment groups via block randomization (6 blocks, 7 kids per block): 1) simulated disbudding (SHAM), or thermal disbudding after administration of 2) 0.05 mg/kg IM xylazine (X), 3) 1 mg/kg oral meloxicam (M), 4) 4 mg/kg SQ buffered lidocaine (L) 5). xylazine + buffered lidocaine (XL), 6) xylazine + oral meloxicam (XM), and 7) xylazine + oral meloxicam + lidocaine (XML). All pain agents were administered 20 min prior to disbudding. Photographs of kids' faces were taken at six time points: baseline (T1 – before administration of pain agents), pre-blood collection (T2), pre-disbudding (T3), post-disbudding (T4), and directly before (T5) and after (T6) mechanical nociceptive testing (4 h post-disbudding). Data were analyzed using the Mixed and Genmod procedure of SAS software.

Four trained raters scored the kid photographs over two sessions using four FAUs: orbital tightening, ear position, lip tightening, and nostril dilation. The ICCs were as follows: orbital tightening (0.79 & 0.84), ear position (0.67 & 0.61), nostril dilation (0.45 & 0.56), and lip tightening (0.45 & 0.56) for the first and second session, respectively. Prior to disbudding (T1-T3), the odds of X, XL, XM, and XML scoring in a higher grimace category for orbital tightening were 79.1, 164.80, 128.0, and 86.0 to SHAM, respectively (all $P < 0.001$). For all times points (T1-T6), the odds of X, XL, XM, and XML scoring in a higher grimace category for orbital tightening were 43.5, 89.0, 82.8, and 71.2 to SHAM (all $P < 0.001$), respectively. The odds of X, M, L, XL, XM, XML scoring in a higher grimace category for ear position were 23.1, 5.1, 12.2, 23.6, 32.1, and 45.9 to SHAM (all $P < 0.05$), respectively. The odds of X, L, XL, and XML scoring a higher grimace category for lip tightening were 8.3, 12.3, 15.9, and 16.0 to SHAM (all $P < 0.05$), respectively. The odds of XM scoring in a higher grimace category for nostril dilation was 5.89 to SHAM ($P = 0.0005$). Results suggest that orbital tightening and ear position may be promising FAUs for a GKGS. Application of some pain mitigation treatments (X, XL, XM, and XML) alone induced a change in orbital tightening, while all FAUs changed in response to pain mitigation treatments (X, L, XL, XM, and XML) together with disbudding.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 3

CHAPTER 4

CHAPTER 1: LITERATURE REVIEW

1. Introduction

Neonatal livestock animals routinely undergo procedures such as surgical castration and thermal disbudding within the first week of life [1][2]. The purpose of these procedures is to facilitate management of livestock animals within a food production setting. For instance, male piglets are surgically castrated to ensure high meat quality through the elimination of boat taint, reduce levels of aggression for the safety of pen mates and caretakers, and prevent unwanted breeding [3]. For similar reasons, goat kids within a dairy production setting are disbudded for safety, meat quality, and the prevention of housing damages [4]. It is uncommon for piglets and goat kids to receive an analgesic or anesthetic agent for pain relief for these procedures in the US [1][2]. An analgesic provides pain relief, while an anesthetic blocks pain through the loss of physical sensation [1][2]. However, extensive research has determined that both surgical castration and thermal disbudding are painful for animals and therefore both constitute an animal welfare concern [5].

Each year the U.S. markets over 115 million pigs, approximately half of which are male and nearly all of these are surgically castrated [1][6]. The American Veterinary Medical Association (AVMA) recommends swine producers to avidly use practices that minimize pain due to castration [7]. Management practices are also expected by the British Veterinary Association and Goat Veterinary Society to reduce the pain caused by disbudding [8]. In an extended effort to eliminate pain during and after these procedures, these organizations highly encourage the implementation of pain-relieving protocols to uphold animal health and well-being [8][9].

The AVMA stated that piglets castrated after 14 days of age should be provided with an analgesic, anesthetic, or a combination of the two [7]. Disbudding under UK legislation is considered a veterinary surgery and anesthesia should be used [4]. Even highly recommended, the use of pain agents in animal production has faced opposition from producers based on increased cost, labor, and safety risks [10]. To determine what pain agents are reliably effective in managing pain in all regards, scientists must first identify and recognize pain in animals [11]. This presents a major challenge as pain is an individual experience and can only be inferred in non-verbal subjects [12]. Therefore, scientists must rely on pain-associated indicators. Research has focused on assessing pain through a variety of indicators associated with the behavior, physiology, and health of animals [13]. An animal's response towards nociceptive drugs in part with these indicators has also been investigated [14]. Since pain assessment is complex, the use of multiple indicators is necessary to make informed decisions as to whether an animal is experiencing pain and the level of severity [11]. This literature review focuses on current knowledge regarding pain assessment in neonatal pigs and goats.

2. Animal welfare

Animal welfare is a field of science used to develop standards of animal care and guide people on how their actions affect the animals under their care [17]. The World Organization for Animal Health defines animal welfare as the "physical and mental state of an animal in relation to the conditions in which it lives and dies" [18]. This essentially refers to animal's quality of life while alive up until the moment it is processed for food, dies of natural causes, or must be euthanized [17][19][20]. The interactions between animals and their caretakers (i.e. human-animal relationship) throughout all phases of life can be positive, neutral, or negative resulting in long-lasting effects that greatly affect animal welfare [21][22][23]. Thus, animal welfare is an essential component in all aspects of animal research, teaching, and testing [24]. In the U.S. the Animal Welfare Act (AWA) of 1966 is currently the only federal law enforced by the USDA Animal and Plant Health Inspection Services that regulates the treatment of animals in research, exhibition, transport, and by dealers, requiring minimum standards of care and treatment [24]. However, the AWA does not cover livestock animals in any regard.

The criteria used to assess animal welfare are multi-dimensional [20]. The three spheres model is acknowledged as a central framework for assessing animal welfare integrating three ethical concerns – biological functioning, natural living, and affective state [19][25]. Biological functioning refers to the physical health of an animal in terms of health, growth, and reproduction [19][25]. Natural living exemplifies the opportunities (e.g. substrates for foraging) given to an animal to lead a natural way of life and their ability to express instinctual behaviors [19][25]. Lastly, affective state denotes the underlying emotions animals are feeling, influencing their mood [19][25]. Positive affective states of comfort, contentment, and pleasure should be enhanced by eliminating negative affective states such as fear, stress, anxiety, and pain [19][25].

Researchers have focused on how the resources provided to an animal will affect the animal in relation to the three spheres [20]. Thus, animal welfare can be assessed through management (i.e. management-based measures) and environmental (i.e. resource-based measures) inputs and animal-based (i.e. animal-based measures) outputs [17][20][26]. Inputs include core areas of production such as housing, stockmanship, nutrition, breeding, transportation, slaughter, and euthanasia, while outputs include animal behavior, production, health status, and mental state [20][27]. In addition to inputs and outputs, an animal's natural history, normal behavioral, and interests when given the opportunity to choose provide reliable information on the quality of life an animal is experiencing [20][28].

In 1964, publication of Animal Machines by Ruth Harrison began the era of animal welfare concern within agriculture, as she exposed the living conditions of animals within large scale intensive farms [24]. The following year, the idea that animals are sentient beings was proposed in the Brambell Report in 1965, which ultimately led to the codification of animal welfare principles known as the Five Freedoms [24][27][29]. The Five Freedoms described the ideal state of animal and specified that animals should be free from: thirst and hunger; discomfort; pain, injury, and disease; fear and distress; and free to express normal behavior [29]. Since the Five Freedoms, as increasing emphasis has been placed on the promotion of positive welfare states and the Five Domains were established distinguishing nutrition, environment, health, behavior, and mental state [27].

As sentient beings, animals experience the unpleasant physical and emotional aspects of pain [28][30]. Pain is associated with poor animal welfare as it negatively impacts an animal's quality of life [28] [31][32]. Studies have shown that pain can induce abnormal behavior, avoidance responses, high frequency vocalizations, an increase in immunological and inflammatory biomarkers, and changes in facial expression [9][12] [13][14][28][33][34]. Since pain cannot be fully eliminated within a food production setting, scientists are working towards developing reliable methods of pain assessment to improve pain management for food animals [35]. However, pain research has many challenges including accurate identification of pain, understanding of baseline and pain-associated responses, reliability of subjective ratings, and lack of knowledge on the specificity of nociceptive drugs [9][28][35].

Nonetheless, the assessment of pain plays a critical role in establishing animal welfare standards and determining what is required of caretakers to safeguard the well-being of their animals. Pain management falls under a management-based requirement for procedures such as surgical castration and disbudding [17][26]. Scientists use these management-based requirements to assess how management practices affect animal welfare, to test practices that have the potential to improve animal welfare, and examine how standards are upheld once in effect [17].

3. What is pain?

The International Association for the Study of Pain (IASP) defines pains as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" [9][28][33][36][37]. Hence, pain is a complex experience encompassing both a physical and emotional response [28][30][37]. Pain is cognitively integrated and dependent on the level of consciousness of the animal, an animal needs to be aware of itself and its surroundings to process the painful experience [13][28][36]. Therefore, how an animal perceives and responds to pain varies among subjects [9][28]. Pain itself can differ in terms of location, duration, intensity, and modality [28]. Animal-related factors such as age, health status, species, breed, and individual threshold all affect how an animal experiences pain [9].

Scientists categorize pain based on the duration of the pain state, cause and anatomical location of the tissue damage, and the presence of inflammation [9][28][33][36]. Nociception is the sensation of pain and is defined as the "neural process of encoding and processing noxious (e.g. tissue damaging) stimuli" [12][33][36]. Nociceptive pain arises from the activation of pain receptors called nociceptors and is described as short-lasting [33][36]. This type of pain is adaptive, with a biological function to alert and protect an organism, activating avoidance responses [33][38]. Sneddon et al. [39] proposed a list of criteria suggesting that animals who satisfied all criteria are capable of experiencing pain. The list includes criteria that is analogous to how humans process pain such as procession of nociceptors, nociceptive pathways to the brain, brain structures similar to the human cerebral cortex that controls and alters the sensation of pain, and positive responses to pain agents [39]. Pain is referred to as the subjective component (i.e. affective state), while nociception refers to the physiological process [33][39].

In addition to being an individual experience, the assessment of pain in animals presents more of challenge given their inability to verbally communicate [33]. Furthermore, as prey species, food animals may not overtly express when in a pain state as a protective mechanism against predators [28]. Natural evolution has enabled this protective mechanism to become instinctual, as concealing behavioral indicators of pain aids in survival [40]. Consequently, the presence and severity of pain may be underestimated in animals [35]. However, pain assessment in neonatal livestock species may be advantageous as neonates have the tendency to overtly express certain behaviors to obtain maternal care [41].

4. Nociceptive pain – mechanisms and function

Overall, the nociceptive pain pathway is similar among the mammalian species [33]. Pigs are a primary model used to study nociceptive pain, as this species have neuroanatomical structures to essential for pain perception similar to humans, specifically nociceptors and nociceptive pathways connected to the central nervous system [12][14]. The distribution, axonal excitability, and conduction velocities of pig nociceptors are analogous to those in humans [33].

The body is equipped with two types of nociceptors, both unimodal and polymodal, that are located on the peripheral ends of sensory nerves [33][36]. Unimodal receptors are sensitive to one type of stimulus, while polymodal are sensitive to multiple stimuli [33][36]. The body receives a mechanical, chemical, or thermal stimulus and its intensity determines the ability of an animal to detect it [33][36]. Once the stimulus reaches a specific threshold, it is converted into an action potential by the nociceptors [33][36]. The action potential produces a signal that travels along sensory afferent nerve fibers along the dorsal horn of the spinal cord to the brain [36]. Myelinated A-delta fibers and unmyelinated C-fibers are the two primary afferent nerve fibers essential in the transduction of signals from the peripheral ends of sensory neurons to central nervous system [33][38]. Myelinated A-delta fibers quickly conduct signals of low and high intensity (e.g. mechanical and thermal stimuli), translating the sensation of a rapid “stabbing” pain [33][38]. The fast conduction of Myelinated A-delta fibers function as a warning to the alert the animal to withdraw from the stimulus [38]. Unmyelinated C-fibers conduct signals of high intensity polymodal receptors (e.g. mechanical, thermal stimuli, and thermal) for a “slow burning” pain [33][38].

Lastly, the brain processes the signal to alert the organism of the tissue damage [36]. Within the mammalian brain, the somatosensory cortex controls how pain is perceived [33]. At the site of tissue damage, inflammatory mediators are released in response to the activation of the pain pathway [33][36]. The transmission of pain can either be amplified or inhibited by neurotransmitters such as substance P and prostaglandin (PGE_2) released by primary afferent axons [33][36]. The level of amplification or inhibition is dependent on the strength of the original stimulus [36]. Since Substance P and PGE_2 influence the perception of pain, they are commonly used as pain biomarkers [12]. The synthesis and release of neurotransmitters are controlled by neuromodulators [36].

Nociceptive pain serves a vital biological function [33]. It ultimately functions to protect the body by alerting an animal of potential danger [33][39]. It is an important aspect of evolutionary biology as it plays an essential, advantageous role towards survival [40]. Evolution has integrated pain as a mechanism to activate the allostatic processes within the body that restore biologic homeostasis [40]. Nociceptive pain enables an animal to learn behaviors used for avoiding and recognizing dangerous situations, to minimizing damage to the body [33][40]. Additionally, nociceptive pain helps maximizes recovery by allowing an animal to obtain the appropriate time to heal from damaging injuries [28].

5. Types of nociceptive pain

Nociceptive pain is a type of physical pain, as it is defined by actual or potential tissue damage [9][28][34[36]. It requires activation of nociceptors by a mechanical, chemical, or thermal stimulus [33]. Nociceptive pain can be categorized as acute or chronic pain [33].

5.1 Acute pain

Acute pain is specific in cause and temporary, not lasting longer than the expected healing period [34][36]. Broken bones, cuts, burns, and elective surgeries are common causes of acute pain [38]. The purpose of acute pain is to signal a risk and quickly alter an animal's behavior to avoid the painful stimulus and optimize conditions for healing if necessary [38]. Although acute pain does not persist beyond the normal time of healing (e.g. up to months), it can vary in intensity from mild-to-moderate or severe-to-excruciating [38].

5.2 Chronic pain

Chronic pain assessment faces further challenges. Chronic pain can develop from acute pain [38]. It is not temporary and persists longer than the expected healing period (e.g. from months to several years), potentially never fully healing and as a result may be considered a disease state [33][34][36][38]. The underlying cause of chronic pain is difficult to define, as it may originate from damage to the peripheral or central nervous system [34][42]. Chronic pain serves no biological function, as it is maladaptive with no purpose towards aiding survival and may lead to alterations in mood (e.g. depression and anxiety) [33][34][38] Osteoarthritis, degenerative joint disease, stomatitis, and degenerative disc disease are examples of conditions associated with chronic pain states [38].

5.3 Somatic pain

Chronic and acute pain can be further classified into somatic and visceral pain. Somatic pain can be easily localized and originates primarily from a location on the wall of the body cavity [33][36]. It is commonly caused by a minor injury, surgery, or infection [36]. A stimulus causing the onset of somatic pain activates the A-delta fiber nociceptors as they are predominately present on the cutaneous level [33][40]. Somatic pain is described as "escapable" pain, working as a threat indicator and leading to the "fight or flight" response"[40].

5.4 Visceral pain

Visceral pain originates from deep within the viscera of the body cavity and as a result may be poorly localized [33][34][36][40]. The cause of visceral pain can arise from damage to a particular organ or group of organs, evoking activation of the C-fiber nociceptors located in the viscera [33][40]. Visceral pain is described as “inescapable” pain, expressed with passive coping strategies leading to “sickness behavior” (e.g. nausea, vomiting, and increase in heart rate and blood pressure) [40].

6. Management practices that cause pain for neonatal pigs and goats

In June 2019, the USDA National Agricultural Statistics Service, reported that US farms consisted of 75.5 million hogs and pigs [43]. On the farm, piglets undergo several management procedures within the first week of life, commonly referred to as “piglet processing” [12]. These practices allow for pig identification, reduction of injurious and aggressive behavior, improvement of product quality, and the establishment of facility management standards in large-scale production systems[12]. However, in the US it is common for trained staff to not provide piglets with an analgesic or anesthetic agent for pain relief before or after these procedures [7].

“Piglet processing” procedures vary across farms in terms of restraint methods, who performs the procedure, time of execution, whether all procedures are completed all at once etc. [1]. Iron injections, surgical castration, tail docking, teeth resection, and identification (i.e. ear notching and tagging) are some of the procedures performed dependent on management protocol [12] [33]. All of these procedures are painful to varying degrees, as they cause somatic and visceral tissue damage and induce behavioral responses related to acute or chronic pain [12] [44].

6.1 Surgical castration

Surgical castration is one of the most common practices which cause pain in piglets [14]. It serves to reduce aggression, prevent unwanted breeding, and prevent production of boar taint [1]. Boar taint is attributed to a combination of compounds (i.e. androstenone and skatole) causing an unpleasant meat odor and flavor and ranging in prevalence from 1-30% depending on breed and individual differences [1]. Within the EU countries, it is estimated that 80% of the 250 million male piglets born are castrated each year [35]. Results from the PIGCAS (Titled: Attitudes, practices and state of the art regarding piglet castration in Europe) project concluded that out 26 EU countries, the rate of castration was 90-100% in 19 countries [1][45]. This echoes the prevalence of castration in the US [1]. The AVMA recommends producers to castrate piglets between 4-14 days of age and suggests that pain mitigation should be provided if piglets are castrated after 14 days [7]. The advantages of performing these procedures early in life include easy restraint of pigs given their small size, unmatured reproductive organs and sensory systems which can reduce bleeding and pain, and antibodies from the dam's colostrum which may help promote healing and prevent infection [1][46].

Surgical castration is the removal of the testes, requiring one or two incisions of the scrotum to externalize the testes [12]. During surgical castration, piglets can be restrained by suspension of the hind legs, between an individual's legs, or using a steel castration device or v-trough [7][47]. Testes can be removed by cutting or pulling out the spermatic cord following the application of a disinfectant (e.g. iodine) [7]. Taylor and Weary [48] determined that severing and pulling the spermatic cord was the most painful part of the procedure followed by the scrotal incision.

After castration, piglets exhibit abnormal behavior such as decreased locomotion, alternate lifting of hind legs, abnormal postures, spasms, and excessive head turning towards the surgical cite [1][12]. An increase in desynchronized maintenance behavior (e.g. feeding, drinking, exploring, grooming, and walking) has been observed for up to 6-8 h post-castration [1]. Castrated piglets also elicited vocalizations of higher peak frequency (i.e. screams vs. grunts and squeals) with longer duration than sham-castrated and pain-medicated piglets [12]. Concentrations of cortisol, a physiological indicator of stress, were also greater in castrated piglets for 2 h post-castration compared to sham-castrated piglets [1].

There is limited knowledge on chronic pain or hyperalgesia caused by castration [1]. However, surgical castration can lead to potential complications including hemorrhaging, edema, infection [7]. Additionally, there is evidence to suggest that castrated piglets have suppressed immunity, with an increase in inflammation and pneumonia [7]. Immunocastration (e.g. Improvac), raising intact males, chemo-castration, and genetic selection are alternatives to surgical castration being explored [1][35]. Immunocastration immunizes pigs against the gonadotropin releasing hormone (GnRH) and inhibits testicular function, requiring two rounds of injections 4 weeks apart [1]. Unlike immunocastration, chemo-castration is the local destruction of testicular tissue by chemical (e.g. formaldehyde, lactic and acetic acid) injection [1].

6.2 Tail docking

Tail docking is performed on more than 90% of piglets in both the US and the EU [35]. Tail docking is the partial removal of the tail and it is common practice to leave a 1.9-2.54 cm (0.75-1.0 in) stub, the equivalency of covering the vulva in females and the same representative length in males [12][47][49]. Clippers, cautery irons, knives, scalpels, or scissors can be used to remove part of the tail [12]. The reduction of tail biting is the main reason of this procedure [49]. Tail biting is defined as an abnormal behavior with negative implications for animal welfare and profitability [49]. It is difficult for producers to identify the underlying cause of tail biting given numerous risk factors such as environmental conditions (e.g. stocking density, ventilation, and lack of environmental enrichment), genetics, nutrition, sex, and health status [49].

Tail biting has also been referenced as a redirected exploration behavior [49]. Thus, environmental enrichment of novel objects (e.g. chains and rubber toys) and substrates (e.g. straw and peat) have been heavily investigated, as they may provide pigs the opportunity to perform natural behaviors of rooting [49]. Objects with ingestible, chewable, and destructible properties are highly preferred by pigs [49]. Social groups with low stocking densities and a balanced number of females have also been suggested to reduce the incidences of tail biting [49].

Evidence of acute pain responses from tail docking have been reported, mainly related to the method used to dock the tail. Cortisol concentrations were higher in piglets tailed-docked using cutting pliers in comparison to a cautery iron [49]. Behavioral changes including tail jamming, tail wagging, and posterior scooting are observed more frequently in tail-docked piglets compared to sham-handled piglets [49]. Tail-docked piglets also vocalize more (grunts and squeals) and produce higher peak frequency vocalizations [49]. Tail docking also can lead to neuropathic injuries and increased sensitivity which may be associated with chronic pain [33] . Neuromas (also referred to as "pinched nerves" or "nerve bundles") can develop on tail stumps, with neuromatous tissue developing as early as one month post-tail docking and leading to traumatic neuroma development at 16 weeks [33][49].

6.3 Teeth resection

Piglets are born with eight sharp canine teeth referred to as "needle" teeth [50]. Piglets use their needle teeth to compete against litter mates for access to functional teats [12]. Teeth resection can help minimize injuries to litter mates (e.g. facial lacerations) and the sow's udders [12][50]. Needle teeth can be clipped using wire cutters or other cutting tools or grinded using an electric or battery-operated rotating grindstone [47]. Marchant-Forde et al. [51] found that grinding took longer and resulted in higher cortisol concentrations, reduced growth rates, and vocalizations of longer duration compared to sham-handled piglets. However, others found no differences between the two methods related to adrenocorticotrophic hormone (ACTH) and cortisol levels [12].

Abnormal "chomping/champing" behavior and an increase in sleeping and lying have been observed in piglets after this procedure [47][50]. Piglets have also experienced tissue damage that may lead to long-term pain effects. Both teeth clipping and grinding can induce severe lesions such as exposure of the pulp cavity, fractures, hemorrhaging, and abscesses [33]. Given inconsistencies across studies, justification of teeth resection remains questionable [50][51]. There is evidence supporting that piglets with needle teeth may have no effect on face and udder lesions, while others report benefits of teeth resection on preweaning mortality and growth rates [51]. It has been suggested that piglets with intact teeth induce sow injuries, causing sows to become restless and increasing piglet crushing as the sow changes positions [47][50]. The partial removal of needle teeth (e.g. 1/3 of the tooth), leaving teeth intact for only low-birthweight piglets, or only performing this procedure when evidence of facial and udder lesions are evident have been proposed as alterative solutions [47][50].

6.4 Piglet identification

Ear identification is essential for good record-keeping (e.g. health records) and traceability of the pig throughout the farm and products (e.g. assurance of quality and public health) [47][50][52]. Ear notching and tagging, and transponders are methods used for pig identification. The AVMA recommends ear notching within the first week of like, while in Canada, the National Farm Animal Care Council (NFACC) requires that ear notching be performed prior to 14 days of age [3]. Ear notching is performed using a "V-notcher", cutting out a triangular section of the ear [47]. The right and left ear represent the litter number and individual pig, respectively. However, ear notching is not commonly performed in large scale production settings, as ear notching is time consuming and painful for piglets [50].

Ear tags are pierced onto the ears and carry a visual identification number [47][50]. Transponders are injected under the skin (e.g. base of the ear) and function using an electronic tracking system [47][50]. An advantage to tags and transponders is their easy and quick application that may be less stressful and painful for the pigs and caretakers [3][47][50]. However, tags can be torn off and transponders can migrate [47].

Research on pain caused by pig identification procedures is limited [47][52]. Nevertheless, pigs have shown signs of discomfort, inflammation, and infection at identification sites [50]. In addition, ear notched piglets elicited more vocalizations (i.e. grunts and squeals), head shaking, and escape attempts compared to sham-handled piglets [47]. Marchant-Forde et al. [51] found that ear-notched piglets evoked calls of higher peak frequency, produced greater cortisol concentrations at 4 h post-ear notching, and had worsened wound scores than ear-tagged piglets. Ear notching also took longer than ear tagging [51]. Moreover, in a study comparing ear-notching, tagging, and transponders, pain-related behaviors (i.e. head shaking, ear and abdominal scratching, huddling, and shivering) were higher for notching and tagging and sound pressure (dBA) and lactate levels were highest for notching compared to transponders [52].

6.5 Disbudding

Thermoregulation, protection from predators, and establishment of dominance within a herd are advantages horns provide to goats in the wild [4]. However, horns on dairy goats are removed in a process called disbudding which destroys the corium from which the horn grows [4]. Within a dairy production system, horns are a risk for the safety of pen mates and caretakers. Additionally, goats with horns require extra space, can destroy housing facilities, and reduce profitability due to carcass bruising [2]. There are several methods used to disbud goat kids such as caustic paste, clove oil, cryosurgical (i.e. liquid nitrogen), and thermal cautery [53][54]. Thermal cautery is most commonly used by producers and veterinarians as the corrosive action from caustic paste can rub onto pen mates or other body parts, clove oil lacks FDA approval, and liquid nitrogen is more painful [4][53].

It is highly recommended that goat kids be disbudded between 2-7 days of age, as developing horn tissue begins to attach the skull around 3 weeks of age [4]. Dehorning differs from disbudding as it is the removal of the horns once the horns have attached to the skull [55]. Prior to cautery, hair around the horn buds should be removed [4]. The iron is heated up (295-326°C) and placed over the horn bud for a maximum of 4-7 s using a gentle rocking motion as the kid is being restrained [4][56]. Done incorrectly, thermal disbudding can cause third degree burns, bacterial infections, inflammation, and subcutaneous damage [57]. Poor technique can lead to additional adverse outcomes such as seizures, fractured skulls, anesthetic overdose, and even death [4].

Changes in maintenance behavior such as feeding, grooming, biting, running, and exploration have been used to evaluate pain in goats following the disbudding procedure. Disbudded kids spent less time standing, feeding, and drinking than medicated kids (e.g. 2% lidocaine and flunixin meglumine) kids [58]. Goat kids disbudded without any pain mitigation exhibited more head shaking, rubbing, and scratching than sham-disbudded kids [57]. Disbudded goats also had greater cortisol concentrations and struggled and vocalized more than sham-disbudded kids [2].

7. Pain Assessment

For humans, verbal self-report is considered the “gold standard” for pain assessment [12]. As animals are non-verbal, there is as of yet no “gold standard” for assessment of their pain [12][33]. Routine management procedures that cause pain in animals have been the main focus of study for pain assessment in neonatal livestock [12]. Using these procedures as experimental models, neonates receiving the full extent of a procedure (i.e. negative control) are compared to neonates who experience only handling procedures (i.e. positive control) [12][59]. Additionally, neonates given an analgesic or anesthetic agent for pain relief have also been compared to positive and negative controls [12].

Pain indicators are assessed before, during, and/or after these procedures [12]. Pain indicators are animal-based measurements enabling some insight into the nature and severity of the pain animals are experiencing in terms of how they respond [17][26][33]. Animals’ responses to pain can be evaluated using behavioral, physiological, and clinical indicators [12][33]. Although there is no consensus on which type of indicator is better than others, pain indicators should be robust, reliable, sensitive, and valid [12][26].

Reliability is the ability for a pain indicator to be reproducible over time and in different experimental settings [12][60]. Reliable indicators should meet a level of agreement (correlation) between (inter) and within (intra) observers [12][26][60]. Observer bias should not influence the reliability of an indicator [12][26]. Sensitivity refers to the ability of an indicator to detect pain consistently at both low and severe levels [12]. Sensitive indicators should consider species-specific and individual (e.g. sex, age, and health status) characteristics [12][26]. Lastly, validity is how well an indicator captures the measurement of interest [12]. Valid pain indicators measure pain only, not other negative affective states such as fear, stress, and anxiety [12].

7.1 Behavioral indicators of pain

Deviations from their normal behavioral patterns are considered as a significant indicator of pain [11]. Animals perform daily patterns of normal behaviors (i.e. behaviors seen under natural conditions) such as drinking, feeding, sleeping, walking, grooming, socializing, and exploring [11]. Normal behavioral patterns are also referred to as behavioral baseline (e.g. no pain state) for pain assessment [61]. Time budgets are used to determine what proportion of the animal's time is spent performing certain behaviors [33][61][62]. Prior to a painful procedure, behavioral time budgets can be recorded and then compared to time budgets after the procedure to assess if there are any deviations from baseline behaviors. In addition to baseline behaviors, pain-specific behavior (e.g. huddling, spams, trembling, tail jamming, rump scooting, prostration, and isolation) after the pain procedure can be evaluated [12]. Retrospective behavioral assessment assesses past behavior (e.g. video recordings) and allows animals to be observed with minimal disturbance and handling stress [63]. However, a disadvantage to this method is not able to provide animals with immediate pain management if necessary [64].

Sutherland et al. [65] scan-sampled piglets at 1-min intervals for 60 min before and 90 min after tail docking. Tail-docked piglets spent more time scooting and sitting than sham-handled piglets [65]. Castrated piglets also showed more pain-specific behavior such as huddling, spasms, and trembling [66]. Compared to sham-handled and medicated piglets (i.e. CO_2 and Banamine) 30 min post-castration, castrated piglets given no pain relief spent more time lying and isolated from their littermates [67].

Piglets display escape behavior during painful procedures [12]. Marchant-Forde et al. [51] defined an escape attempt as "a body movement carried out to effect an escape". In addition to escape attempts, the number of kick bouts, and the number of individual kicks within each bout were recorded [51]. They reported that piglets castrated by method of cutting and tearing had a greater number of escape attempts than the control group [51]. Walker et al. [68] compared the "reaction behavior" of castrated piglets from three treatment (no anesthesia, isoflurane (ISO), and isoflurane/N_2O (ISO/N_2O)) groups using a 4-point scale (0 = no movement, no vocalization, 1 = 1 movement, 2 = vocalization and slight movement, and 3 = strong vocalization and strong movement) [68]. The mean score values of castrated piglets with no anesthesia was 7.7, whereas ISO and ISO/N_2O, scored 0.6 and 0, respectively, indicating that anesthesia via ISO and ISO/N_2O greatly reduced reaction behavior[68].

Leidig et al. [69] developed a similar scale including individual scores for intensity and duration for "defense behavior". Behavioral intensity used a 5-point scale (0 = no movement, 1 = movement of 1 limb, 2 = more than 1 limb, 3 = participation of vertebral column, and 4 = high intensity), while behavioral duration was measured along a 4-point scale (0 = no movement, 1 = 1 single movement, 2 = repeated movements, and 3 = continuous movement) [69]. Castrated piglets had the highest defense behavior scores for both duration and intensity compared to sham and piglets given Procaine, an anesthetic [69]. Hanasson et al. [10] used a visual analogue scale (VAS) to judge low to high intensity resistance movements ranked on a 1-4 scale. Piglets medication with lidocaine and lidocaine in combination with meloxicam produced the least amount of resistance movements compared to controls [10].

The variability in terminology and inconsistency in defining behavior during painful procedures can lead to unreliable assessment of escape behavior within different experimental and farm settings. Escape behavior should be clearly defined in terms of what anatomical body parts are moving (e.g. front vs. hind legs) and elements and other body part movements that are observed as a result of limb movements (e.g. skin wrinkling and participation of neck) for consistent measure.

7.2 Vocalization indicators of pain

Vocalizations are the main mode of communication among pigs [33]. The characteristics of pig vocalizations remain consistent even through years of domestication [70]. Pigs communicate using three different types of calls: grunts, squeals, and screams [12]. Vocalizations are considered useful pain indicators as structures in the brainstem receive important sensory, emotional, and homeostatic information from primary brain structures such as the sensory cortex, limbic system, and cingulate cortex control, respectively [70]. With the use of specialized equipment (e.g. STREMODO) and software (e.g. RavenPro), researchers have been able to provide detail analysis on vocalization parameters such as call duration, rate, peak frequency, energy, and power [12][51][71].

Duration and peak frequency have been studied extensively and are considered reliable indicators of pain. Peak frequency (Hz) is defined as the loudest frequency within a call, with low (< 1000 Hz) and high (≥ 1000 Hz) classifications [72]. Peak frequency describes the loudest frequency of a single event in each call, which is considered more accurate than average frequency (e.g. main and weighted frequency) [51][73].

Marx et al. [73] determined that vocalization parameters for screams are significantly different than grunts and squeals. Call energy for grunts, squeals, and screams were low, higher, and highest, respectively [74]. Castrated piglets elicited more calls of higher frequency and longer duration compared to sham-handled piglets [75][76]. When given lidocaine, castrated piglets produced calls of lower intensity compared to piglets given meloxicam and sham-handled piglets [10]. In a similar study, squeals from castrated piglets were of longer duration and higher intensity compared to meloxicam-medicated piglets [77]. However, it is important to acknowledge that piglets are highly vocal when being handled, which should be taken into consideration when assessing vocal responses [33].

7.3 Physiological indicators

A physical and psychological stressor like pain activate the hypothalamic-pituitary-adrenal axis (HPA) [12]. The HPA works towards increasing metabolism and reducing inflammation to help the body maintain homeostasis [33]. The activation of the HPA causes the hypothalamus to release corticotrophin-releasing hormone (CRH) and stimulates the anterior pituitary to secrete the adrenocorticotrophic hormone (ACTH), which act on the adrenal glands [12][33]. Cortisol is a glucocorticoid measured from blood plasma and is the body's main stress hormone produced by the adrenal glands [38][33][61]. Castrated piglets tend to have higher cortisol levels compared to sham-handled piglets [66]. Piglets receiving lidocaine before castration had lower cortisol levels than castrated piglets without pain-reliving medication and meloxicam medicated piglets [77].

A list of other common physiological pain indicators in pigs include lactate, catecholamines, substance P, and adrenocorticotrophic hormone (ACTH) [12][33]. Substance P is a neurotransmitter released by the primary afferent nerve fibers and is considered a potential pain biomarker given is location at the site of tissue damage [12][36]. Sutherland et al. [67] found that substance P was greater in piglets given CO_2 compared to shamed, castrated with no pain agent, and Banamine medicated piglets.

Physiological measurements are invasive and require additional restraint and specialized training and equipment, adding stress onto pig and caretakers compared to behavioral measurements [64]. Additionally, caution must be taken when interpreting levels of cortisol and catecholamine concentrations as they lack pain specificity and can be become elevated due to general arousal [1]. For example, there is evidence that cortisol may be directed related to the length of the surgical castration procedure, rather than the actual pain inflicted [1].

7.4 Clinical indicators of pain

The autonomic nervous system (ANS) is key in regulating and maintaining bodily homeostasis when an animal is experiencing stressful and painful conditions [33]. In part with the ANS, the sympathetic adrenomedullary system helps mobilize the body and activates the "fight-or-flight" response [12]. Autonomic changes can be measured indirectly as a result of the ANS being activated [33]. Examples of autonomic changes, also referred to as clinical indicators, are heart rate, respiratory rate, blood pressure, and temperature (e.g. skin or rectal) [14][33].

As the sympathetic adrenomedullary system begins to respond, blood vessels begin to dilate to direct blood towards muscles and organs in preparation for "fight-or-flight" [12]. Thus, skin temperature is considered a good indicator of pain. Castrated piglets experienced elevated ear temperatures compared to piglets given lidocaine or meloxicam [10]. Eye and rectal temperature was greater in castrated piglets for up to 4 h post-castration [12].

Like physiological indicators, clinical parameters are also invasive and require additional restraint and specialized training and equipment [64]. Caution should also be taken when interpreting clinical increased clinical responses (e.g. heart rate) as general arousal and excitement could be a probable causes [1].

8. Nociceptive drugs

It is important that scientists and veterinarians learn how to control and minimize pain because pain cannot be fully eliminated [13][35][37]. The French National Institute for Agriculture Research (INRA) has developed a three-step approach – Suppress, Substitute, and Soothe, an analogy to the 3Rs (Reduction, Refinement, and Replacement) [35]. This approach aims to review and restructure existing solutions by suppressing painful stimuli, substitute for procedures that cause the least amount of pain and stress, and finally soothe pain that is unavoidable [35].

Validation of pain assessment methods can lead towards the development of licensed drugs effective in treating pain that are species-specific [33][35]. In the list of criteria proposed by Sneddon et al. [39], “a reduction in adverse behavioral and physiological effects after administration of analgesics or painkillers” is considered a key factor to examine when assessing pain in animals. The rational of using nociceptive drugs is supported given that non-human mammals process nociceptive pain similar to humans in terms of physiological and behavioral responses [78]. Xylazine, meloxicam, and lidocaine are commonly nociceptive drugs used on piglets and goat kids.

8.1 Xylazine

Xylazine is an alpha-2 adrenoceptor agonist [78][79]. It acts as an analgesic by binding to alpha-2 receptors located in the central nervous system (CNS) [78]. The specific location of alpha-2 adrenoceptors in the CNS is relatively close to brain and spinal cord structures that process pain information [78]. Within veterinary medicine, xylazine is primarily injected intramuscularly or intravenously and used to sedate patients and relax muscles [78]. Xylazine is less effective on pigs, but is commonly used in ruminants with extreme care as risk of cardiovascular depression, dyspnea, and arterial hypoxemia are high [80]. Goats may take up to 1 h to fully recover from the analgesic effects of xylazine after injection, with an initial effect starting within 10 min [81].

8.2 Meloxicam

Meloxicam is non-steroidal anti-inflammatory drug (NSAID), with anti-inflammatory, analgesic, and antipyretic (i.e. fever reducer) properties [78]. As an NSAID of the oxicam class, meloxicam decreases the synthesis and release of prostaglandin by inhibiting the cyclooxygenases (COX-2) [77][78]. It can be intravenously or subcutaneously injected or given orally [77][82]. For ruminants and pigs in the U.S., meloxicam requires an extra label drug use (ELDU) under the Animal Medical Drug Use

Clarification Act (AMDUCA) [82][83]. However, meloxicam is licensed for pigs in the E.U. and Canada to relieve post-operative pain in pigs [77][82]. Meloxicam has not been licensed for goats, but results have shown meloxicam activates within 30 min. and has long-lasting effects on ruminants (e.g. 24 h) [83].

8.3 Lidocaine

Lidocaine inhibits the primary afferent nerve fibers (i.e. myelinated A-delta fibers and unmyelinated C-fibers) from eliciting action potential and preventing the transmission of noxious stimuli to the brain [78][82]. Thus, as a local anesthetic, lidocaine can provide complete analgesia to the application site, blocking pain to myelinated A-delta fibers first, followed by the unmyelinated C-fibers through subcutaneous or intramuscular injection [78]. Lidocaine has acidic properties and it has been suggested that an injecting lidocaine is irritating and aversive, but buffering may reduce these side-effects [2]. Lidocaine is classified as a short-acting local anesthetic, with an initial effect within 5-10 min. and maximum effect lasting about 1 h [78][82]. In piglets, lidocaine has been injected into the scrotum, testes, and spermatic cord showing positive responses [10][77]. Unlike calves, goats require four injections of lidocaine to block the cornual branch of the infratrochlear and lacrimal nerve in both horn buds [4].

9. Facial grimace

The grimace scale identifies changes in facial expression induced by post-procedure acute pain [15]. The grimace scale was originally developed to assess pain in humans where verbal communication was limited (e.g. cognitive impaired) or nonexistent (e.g. infants) [15]. Across mammalian species, facial movements are evolutionarily conserved with similarities among human and non-human mammals [84]. Human facial expressions have been extensively studied as psychological and emotional indicators providing information on specific temporal and stimuli responses [84]. There is much evidence to suggest mammals have a wide range of involuntary and voluntary facial movements dependent on species [84]. However, in comparison to humans, animals have less voluntary control over facial movements, especially motor behavior [84]. Nonetheless, facial expressions are advantageous as they provide species-specific patterns and account for individual variation [84]. Furthermore, it is non-invasive, can provide real-time assessment, requires minimal training, and specialized techniques are not essential [84].

The Mouse Grimace Scale (MGS) was the first nonhuman animal grimace scale validated for non-human animals [15]. The MGS uses a simplified version of the Facial Action Coding System (FACS), termed Facial Action Units (FAUs), which are involuntary muscle movements in the four major regions of the face (eyes, ears, mouth and jaw, and nose and cheeks) specific to pain states [84]. Langford et al. [15] complied a set of mouse images categorized as "no pain (baseline)" and "pain" to devise five FAUs analogous to those observed in humans. Orbital tightening, ear position, nose bulge, cheek bulge, and whisker change were scored on a 3-point scale (0 = no present, 1 = moderately visible, and 2 = severe) [15]. Images were also given a global pain score of "pain vs. "no-pain" as a measure of accuracy [15]. For the MGS, 64 images were taken at 2 time points (pre and post) and an image for each mouse at each time point was selected based on quality to be scored by 7 trained raters [15]. Score averages (i.e. average of 5 FAU scores) resulted in high inter-rater reliability, with an average intra-class correlation coefficient (ICC) of 0.90 and global accuracy of 72% and 81% for one rater [15].

Miller and Leach [85] determined that MGS scores varied between live and retrospective assessment and recommended that differences between sex and strain should be considered. Following the MGS, grimace scales have been developed for rats, rabbits, horses, cattle, sheep, piglets, and cats using similar procedures as the MGS [59][86][87][88][89][90][91]. The Rat Grimace Scale (RGS), used the same FAUs as the MGS except combined nose and cheek bulging into one FAU as the nose and cheek muscles move congruently (i.e. nose/cheek flattening) [86]. Score averages f resulted in high ICCs (i.e. 0.86 for nose/cheek flattening to 0.96 for orbital tightening) and 81.6% accuracy of global pain scores [86]. Scores for the Lamb Grimace Scale (LGS) were significantly higher after tail-docking compared to sham tail-docking [92]. All sheep underwent surgery (i.e. unliteral tibia osteotomy) for the development of the Sheep Grimace Scale (SGS) concluding increased scores up to 3 days post-surgery and high overall ICC (0.92) and accuracy (68.2%) [91].

Gottardo et al. [59] was the first to assess facial expressions as a pain indicator in piglets (sham, castrated, and tetracaine hydrochloride, meloxicam, ketoprofen, and tolfenamic acid medicated) with only 2 FAUs (i.e. orbital tightening and cheek tension) and an overall pain score. Images were taken at 6 time points (i.e. pre-castration, post-castration, and 10, 20, 60, 180 min post-castration). Three trained raters scored the FAUs using the same 3-point scale as the MGS, except score 9 was added to represent "don't know/no assessable by the observer." Images were also scored for "overall pain" based on the whole face [59]. Results indicated no difference among treatments and only a high ICC (0.83) for orbital tightening [59]. Di Giminiani et al. [16] used the Piglet Grimace Scale (PGS) to evaluate castrated and tail-docked piglets. The PGS in this study included 10

FAUs including novel FAUs such as temporal tension, upper lip contraction, and snout plate changes [16]. Orbital tightening was the only FAU with a significant change for only tail-docked piglets using median score values and all FAUs obtained high ICCs, except lip contraction, nostril dilation, and lower jaw profile [16]. Viscardi et al. [93] used the PGS to evaluate meloxicam and EMLA for piglet castration resulting in moderate reliability, high PGS scores up to 5 h post-castration, and significant correlation between scores and behavioral activity.

Of all the FAUs examined, orbital tightening appears to be most reliable and easily scored. It is assumed that eye tightening protects sensitive areas of the face from a painful stimulus or attack [84]. Ear position has also shown to be informative, as backwards or low positions (e.g. flattened) represent negative situations, forward facing positions are associated with attentiveness [84].

9.1 Refinement of the grimace scale

Even with successful results, future research on the grimace scale requires improvement for further validation. The establishment of a robust facial expression baseline (i.e. no pain state), image quality and angle (e.g. frontal vs. profile view), separating responses (e.g. pain vs. handling and pain agents), reducing observer subjectivity, consistent score analysis, and specification of different morphological (e.g. muscle actions) and external features (e.g. breed, sex, age, and etc.) should be considered [16][33].

Kinematics is a field of mechanics and represents a novel method approach for behavioral assessment to improve animal welfare in terms of affective states [30]. It focuses on locomotion by defining points of movement in a coordinate system and tracks how the points change over time and space [30]. Kinematic-like techniques have been used to categorize and score grimace in lambs [30][92], where by markers were placed on specific locations corresponding 6 LGS FAUs on lambs – left eye aperture, right eye aperture, nose angle, mouth angle, left ear angle, and right tear angle [92]. Mouth features and orbital tightening were the only two FAUs that displayed significant quantitative variation [92]. However, the researchers in that study noted inconsistent camera angles relative to head movement and difficulties quantifying depth perception with superimposed length measurements on 2D images [92]. Unlike a human observer, the measuring software lacked the ability to intuitively correct for camera angle and depth perception [30][92]. A 3D optical system has been recommended as a possible solution towards these challenges [30] given its potential to provide fine, sensitive details attributed to changes in facial features, and supporting the validation of the grimace scale [30].

The application of landmark-based geometric morphometrics has been proposed as a 3D solution to objectively assess changes in facial expression, but as of yet has only been used on cats [94]. Landmark-based geometric morphometrics can evaluate shape variation and how it correlates with other variables [95]. This approach is predominantly used in the field of biology to enable quantitative descriptions of organisms [95]. Landmarks can be placed on 2D or 3D images to annotate locations of biological significance, where the landmark coordinates represent the organism's shape [95]. Through a single principal component analysis, Finka et al. [95] were able to quantify facial expressions in relation to pain in cats. However, the cat study used 2D images in both frontal and lateral angles of cat faces [95]. Unlike 3D, 2D images do not retain the geometric integrity of landmarks, so measurements (e.g. distance) can be greatly misinterpreted depending on the angle in which the images were taken. A key construct of 3D landmark-based geometric morphometrics is that all landmarks must be placed on the same location and orientation for proper analysis.

A combined application of landmark-based geometric morphometrics and photogrammetry would allow for a three-dimensional system providing a more robust quantification of differences in shape. Photogrammetry is a computational method for 3D reconstruction built from a series of overlapping 2D images captured from a series of angles with the object of interest in focus [96]. Photogrammetry is also referred to as structure from motion, with an advantage over 2D given its ability to automatically solve for geometric integrity, camera position and orientation, and account for targets with known 3D coordinates [96]. Applying photogrammetry to landmark-based geometric morphometrics would require the construct of placing landmarks in the same location and orientation for proper analysis. Within livestock farming, novel 3D techniques are of great interest [97]. Producers perform live assessments on pig conformation to determine carcass value and viability, but subjective opinion is not fully reliable [97][98]. The measurement of cross-sectional areas, volume, and depth are possible with 3D, with differences of adjusted by color and background [96][97]. Scientists have been successful in assessing pig conformation through automatic systems of pig weighing and shape assessment [96][97][99][98][100].

10. Proposed studies

Management procedures, such as castration and thermal disbudding, can cause pain in neonatal animals. Behavioral, physiological, and clinical indicators of pain have been used to evaluate pain caused by these procedures. Neonates deviate from their normal baseline patterns after the procedures indicating that pain negatively impacts the well-being of neonates. Therefore, this leads us to develop, evaluate, and refine the grimace scale as a novel method of acute pain assessment. The next chapter of this thesis focuses on vocalization and struggle behavior of piglets during castration as an initial assessment of pain caused by the procedure. The third chapter presents an attempt to refine the piglet grimace scale using photogrammetry and 3D landmark-based geometric morphometrics. The final chapter describes a project to develop a goat kid grimace scale.

CHAPTER 2: STRUGGLE BEHAVIOR AND VOCALIZATIONS OF MALE PIGLETS DURING CASTRATION

Maria Lou[1, 2], Beth Ventura[1], John Deen[3], and Yuzhi Li[1,2]

[1]Dept. of Animal Science, University of Minnesota, St. Paul, MN.
[2]West Central Research and Outreach Center, University of Minnesota, Morris, MN.
[3] Dept. of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN

Synopsis

The objective of this study was to assess acute pain during castration through behavioral indicators. Eighty-eight piglets were randomly allocated to one of two treatments: surgical castration (C; n=43) and sham-castration (S; n=45). Within 24 hours after birth, identical procedures were followed for both treatment groups, except sham piglets were not castrated. Struggle behavior (curl ups, leg kicks, and body flailing) and vocalization parameters (duration and peak frequency) were analyzed. Castrated piglets kicked more frequently than sham piglets (28.8 vs. 21.3 kicks/min, SE=0.09; P=0.02). Additionally, 51.2% of castrated piglets displayed body flailing, whereas only 4.4% of sham piglets displayed the same behavior (P=0.03). Castrated piglets also responded with more high frequency (≥1,000 Hz) calls than sham piglets (23.6 vs. 18.6 calls/min, SE=0.26; P=0.04) and high frequency calls tended to be of longer duration for castrated piglets (0.45 vs. 0.27 sec/call, SE=0.04; P=0.08). Results indicate that surgical castration increased the frequency of leg kicks, body flailing, and high frequency calls compared to sham-castration, suggesting these may be useful behavioral indicators of acute pain in piglets.

Key words: pig, pain, routine procedures

Introduction

Each year the U.S. markets over 115 million pigs, approximately half of which are male [6]. In the U.S., nearly all male piglets are surgically castrated without an analgesic or anesthetic agent for pain relief [1]. Surgical castration serves to reduce aggression and improve meat quality by eliminating boar taint [3]. However, research has shown that surgical castration is painful for pigs and therefore constitutes an animal welfare concern [1]. The first step in addressing this concern is to better identify and recognize pain in animals, which can be pursued in part through identification of multiple pain-associated indicators.

Pain assessment in prey species is a complex issue in part because they may not overtly express when they are in a pain state as a protective mechanism against predators [28]. Through natural evolution, this protective mechanism has become instinctual, as concealing behavioral indicators of pain aids in survival [40]. As a result, pain measures (i.e. behavioral, physiological, and clinical) may underestimate the presence and severity of the pain experienced by the animal [35]. Previous research has shown that pain assessment in neonatal livestock species may be more straightforward, as neonates in distress more overtly express certain behaviors so as to elicit maternal caregiving [41][102].

Proper pain management rests on the establishment of reliable pain indicators, which in turn requires an in-depth understanding of pain experienced before and after the procedure [35]. Research on the identification of pain associated with routine management procedures has focused on vocalizations and behaviors before, after, and even during the procedure [12]. Key vocalization parameters of interest included total number, duration, and peak frequency of calls [75]. In addition to vocalizations, deviations from normal body movement behavior, including escape attempts, have been acknowledged as important indicators of pain [11].

Results for both vocalizations and escape behavior are consistent when comparing piglets within different experimental settings, i.e. non- and sham-treated piglets [12]. Surgically castrated piglets had a higher total number of escape attempts and total number and peak frequency of vocalizations compared to the sham-handled and lidocaine treated piglets [51][103]. Surgical castration also elicited the highest defense behavior scores for intensity and duration and stress calls of the longest duration [69]. When comparing analgesia treated piglets, lidocaine reduced the duration and intensity of resistance behavior and vocalizations in castrated piglets [10]. Similarly, nitrous oxide reduced the total length of vocalizations and frequency and intensity of agitation scores [68]. In addition to deviations during castration, piglets express consistent behavioral and physiological changes after castration with some lasting for up to 72 hours, increasing the concern for their welfare [12].

In part with learning how to identify pain in pigs, it is necessary for observed behaviors to be clearly defined to ensure pain is being assessed consistently within different experimental and farm settings. However, there exists some variation within existing literature in this respect. For example, escape behavior that has been variably termed as avoidance, resistance, agitation, reaction and defense behavior(s), which may lead to inconsistency with its categorization and assessment [10][69][68][104]. Moreover, the literature as of yet lacks clear description on which anatomical body parts are moving, features that may be seen on the body as a result of limb movements, or which the movements that should be consistently observed to be considered escape behavior. Previous work have assessed agitation, reaction, and defense behavior using ordinal scales for intensity and duration, but what specifically constitutes a limb or continuous movement is unclear [68][69][104].

Collectively, pain indicators of struggle behavior and vocalizations appear to be useful in making an informed decision on whether an animal is in pain [11]. To improve how pain is assessed in castrated piglets, the establishment of better-defined struggle behaviors will help future studies consistently apply and describe these indicators, particularly when comparing multiple indicators or pain-relieving agents. Therefore, the objectives of this study were to assess acute pain of male piglets during castration through struggle behavior and vocalizations, and to provide discrete behavioral descriptions.

Materials and Methods

The protocol for this study was approved by the University of Minnesota Institutional Animal Care and Use Committee (#1904-36967A).

Animals and Facilities

This study was part of a larger project to refine the piglet grimace scale [16][93] and as such piglets were followed for 48 h after birth for the collection of additionally parameters not reported here. Data were collected in May 2019 at the University of Minnesota's West Central Research and Outreach Center in Morris, MN. Male piglets (n=88, Yorkshire x Landrace x Duroc) born to a total of 16 sows were enrolled in the study. Sows and their litters were housed in 16 individual farrowing stalls within a temperature-controlled room, controlled at approximately 20°C within the thermoneutral zone of the sow. Farrowing stalls were equipped with supplemental heat lamps in the creep area for piglets, a feeder for the sow, and two nipple drinkers, one for the sow and the other for the piglets. Sows and piglets remained together until weaning at 21 days. Cross fostering only took place between litters that completed the larger grimace scale project (48 h after birth), i.e. no enrolled piglets were crossed-fostered in this study. After castration, birthweights were recorded and piglets were marked with a number (1 to 10) using a Paintstik Livestock Marker (LA-CO Industries, INC., Elk Grove Village, IL) for identification. Piglets were not fully processed (tail docked, needle-teeth clipped, and iron injected) until 48 h after birth.

Experimental Design and Piglet Enrollment

An even number of male piglets from each litter was randomly allocated into one of two treatments using a generalized randomized block design: surgical castration without the use of analgesia (C) and sham-castration (S). Piglets were assigned to a treatment group using an alternative sorting method so as to address treatment order within litter. The alternative sorting method consisted of litters being randomly assigned a number using a random number generator in Microsoft Excel (Version 16.27) (Microsoft Corporation, Washington, US), where an even and odd number specified whether the first piglet would be castrated or sham-castrated, respectively. Treatment assignment was alternated thereafter. Both treatments were equally represented in each litter, with a range between two and ten male piglets per litter. All male piglets passed a visual health inspection prior to study enrollment.

Treatment Procedures

Within 24 hours after birth (avg. birth weight = 1.72 kg ± 0.78 SD), identical restraint handling and iodine disinfection procedures were followed for both treatment groups, except sham piglets were not castrated. Piglets were castrated or sham-castrated by two trained castrators and restrained by two trained handlers for the entire study. Piglets were castrated (or sham-castrated) each day between 0800 and 1200hrs over an 11-day period. On treatment day, a cart was used to move piglets out of the farrowing room and into the lobby of the farrowing barn. Piglets were restrained by their hind legs and held upside down. For castration, a scalpel incised the scrotum to externalize the testicles with two vertical incisions, the spermatic cord was cut, and iodine was applied to the surgical site following farm protocol. Sham-castration was defined as using the dull end of the scalpel to mimic the motion of incision and testicles were manipulated over the skin to simulate removal of testicles followed by iodine application.

Data Collection

Struggle behavior and vocalizations were collected via continuous video and audio recording using a single camcorder (Canon VIXIA-HF R82, Canon, USA) during the treatment period. The start and end of the treatment period were defined as the first application of the scalpel to the application of the iodine over the scrotum, respectively [75]. Video and audio analysis was performed by a single trained observer to ensure consistency of analysis.

Struggle Behavior

Struggle behavior was classified as one of three types: curl ups, leg kicks, and body flailing. An ethogram (**Table 1**) was used to identify these struggle behaviors. The video footage was viewed continuously, and the number of leg kicks and curl ups for each piglet during the treatment period was recorded. Body flailing was recorded as a binary categorical variable, i.e. as present or absent during the castration period.

Vocalizations

The duration and peak frequency of each call was analyzed using the Raven Pro: Interactive Sound Analysis Software (Version 1.5) (Cornell University, Ithaca, NY), as used in a previous study [51]. Audio files were converted from MP4 to MP3 files using WonTube Free Video Converter (Version 2.0.6) (Wondershare Software Co., Ltd), as MP4 files were not accepted by the sound analysis software. The duration of each call was calculated by the software using the waveform. Peak frequency (Hz) is defined as the loudest frequency within a call and was calculated by the software using the spectrogram [72]. Peak frequency was classified as either low (< 1000 Hz) or high (≥ 1000 Hz) in accordance with previous work [48][75][76].

Body Weight

Piglets were weighed individually after castration and again at weaning.

Statistical Analysis

Data were analyzed using the Glimmix Procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC), except for body flailing. Within the Glimmix Procedure, the Gaussian, Poisson, or negative binomial regression was used to fit the distribution of the data. Vocalization data were log-transformed to achieve normal distribution. Treatment was the fixed effect, and castrators, handlers, and litter were random effects. Vocalization and struggle behavior (curl ups and leg kicks) were dependent variables. Curl ups, leg kicks, and vocalizations were divided by the duration of castration to adjust for duration differences between treatments and presented as frequency (#/min). As a binary categorical variable, body flailing was analyzed using the Proc Logistic Procedure with treatment and duration of castration as fixed effects. In all cases, piglet was the experimental unit. Least-square means were compared with the Tukey-Kramer test. Statistical significance was set at $p \leq 0.05$ and tendencies at $p < 0.10$.

Results

Neither birth nor weaning weight differed between treatments. However, there was a difference in treatment duration, as castration took longer than sham-castration ($P<0.001$) (**Table 2**).

Struggle Behavior

Curl up frequency did not differ between treatments (**Table 2**). However, castrated piglets kicked more frequently than did sham piglets ($P=0.02$). Additionally, 51.2% of castrated piglets displayed body flailing, whereas only 4.44% of sham piglets displayed the same behavior ($P=0.03$).

Vocalizations

No difference was found for the number or duration of low frequency calls during treatment procedures (**Table 2**). However, castrated piglets responded with more high frequency calls than sham piglets ($P=0.04$) and high frequency calls tended to be of longer duration for castrated piglets ($P=0.08$).

Discussion

Pain is an indicator of poor animal welfare and challenges remain in reliably and consistently identifying pain in animals [32]. Deviations from normal behavior are promising candidates for robust indicators of pain [39]. The objective of this study was to provide a more discrete description of struggle behavior and vocalizations so as to assess acute pain of male piglets during castration. Leg kicks, curl ups, and body flailing were selected to more discretely define struggle behavior based on previous work [51]. Leg kicks were selected with consideration that they are relatively easy to identify from video recordings as discrete and frequent events. Our results indicate that castrated piglets kicked more than sham-castrated piglets. These results coincide with an earlier study that found piglets castrated without the use of analgesia carried out more escape attempts and leg kicks than piglets in the control group [51]. Our results, together with previous work [51], suggest that castrating piglets without analgesia results in more frequent leg kicks compared to sham-handled piglets.

Furthermore, more than half (52%) of the piglets castrated in the present study displayed body flailing compared to only 4.4.% in the control group. This study defined a body flailing event as incorporating all or some participation of the vertebral column, front legs, neck, and head. This is similar to the description given in these two previous studies, where the highest defense and agitation scores required both vertebral column and limb movement [69] [104]. Castration without anesthesia (Procaine) produced the highest defense behavior scores, on average a 2.7 (3 = continues movement) and 4.0 (4 = high intensity) for duration and intensity, respectively [69]. The same results were seen for agitation scores, where castrated piglets without analgesic (nitrous oxide) elicited the highest agitation scores [104].

Using curl ups as an indicator of castration pain in piglets restrained upside down remains a challenge, as these movements are difficult to quantify and interpret. In this present study, a single curl up was recorded when the body completed and released a full bend into an L-shape, without consideration of the duration held in the L-shape position. Interpretation of curl up frequency and duration remain unclear, i.e., whether short, frequent curl ups may tell us something different than fewer, but longer-sustained curl ups.

This challenge in interpretation may explain why no differences were observed with curl up indicators in this study. Alternatively, the lack of treatment effect could relate to energy cost of this particular movement. Piglets are born with a limited amount of energy reserves, which initially support the thermoregulatory and basic locomotive functions critical for survival immediately after birth [105]. In this study, piglets were castrated 24 h after birth, when not all coordinated movements and reflexes have fully developed [106]. Given that piglets were inverted during castration, a curl up for this study is considered a coordinated movement requiring extensive energy, full control of the vertebral column, and potentially the front limbs to support this position. It is possible that because of lack of energy and complete maturation of neuromotor function, piglets may have been unable to fully express curl up behavior at this age.

Vocalization parameters of duration and peak frequency are considered well-established and validated indicators of pain [70]. Peak frequency in particular is considered a key indicator of pain, as it quantifies the frequency of a single event in each call [51][73] Many studies have found significant differences between piglet calls using such indicators applying specialized equipment and software to identify three types of pig calls: grunts, squeals, and screams [12]. Calls of high peak frequency (> 1000 Hz) are classified as screams, as they evoke a maximal vocal response from pigs possibly in pain [73]. In this study, castrated piglets produced more calls of high peak frequency than sham-castrated piglets and these high peak calls tended be of longer duration. These results correspond with previous literature suggesting that calls of high peak frequency and of longer duration are more frequent in piglets castrated without a pain-relief agent, indicating that piglets are experiencing pain [12][73].

It should be noted that this study was limited by certain factors. The study farm's protocol of holding piglets upside down for castration may have introduced some difficulty in separating behavioral responses between restraint and treatment when assessing curl ups. Research has shown that restraining animals upside down is more aversive than upright restraint [63]. Moreover, behavior research has shown that any handling and manipulation causes stress and discomfort leading to further difficulty in assessing responses [107]. In this study, prior to recording struggle behavior and vocalizations an effort was made to keep handling to a minimum as all remaining processing procedures were put off until the completion of the study and piglets were only handled previous to treatment when placed into the holding cart.

In our study, the sham-castration procedure took less time than castration and it is possible that differences in duration between treatments could have affected struggle behavior and vocalization responses. We controlled for this by adjusting for treatment duration in calculating the frequency of leg kicks, curl ups, and vocalizations. The large difference in body flailing may have also been attributed to castrated piglets being handled for a longer period of time than those that were sham-treated. Thus, the duration of castration was included in the statistical model to hold castration duration constant when testing treatment effect in an effort to control for differences in castration duration. Future research should nonetheless ensure similar durations between sham and treatment procedures.

Lastly, since piglets were moved by litter on treatment day it is important to recognize that differences in behavioral responses between littermates may be a result of the order in which the piglets were tested. Key differences may be seen between the first and last piglet given visual, auditory, and olfactory cues from other littermates [75]. However, this issue was addressed by using an alternative sorting method in randomly assigning the first piglet within a litter as either castrated or sham-castrated and the alternative treatment thereafter. Therefore, each treatment was equally represented in each litter.

Conclusion

Results of this study suggest castration without the use of an analgesic increased the frequency of leg kicks, body flailing, and high frequency calls compared to sham-castration. Additionally, the high frequency calls elicited by castrated piglets tended to be of longer duration compared to sham-handled piglets. Given the consistency seen across studies using the same or similar behavioral indicators, this study provides additional support for the reliability of these specific behavioral measures as robust indicators of acute pain in piglets.

Table 2.1 Ethogram[a] of piglet struggle behavior during surgical castration

Behavior	Definition
Curl up	A full bend and release of the vertebral column. Body forms an "L-shape", creating skin wrinkling on side(s) of abdomen. Incorporates all or some participation of the front limbs
Leg kick near	A full bend and release of the front limb(s) creating skin wrinkling
	the underarm and side(s) of abdomen
Body flailing participation	Body moving vigorously side-to-side. Incorporates all or some
	of the vertebral column, front legs, neck, and head

[a]Adapted from [51].

Table 2.2 Struggle behavior and vocalizations of piglets during surgical castration

Variable	Castration	Sham-Castration	SEM[a]	P-Value
Number of piglets	44	44	---	---
Birth weight (kg)[b]	1.81	1.77	0.16	0.48
Weaning weight (kg)[c]	6.49	6.58	0.83	0.84
Duration (s)	33.30	16.98	1.15	**< 0.001**
Struggle Behavior[d]				
Curl ups (#/min)	8.18	8.90	0.78	0.25
Leg kicks (#/min)	28.81	21.31	2.64	**0.02**
Body flailing (%)	51.16	4.44	1.00	**0.03**
Vocalizations[e]				
LF (# calls/min)[f]	20.55	21.46	---	---
Transformed LF Frequency[g]	*2.93*	*2.76*	*0.27*	*0.63*
Duration of LF (s/call)	0.32	0.30	---	---
Transformed LF Duration[g]	*-1.12*	*-1.19*	*0.07*	*0.24*
HF (# calls/min)[h]	23.57	18.55	---	---
Transformed HF Frequency[g]	*2.70*	*2.01*	*0.37*	***0.04***
Duration of HF (s/call)	0.45	0.27	---	---
Transformed HF Duration[g]	-0.77	-0.92	*0.08*	***0.08***

[a]Pooled standard error
[b]Piglets weighed after castration.
[c]Weaning weight – Castration (n=39). Sham-castration (n=40). Piglets were weaned at 21 d of age.
[d]Struggle behavior adapted from [51].
[e]Vocalizations adapted from [75].
[f]LF – Low peak frequency calls (< 1000 Hz).
[g]Log-transformed to achieve normal distribution.
[h]HF – High peak frequency calls ($\geq$ 1000 Hz).

CHAPTER 3: THE APPLICATION OF 3D LANDMARK-BASED GEOMETRIC MORPHOMETRICS TOWARDS REFINEMENT OF THE PIGLET GRIMACE SCALE

Maria Lou[1, 4], Samantha T. Porter[2], Jason Massey[3], Beth Ventura[1], John Deen[5], and Yuzhi Li[1,4]

[1]Dept. of Animal Science, University of Minnesota, St. Paul, MN.
[2]Liberal Arts Technologies and Innovation Services, University of Minnesota, Minneapolis, MN.
[3]Dept. of Anatomy and Developmental Biology, Biomedicine Discovery Institute, Monash University, Melbourne, VIC, Australia
[4]West Central Research and Outreach Center, University of Minnesota, Morris, MN.
[5] Dept. of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN

Synopsis

The two objectives of this study were: 1) To evaluate the utility of the Piglet Grimace Scale (PGS) to detect acute pain and 2) To refine the current method of data collection for the PGS using photogrammetry and 3D landmark-based geometric morphometrics. The second objective had two sub-objectives: a) To determine if photogrammetry and 3D landmark-based geometric morphometrics can obtain clear, analyzable images of piglet faces and b) To evaluate 3D landmark-based geometric morphometrics to quantify changes in piglet facial shape. Eighty-eight piglets were randomly allocated to one of two treatments: surgical castration (C; n=43) and sham-castration (S; n=45). Within 24 hours after birth, identical procedures were followed for both treatment groups, except sham-castrated piglets were not castrated. Photographs of piglets were taken at four time points using 11 cameras mounted on a photogrammetry rig: baseline (T1- immediately before castration), post-castration (T2 - immediately after castration), 4 h post-castration (T3), and 22 h post-castration (T4). Four trained raters scored the piglet images using five facial action units (FAUs): orbital tightening, ear position, temporal tension, lip contraction, and nose bulge/cheek tension. 3D facial models were generated for each piglet at each time point using the Agisoft Metashape software. Landmarks corresponding to 3 facial action units (FAUs) (orbital tightening, lip contraction, and nose bulge/cheek tension) were placed onto the models using the Markers feature for geometric morphometric analysis. Data were analyzed using the Glimmix, Mixed, and Genmod procedures of SAS software, and Generalized Procrustes Analysis (GPA) procedure of Morpheus software. The reliability of the PGS was tested by evaluating the agreement among four raters. The intra-class correlation coefficient (ICC) for orbital tightening, ear position, nose bulge/cheek tension,

temporal tension, and lip contraction was 0.68, 0.67, 0.54, 0.40, and 0.28, respectively. For all time points (T1-T4), the odds of castrated to sham-castrated piglets for all FAUs did not differ from 1, suggesting that castration did not change any FAU. Moreover, geometric morphometric analysis did not discriminate differences between the treatment groups or among time points (all $P > 0.10$). These results suggest that orbital tightening and ear position are more reliable than all other FAUs for a PGS, as indicated by the ICCs. However, neither the PGS nor 3D landmark-based geometric morphometrics were sensitive enough to detect pain in piglets post-castration in the current study.

Key Words: castration, pain, photogrammetry, animal welfare

Introduction

The grimace scale is a method of acute pain assessment that identifies and assesses post-procedural changes in facial expression [15]. These pain-induced changes in facial expression are controlled by involuntary muscle movements within the major regions of the face: eyes, ears, mouth and jaw, and nose and cheeks [84]. The grimace scale uses a simplified version of the Facial Action Coding System (FACS) termed Facial Action Units (FAUs) that are specific to pain states such as orbital tightening, ear position, cheek tension, and nose bulge [84][108]. Unlike other methods of pain assessment (e.g. retrospective scoring and physiological and clinical tests), the grimace scale is advantageous as it is non-invasive, provides real-time assessment, requires minimal training, and specialized techniques are not essential [64][84][109]. Following promising results from the first non-human grimace scale, the Mouse Grimace Scale (MGS) [15], grimace scales have been developed for other domestic animal species including rats, rabbits, horses, sheep, and cows [86][87][89][91][108].

There are few existing studies on the Piglet Grimace Scale (PGS) [59]. The PGS was developed as an additional method of pain assessment for male piglets, who are commonly surgically castrated without an analgesic or anesthetic agent [1]. The U.S. markets over 115 million pigs every year and approximately half are male [6]. Nearly all male piglets are surgically castrated due to meat quality and animal management, although extensive research has shown it is painful and has negative implications on animal welfare [1][12][14][33][110]. However, the PGS and other grimace scales alike have certain limiting factors including observer bias, inconsistent angles and lighting in photographs and video recordings, and difficulties in quantifying specific measurements (i.e. volume, length, and depth) with two-dimensional images [109][111]. It has been suggested that further research should focus on reducing responses of animals caused by physical restraint, providing effective angles and uniform lighting sources, using high resolution images, establishing of a robust behavioral baseline, conducting formal training, and increasing sample sizes [16][59] [93].

A three-dimensional optical system has been proposed as a possible solution given its ability to provide objective descriptions (e.g. shape) and sensitive measurements [30][112]. One way to address the issue of refining the PGS is through the application of photogrammetry and landmark-based geometric morphometrics, that when combined provide an objective approach to pain assessment by providing a quantitative description of shape variation [95]. These methods of data collection and analysis are non-invasive, reduce labor intensity, and are less stressful for pigs and caretakers than sampling for physiological parameters. Photogrammetry, also referred to as structure from motion, is a computational method for 3D reconstruction built from a series of overlapping 2D images [96]. Novel 3D techniques have gained much interest within livestock farming [96][97][98]. Unlike live visuals and 2D images, 3D data allow one the measurement of cross-sectional areas, volume, depth, and are able to account for differences in color and background [97]. Studies have focused on both 2D and 3D image analysis for automatic pig weighing and assessment of animal shape [97][98][99][100] [113].

Landmark-based geometric morphometrics uses a set of landmarks placed onto a 3D model that annotates locations of biological significance [95]. These landmarks providing the ability to measure animals and specific body parameters [95]. The coordinates of these landmarks (2D or 3D) are representations of the subject's shape and are aligned to eliminate all factors not related to the shape of interest [95]. The surface's natural texture can also be incorporated depending on the method of scanning [95]. Currently, 3D landmark-based geometric morphometrics have not been used to identify and quantify changes in facial shape in piglets, but a study on the domestic cat found significant differences in coordinates associated with pain using 2D images [94].

This study aims to improve the assessment of pain in castrated piglets by refining the piglet grimace scale through the application of photogrammetry and 3D landmark-based geometric morphometrics. The objectives of this study were to evaluate the utility of the PGS to detect acute pain and to apply photogrammetry and geometric morphometrics to quantify changes in piglet facial shape.

Materials and Methods

The protocol for this study was approved by the University of Minnesota Institutional of Animal Care and Use Committee (#1904-36967A).

Animals and Facilities

The animal trial was conducted at the University of Minnesota's West Central Research and Outreach Center in Morris, MN in May 2019. Male piglets (n=88, Yorkshire x Landrace x Duroc) born to a total of 16 sows in a confinement farrowing barn were used for this study. Sows and their litters were housed in 16 individual farrowing stalls (150 cm x 210 cm) on slatted floors. Each farrowing stall was equipped with two creep areas (46 cm x 210 cm/area) with a supplemental heat lamp in one creep area of the stall for piglets, a feeder for the sow, and two nipple drinkers. All sows farrowed naturally without artificial induction. Piglets were not cross-fostered or fully processed (tail docked, needle-teeth clipped, and iron injected) until completion of data collection, 48 h after birth.

Birthweights were recorded and piglets were marked with a number (1 to 10) using a Paintstik Livestock Marker (LA-CO Industries, INC., Elk Grove Village, IL) for identification after castration or sham-castration (see *Experimental Treatment and Design*). Sows and their piglets remained together in their farrowing stalls until weaning at 21 days after farrowing. Room temperature in the farrowing barn was controlled at 20°C, within the thermoneutral zone for lactating sows as much as possible by thermostats that operated exhaust fans and heaters. Light period was set at 8 h daily starting from 0700 h.

Experimental Treatment and Design

The experimental treatment was surgical castration without the use of analgesia (C) vs. sham-castration (S). A generalized randomized block design was employed, with litter serving as block. Within each litter, an even number of healthy male piglets were selected for the study based on visual assessment. Piglets were assigned to each treatment group using an alternative sorting method to balance treatment order within and among litters. The alternative sorting method consisted of litters being assigned a random number generated by Microsoft Excel (Version 16.27) (Microsoft Corporation, Washington, USA), where an even and odd number specified whether the first piglet within litter would be castrated or sham-castrated, respectively. Treatment assignment within each litter was alternated thereafter. Both treatments were equally represented in each litter, with a range between one to five male/piglets/treatment/litter.

Treatment Procedures

Castration or sham-castration of 88 male piglets was performed over a one-week period, with piglets receiving treatment in order as sows farrowed naturally. Identical handling and iodine disinfection procedures were followed for both treatment groups, except sham piglets were not castrated. Piglets (avg. birth weight = 1.72kg ± 0.78 SD) were castrated or sham-castrated within 24 h post-birth by a trained castrator and restrained by a trained handler each day between 0800 and 1200h. On treatment day, selected male piglets were moved out of the farrowing room by litter using a cart and moved into the lobby of the farrowing barn. Piglets were restrained by their hind legs and held upside down. For castration treatment, a scalpel incised the scrotum to externalize the testes with two vertical incisions, the spermatic cord was cut, and iodine was applied to the surgical site. Sham-castration was conducted as using the dull end of the scalpel to mimic the motion of incision and testicles were manipulated over the skin to simulate removal of testicles followed by iodine application.

PGS Photogrammetry Rig and Recording

Photographs of piglets were taken at four time points (**Fig. 1**): pre-castration (T1 - immediately before castration), post-castration (T2 - immediately after castration), 4 h post-castration (T3), and 22 h post-castration (T4) as in previous work [16][59][93]. In an effort to maintain a consistent piglet position and orientation, piglets were placed into a piglet cradle (29.2 cm x 2.5 cm x 17.8 cm), specifically designed for the pigs used in this study. Piglets were placed into the cradle for photographs at each time point for a maximum of 20 seconds until a still image of the piglet was captured.

For 3D reconstruction of piglet models, a pilot test was conducted using a frozen dead piglet to determine the minimum of number of cameras needed to capture pig face images from all required angles. The pilot test determined a total of 11 cameras would be sufficient. Thus, a photogrammetry rig (119.4 cm x 116.8 cm) was built to install 11 cameras (Canon EOS Rebel T5, Canon, Tokyo, Japan) to take photographs of the piglets (**Fig. 2**). The photogrammetry rig was constructed from metal t-slot framing and fittings (McMaster-Carr, Elmhurst, Illinois, USA) and fitted with two ESPER TriggerBoxes (ESPER LTD, Nottingham, UK), connecting all cameras to a laptop (Hewlett-Packard, Palo Alto, CA, USA). The rig was also equipped with two 400L LED task lights (Southwire, Carrollton, GA, USA) for uniform lighting, as flash was shut off to minimize disturbance of piglets. Smart Shooter (Version 3.28) (Kuvacode Oy, Kerava, Finland) was installed on the laptop to control all camera settings for photo quality (JPEG Fine Large), aperture (6.3), shutter speed (1/125), and ISO (3200). For consistency, all camera lenses were set to auto focus and to maximum zoom (55mm). The ground was marked to secure the location of the piglet cradle, which was placed at the center (7.62 cm from center of rig) of the photogrammetry rig with a similar distance to each camera for the entirety of the study. Photos were transferred from the laptop to an external hard drive (Western Digital, San Jose, CA, USA) for further processing (i.e. PGS scoring and 3D models).

Image Processing for PGS scoring

A total of 3,872 images were taken of the 88 piglets in this study. From those images, one image of each piglet at each time point was selected for PGS scoring based on optimal quality, angle (i.e. front-profile only), and lighting in accordance with previous work [16][59][93]. Images that were not in focus or of poor quality were excluded from PGS scoring only, leaving a total of 352 images for final PGS scoring. Each image was assigned a random number generated by Microsoft Excel (Version 16.27) (Microsoft Corporation, Washing, USA) to randomize the order in which they were scored.

PGS Scoring

The following five FAUs adapted from previous Piglet Grimace Scale studies were used for PGS scoring: orbital tightening, ear position, temporal tension, lip contraction, and nose bulge/cheek tension [16][59][93]. Each FAU was scored using a 3-point scale (0 = Not Present, 1= Moderately Present, and 2 = Obviously Present), except lip contraction used a 2-point scale (0 = Not Present and 1= Present). From the 352 images, 120 were selected from the set of 352 using the same randomizing method as described above for PGS scoring. To test the PGS scoring system, four raters with extensive swine background were chosen to score the 120 images. Prior to scoring, raters received formal training, which included instruction on all five FAUs (see below), scoring guidelines, and a practice scoring session of sample images. Raters were then given a PGS Pictorial Guide (**Fig. 3**), a score sheet, and a set of 120 images for scoring. Raters were asked to score images in one sitting in an effort to maintain consistency in scoring. Scores from the 4 raters were used to calculate the interclass correlation coefficient (ICC) to evaluate inter-rater reliability. Among the four raters, one scored all 352 images for analysis of treatment effect on the PGS.

Photogrammetric 3D model processing and landmarking

The set of eleven 2D images for each time point for each piglet was used to generate 3D models of piglets using the software Agisoft Metashape (Version 1.6.2) (Agisoft LLC, St. Petersburg, Russia). Models were processed using the setting described in **Table 1**. Metashape was not able to align the leftmost and rightmost images and models were generated using only 9 photos. Models were scaled using the ‘detect markers’ feature in Metashape on two calibrated 5 cm scale bars (Cultural Heritage Imaging, San Francisco, USA). Markers on the scale bars were placed manually on photos if auto detection failed.

Landmarks corresponding to three PGS FAUs (orbital tightening, lip contraction, and nose bulge/cheek tension) were then placed on the models using the Markers feature in Metashape. These three FAUs were expected to have the largest detectable range in facial shape change given the quality of the 3D models produced. Landmarks corresponding to ear position and temporal tension were not used, as ears were often missing from the processed Metashape models due to their position relative to the cameras and temporal tension lacked sufficient visual appearance. A total of 16 facial landmarks, with 12 representing the three FAUs as done in previous work [94]. The first four landmarks (1-4) correspond to the end points of the scale bars used to generate scale in the models. Landmarks 5-16 (**Table 2**) were placed manually on the textured view of the 3D models using the Marker tool in Metashape (**Fig. 3**).

Statistical Analysis

All data were analyzed using SAS software (Version 9.4; SAS Inst. Inc., Cary, NC, USA). The ICCs for PGS scores were calculated using the Mixed Procedure. The effect of castration treatment on FAUs was analyzed using the Proc Genmod Procedure for non-parametric variables. Dependent variables were grimace scores for each FAU. Treatment and time point were fixed effects, with piglet as the subject for repeated measures. Sham-castration (control) and time point one (T1 - baseline) were used as references to castration treatment and other time points, respectively, in the model. Statistical significance was set at odds ratios with 95% confidence intervals greater than 1. Descriptive data were summarized using the Proc Frequency Procedure.

Geometric morphometrics (GM) data were firstly superimposed by Generalized Procrustes Analysis (GPA) using Morpheus et al. Software (Java Edition) (Florida State

University, Tallahassee, Florida, USA). Missing landmarks were estimated via GPA mean substitution. The Proc GLIMMIX Procedure for a multivariate analysis of variance (MANOVA) was used to test for differences between treatment and time points. Least-square means were compared with the Tukey-Kramer test. Lastly, a Discriminant Function analysis for cross-validation was used to predict group classification. The GM procedures were performed on the entire dataset as well as orbital tightening separately as it has been previously shown to be a reliable indicator of pain [18].

Statistical significance was set at $P \leq 0.05$. Principal component analyses were performed to visualize the data.

Results

Inter-rater reliability

The ICC was highest for orbital tightening (0.68) and ear position (0.67), lowest for temporal tension (0.40) and lip contraction (0.28), and intermediate for nose bulge/cheek tension (0.54).

Facial Action Units (FAUs) scores for the treatments at each time point

The descriptive analysis depicts the percentage of the five FAU scores between the treatments at each time point (**Fig. 4-8**). For orbital tightening, about 70% to 80% of piglets scored 0 at T1 (**Fig 4.**). At T4, about 50% of piglets scored 0. Both treatment groups included all three score types (0, 1, and 2) for orbital tightening at each time point.

More than 50% of piglets scored 2 for ear position at T1 (**Fig 5.**). Less than 30% of piglets from T1 to T3 and about 40% of piglets at T4 scored 0. Similar to orbital tightening, differences in ear position scores were not evident between treatments.

For temporal tension, more than 80% of piglets scored 0 across all time points and both treatments (**Fig 6.**). Five percent and 7% of castrated piglets scored 2 at T1 and T2, respectively. Sham-castrated piglets did not score 2 at any time point except T3, where castrated only scored 0 and 1.

Given its 2-point scale, lip contraction had the least variation in scores among the 5 FAUs (**Fig 7.**). More than 90% of piglets scored 0 for both treatment groups across all time points.

For nose bulge/cheek tension, both treatment groups included all three score types (score 0,1, and 2) across all time points (**Fig 8.**). Differences in nose bulge/cheek tension were not evident between treatment groups at any time point.

Effect of treatment on PGS (Pre- and Post-castration)

During the entire data collection period (T1-T4), the odds of C scoring in a higher grimace category to S for orbital tightening, ear position, temporal tension, lip contraction, and nose bulge/cheek tension did not differ from 1 (**Table 3**), suggesting no treatment effect on PGS.

Application of 3D landmark-based geometric morphometrics on PGS

The principal component analysis (PCA) did not detect any structure within the data. While, differences between treatments were evident across all time points (Wilk's lambda = 0.83186911, F (33,317) = 1.94, p = 0.0021), differences between treatments at individual time points were not detected (P = 0.22 for T1, P = 0.26 for T2, P = 0.96 for T3, and P = 0.92 for T4). No differences within time points alone (P = 0.45). The discriminant analysis likewise found no differences between the treatment groups or time points. Using the more conservative cross-validation classification summary, C piglets were correctly classified at

59.9% and S piglets were correctly classified at 53.3% (**Table 4**). Correct classification for each time point was 25.0% for T1, 18.2% for T2, 33.0% for T3, and 27.3% for T4 (**Table 5**).

Similar results were found when rerunning the above analyses for orbital tightening separately. Visualizing the data through PCAs found no discernable structure within the data. No differences between treatment by time point or individual time points for orbital tightening were found. In the Discriminate Function Analysis, orbital tightening performed better than the entire dataset when comparing treatment groups (C piglets were correctly classified at 59.3% and S piglets were correctly classified at 61.1%) (**Table 6**). However, in discriminating between time points the discriminant function analysis again found no differences (**Table 7**).

Discussion

The first objective of this study was to evaluate the utility of the PGS to detect acute pain. An important consideration made by earlier grimace scale studies was the need to establish a strong behavioral baseline [109]. To improve the reliability of facial expressions as a method of pain assessment, a clear difference between a positive control (i.e. castration without a pain agent) and a negative control (i.e. sham-castration) is necessary as standard for comparison when interpreting responses affected by pain agents (i.e. analgesics and anesthetics) [59]. Thus, this study focused on surgical castration and sham-castration alone, a key difference from previous PGS studies [16][59][93]. A second key difference was the upgrade in the format of our pictorial guide to improve inter-rater reliability, which unlike previous PGS studies it included a real-time photo, sketch, and description for each individual score to help raters understand what specific features should be observed as the severity of pain increases from score 0 to 2. In combination with the pictorial guide, our raters received formal training as well. We observed that orbital tightening, ear position, and nose bulge/cheek tension worked best in terms of inter-rater reliability. However, differences were between treatments were not detected by the PGS nor 3D landmark-based geometric morphometrics.

Inter-rater reliability

Inter-rater reliability among the four raters is essential in determining PGS utility as a measure of consistent scoring over time. According to Koo and Li [60], the ICCs among the four raters in the current study for orbital tightening (0.68), ear position (0.67), temporal tension (0.40), lip contraction (0.28), and nose bulge/cheek tension (0.54) were indicative of moderate, moderate, poor, poor, and moderate inter-rater reliability, respectively. Our ICC results for orbital tightening were promising, but lower than other PGS studies. Gottardo et al. [59] and Di Giminiani et al. [16] observed an ICC of 0.83 among 3 raters and 0.95 among 30 raters for OT, respectively. Moreover, our ICC results for both orbital tightening and ear position also align with grimace scales for other species including mice, rats, rabbits, cats, horses, and cows [15][86][87][88][89][90]. Tightening of eyes is an evolutionary conserved facial expression acting as a protective mechanism, protecting sensitive areas of the face from an attack [84]. Ear position (i.e. backwards or flattened) are commonly expressed during negative experiences [84]. As prominent features of the face, their easy recognition may help facilitate scoring.

Temporal tension was an FAU adapted from Di Giminiani et al. [16], who demonstrated excellent inter-rater reliability. However, Di Giminiani et al. [16] had two treatment groups (i.e. tail docked females and castrated males), where females underwent teeth clipping prior to tail-docking and males were restrained while photographs were taken. Also, observers scored a set of images combining both treatment groups [16]. Temporal muscles move congruently with the ears and involuntary muscle movement can increase during handling where pigs are more likely to vocalize and direct their ears towards alarming auditory signals and after multiple painful procedures [84]. As those images included both treatment groups, where females were exposed to two painful procedures, and images of males were taken during a stressful handling period may have resulted in a strong activation of temporal muscles, making temporal tension more visibly clear to score in that study [16].

Similar to temporal tension, a study of the Horse Grimace Scale (HGS) reported an FAU termed "tension above the eyes", resulting in an ICC of 0.86, but with some scoring difficulties as 21% of images could not be scored in relation to this FAU [88]. In this study, castrated stallions were of mixed breed, age, and color, while horses in the control group were of mixed age and gender, with both groups receiving an NSAID (i.e. Flunixin). Biological differences or analgesic side-effects alone could have facilitated visibility and scoring of altered FAUs. In contract, our set of images only included castration vs. sham-castration with no use of an analgesic or anesthetic agent and images were taken while piglets were in the restraint cradle. In this present study, it is possible that temporal tension muscles were not activated in response to castration, or that fairly light-colored skin of piglets made temporal tension harder to detect without enough visual/texture appearance [98]. For this same reason, we were unable to add landmarks representing temporal tension on the 3D models, which is discussed later.

Lip contraction was also a FAU adapted from previous work [16], although in that study lip contraction was ultimately excluded given that observers scored lip contraction as "don't know" for over 30% of images. Consequently, in an effort to facilitate scoring, lip contraction for this present study was scored on a 2-point scale, but no improvement was observed and indeed it resulted in the lowest ICC. In our study, lip contraction could not be scored in 40% of images. Front-profile images made scoring lip contraction more difficult as seen with horse FAUs (i.e. "prominent strained chewing muscles" and "mouth strained and pronounced chin"), as muscle actions in the mouth/jaw region are more prominent in side-profile images [88]. It should also be noted that an added challenge of scoring lip contraction is misinterpreting lip contraction for piglets vocalizing, as facial and vocal communications are linked [84]. Studies have shown that piglets extensively vocalize when handled and undergo castration, more so piglets that are actually castrated [12]. Lastly, changes in the nose and cheeks have been evaluated independently [15][16][59][87] and collectively [86][88][93]. For the current study, they were combined (i.e. nose bulge/cheek tension) as muscle actions in the nose and cheek region are observed to move congruently in piglets, resulting in moderate inter-rater reliability.

Treatment impacts

Facial expressions can differ in terms of how they are expressed (i.e. sustained vs. subtle) and their duration [84]. Itt is suggested that facial expressions in relation to pain should be seen with more frequency and difficult for still images to detect [84]. However, the Genmod analysis showed that the probability of castrated piglets scoring in a higher grimace category (i.e. 1 and 2 vs. 0) did not differ from sham-castrated piglets for all five FAUs throughout all time points. Comparably, Gottardo et al. [59] reported no significant differences between castrated and sham-castrated piglets for orbital tightening. Di Giminiani et al. [16] reported a significant change between pre- to post-scores in orbital tightening only for tail-docked piglets, but none for castrated piglets most likely because tail-docked piglets were pre-exposed to teeth clipping. Viscardi et al. [93] observed significant interactions among PGS scores (i.e. the sum of scores for each FAU) with higher PGS scores at 0, 3, 4, and 5 h post-castration. However, piglets were exposed to tail-docking prior to castration and treatments involved pain agents (i.e. meloxicam and EMLA cream) and pain agents alone may have caused changes in FAUs [93].

Applying photogrammetry and 3D landmark-based geometric morphometrics to the PGS

To the best of our knowledge, this is the first paper to analyze changes in facial shape in piglets through the application of photogrammetry and 3D landmark-based geometric morphometrics. We hypothesized that the application of these technologies would refine data collection and analysis for the PGS by enabling higher image quality and minimizing human error. In terms of our second objective, photogrammetry proved to be successful in obtaining clear, high resolution images. An added benefit of using a photogrammetry rig is taking multiple simultaneous images, which may help in capturing the nature of pain-related facial expressions, as they should be seen with more frequency, than a single frame can detect. However, scoring difficulties stemmed from certain FAUs being highly visible in a front-profile view (e.g. orbital tightening) and others in side-profile view (e.g. lip contraction).

Geometric morphometrics also proved successful with overall good model quality. Photogrammetry does require surfaces to have sufficient features and textures (e.g. visual appearance) for high quality 3D reconstruction [98]. In our study, parts of piglet faces could not be fully reconstructed in multiple models as enough pixel data was lacking. Future research may improve models by incorporating more cameras, especially in areas above and below the piglet, for a full 360° view. Also, if a restraining device is used as in our study (i.e. piglet cradle), perhaps placing the rig above the ground could help capture all necessary angles. Facial shape differences between treatments across all time points were detected, though when times points were analyzed separately no differences were found. The observed facial shape differences were most likely due to individual variation in piglet's faces. Discriminant analysis was also not able to detect any distinct facial shape differences as piglets were correctly classified roughly 50% and 25% of time for each treatment and time point, respectively. A classification of 70% is considered ideal representing group differences other than natural biological variation. These results indicate that 3D landmark-based geometric morphometries were not able to quantify changes in facial shape of piglets at least under the experimental conditions described. Collectively, results of the current study determined no differences between the two treatment groups possibly supporting that pigs in fact do well in masking signs of pain.

A key construct of geometric morphometric analysis is that all landmarks must be placed in the same location and orientation in every model, so selected FAUs had to be present in all of the models. The eyes were the only FAU fully and clearly reconstructed in all 3D models in the current study. The 3D models provided the ability to annotate each eye with four landmarks. These four landmarks were placed in specific areas in an effort to capture the full range of motion as the eyes tightened. Following the eyes, lips and nose were moderately reconstructed, while the ears and forehead failed to reconstruct for the majority of models. Thus, only the corners of the lips and the two prominent wrinkles of the nose were annotated. For these reasons, orbital tightening performed well compared to the entire data set as castrated and sham-castrated piglets were classified correctly 59% and 61%, respectively.

Currently, measuring distances between points and 2D landmark-based geometric morphometrics have been used to develop a Feline Grimace Scale (FGS) [90][94]. Images of 2D from video-recordings were annotated for both FGS studies [90][94]. Evangelista et al. [90] found significant differences between treatment groups (healthy cats vs. cats with abdominal pain) in terms of linear distance ratios and angles in relation to 5 FAUs (orbital tightening, ear position, muzzle tension, whiskers position, and head position). However, the cats used in this study were a combination of seven different breeds and the cause of abdominal pain was not consistent (i.e. lymphoma, inflammatory bowel disease, pancreatitis, etc.) [90]. Finka et al. [94] were also able to quantify changes in facial expression through a single principal component analysis, indicating distinct shape variation for the ears, muzzle, cheeks, and eyes using cats of mixed breed undergoing ovariohysterectomy. This study also found that T2 (1 h post-surgery) was associated with the largest intensity of pain, yet cats were anesthetized prior to T2. Although both studies were able to detect changes facial shape in relation to pain, differences in breed, coat color, types of painful stimuli, and side-effects of pain agents are factors that must be considered and controlled for when validating a grimace scale [88][109]. Additionally, the use of 2D images is cause for concern particularly for landmark based geometric morphometrics as 2D does not retain the geometric integrity of landmarks like 3D. Distances and angles are unaffected in 3D models when rotated. Finka et al. [94] used both 2D front and lateral images and such factors can be misinterpreted.

It should be noted that our study was limited by certain factors. Extensive research has shown that handling and manipulation alone causes stress and discomfort in piglets, inducing changes in facial expression [63]. Additionally, novel items and events can cause animals to become frightened [107]. In an effort to minimize disturbance and keep piglets calm, future studies should habituate piglets to handlers, restraints, and the presence of technological devices before the commencement of data collection. Moreover, piglets in the current study were castrated within 24 h after birth, following protocols of the research farm. However, this time presented some issues in scoring FAUs, particularly with ears pressed against the head or flipped over and excessive face wrinkling that would otherwise settle with time as the piglets grow. During this early phase of life, pigs experience rapid morphological changes and at 24 h of age, piglets may not have had sufficient time to acclimate themselves to their environment [16]. Previous PGS studies began data collection at age range of 4-5 days, which may have facilitated scoring [16][59][93].

Conclusion

Orbital tightening, ear position, and nose bulge/cheek tension had high ICC values compared to temporal tension and lip contraction for inter-rater reliability, suggesting that orbital tightening, ear position, and nose bulge/cheek tension are reliable FAUs for a PGS in relation to castration. This study provided evidence that the application of photogrammetry and 3D landmark-based geometric morphometrics have the potential to improve data collection and analysis for the PGS by enabling high-resolution images and good model quality for scoring and adequate landmark annotation, respectively. However, differences were not found between treatment groups or across time points by the PGS or 3D landmark-based geometric morphometries, that scoring performed and landmark annotation in neonatal piglets in this duty lacked enough sensitivity in reliably detecting pain. Adjustments are necessary to improve the sensitivity of these technologies to detect pain-related changes in facial shape.

Figure 1. Project Timeline

Camera – time point of pig photograph (time points 1-4)
Red arrow – castration (n = 43) and sham-castration (n = 45)

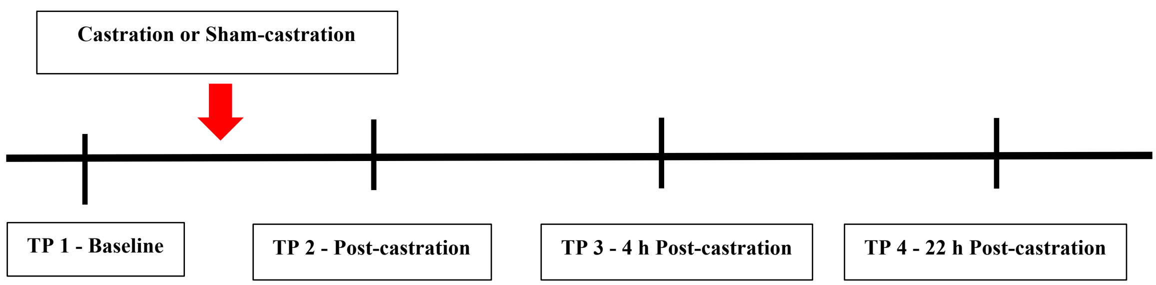


Figure 2. Photogrammetry rig and piglet cradle.

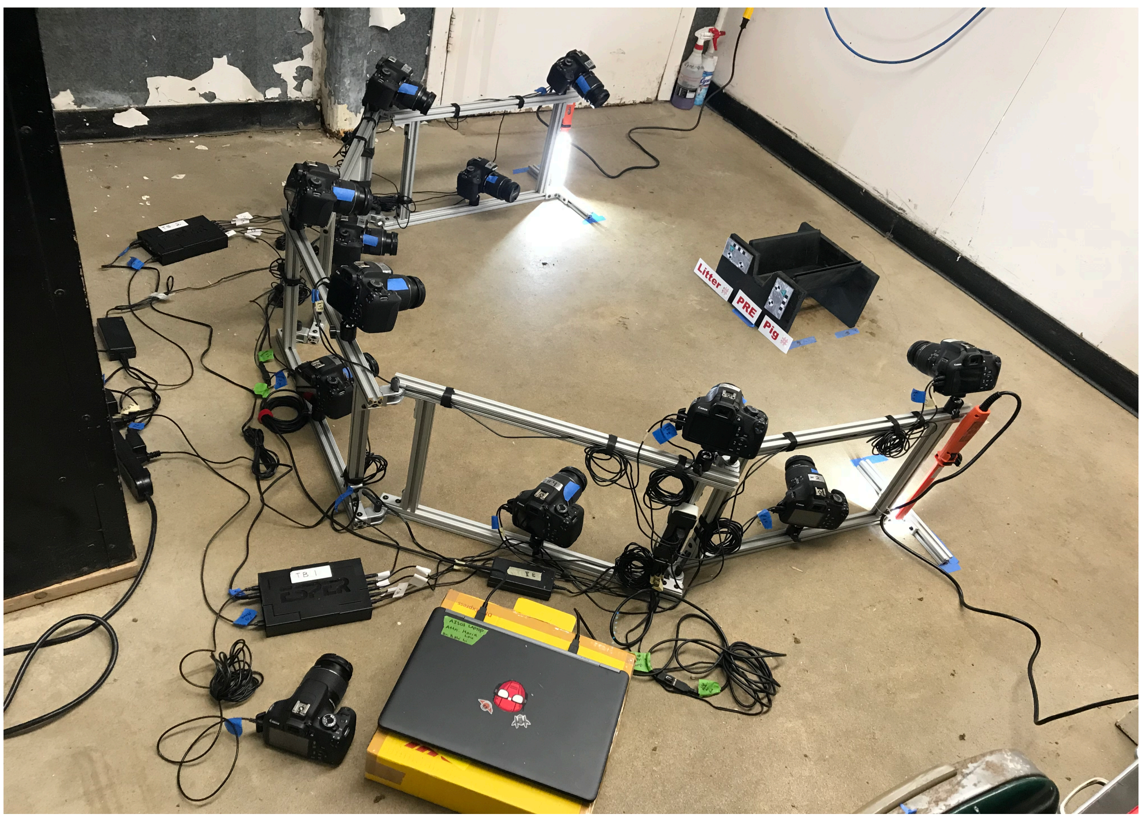
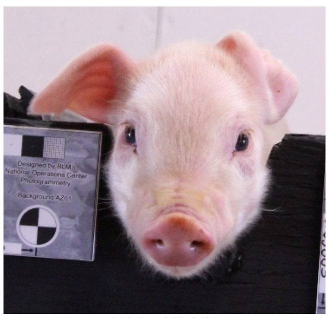
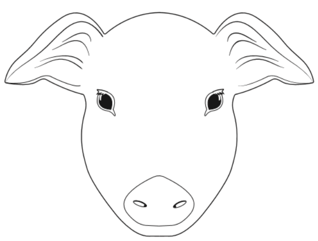
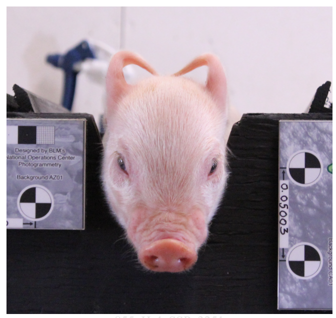
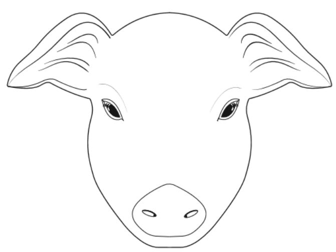
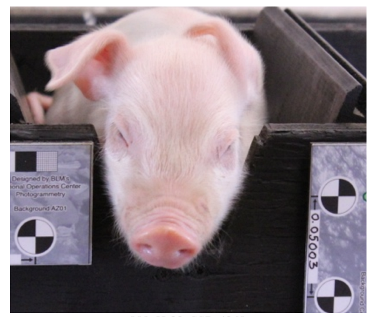
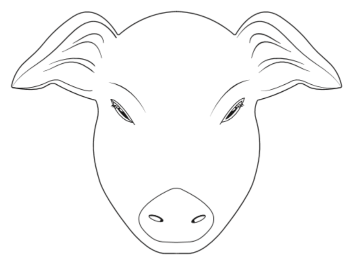


Figure 3. Piglet Grimace Scale, a pictorial diagram of each facial action unit by facial region. Each FAU was scored using a 3-point scale (0 = Not Present, 1= Moderately Present, and 2 = Obviously Present).

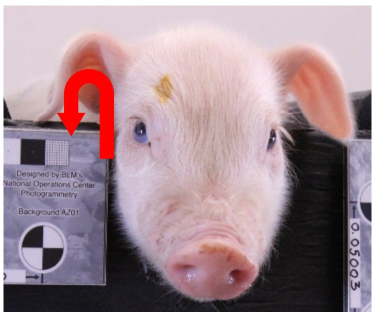
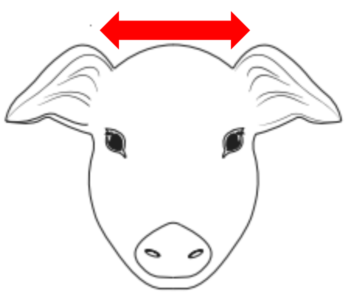
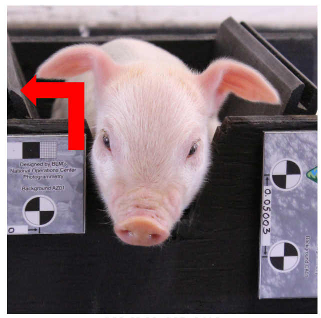
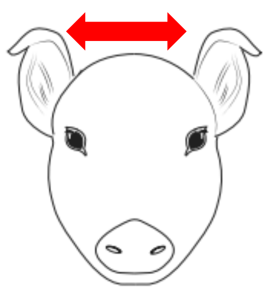
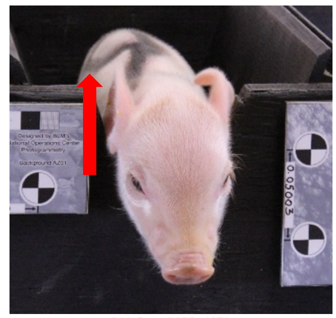
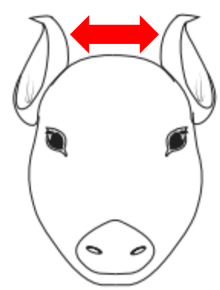
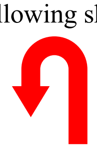
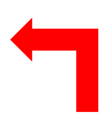
Richard, C. 2020. *Orbital Tightening, Ear Position, Temporal Tension, Lip Contraction, and Nose Bulge/Cheek Tension Digital Illustrations*. Digital Content Library, College of Liberal Arts, University of Minnesota.

Eye Region

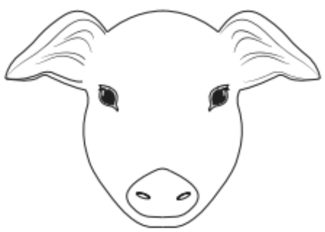
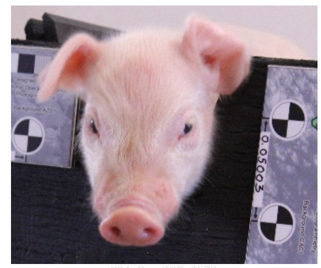
Orbital Tightening		
Not Present (0)	**Moderately Present (1)**	**Obviously Present (2)**
 844_Pre_SSP_2636 	 855_Hr4_SSP_3251 	 889_Hr22_SSP_4268 
Baseline Eyes are wide open, showing a round **circular** shape No tightening of or around the eyelid Opening between eyelids is fully opened (palpebral fissure) If whites of the eyes are visible, they will be the most visible with a score of 0 Pupils are not dilated (eye aperture)	Eyes are halfway closed, showing a **teardrop** shape Partial tightening of and around the eyelid, with medium intensity, described as "eye squeezing". As a result, moderately eyebrow furrowing may be present. Opening between that eyelids is closed halfway Whites of the eyes may be visible Slight increase in pupil dilation **These features may be seen only or more strongly in 1 eye**	Eyes are closed more than halfway, showing almost or full eye closure Eyelid is fully tightened, with high intensity "eye squeezing". As a result, eyebrows may be furrowed significantly Opening between the eyelids is more than halfway closed Whites of the eyes may not be visible at all Pupils fully dilated

		These features may be seen only or more strongly in 1 eye
Note: A score of **9** can be given if the FAU is not visible or if cradle has interfered.		

Ear Region

Ear Position		
Not Present (0)	**Moderately Present (1)**	**Obviously Present (2)**
 890_Hr22_SSP_4277 	 855_Hr22_SSP_3615 	 946_Post_SSPP_4971 
Baseline Ears are fully relaxed, forward, and upright The distance between both the tips and bases of ears is normal Base of ear attached to head will have the following shape:  Inner pinna of ear is partly visible	Ears are drawn back from the forward **baseline** position Ears are pointed **backwards** halfway between the head The distance between both the tips and bases of the ears is decreased halfway, as the ears move from baseline to upwards and back (**Length of red arrow is smaller than in Score 0**) Base of ear attached to head will have the following shape:  Inner pinna is more visible	Ears are fully drawn back from the forward **baseline** position Ears are pointed **backwards** more than hallway behind the head and completely flushed against head The distance between both the tips and bases of the ears is decreased more than halfway (**Length of red arrow is smaller than in Score 1**) Base of ear attached to head will have the following shape:  Inner pinna is fully visible from the sides

	These features may be seen only or more strongly in 1 ear	**These features may be seen only or more strongly in 1**
Note: A score of **9** can be given if the FAU is not visible, ears are flipped, or if cradle has interfered.		

<table>
<tr><th colspan="3">Temporal Tension</th></tr>
<tr><th>Not Present (0)</th><th>Moderately Present (1)</th><th>Obviously Present (2)</th></tr>
<tr>
<td>
866_Hr22_SSP_3702
</td>
<td>
801_Pre_SSP_1860
</td>
<td>
894_Pre_SSP_3879
</td>
</tr>
<tr>
<td>Baseline

Forehead gradient is flat and smooth, with relaxed skin

No tightening of the skin on the forehead or above and in-between eyes

No presence of a wrinkled appearance

No “wrinkled forehead”

Note: Forehead muscles move congruently with ear muscles</td>
<td>Forehead gradient moderately changes, with an increase in wrinkles and tightness

Partial tightening of the skin on the forehead and/or above and in-between the eyes (pre-snout)

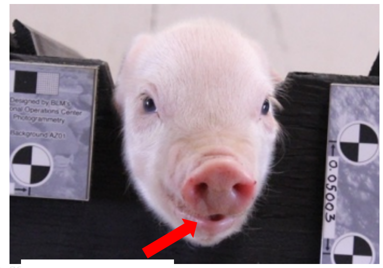
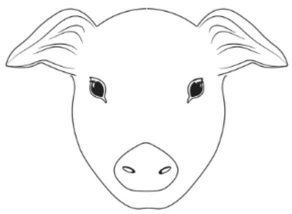
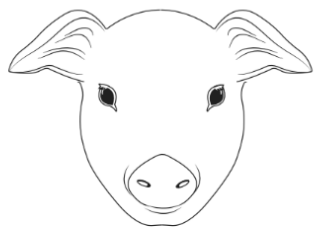
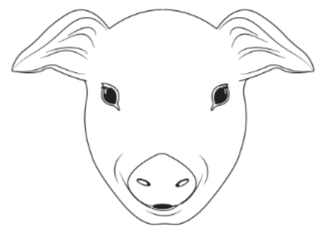
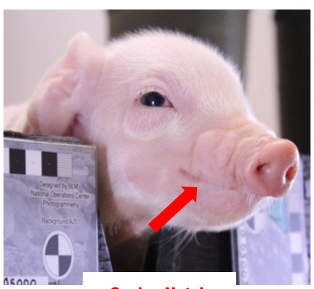
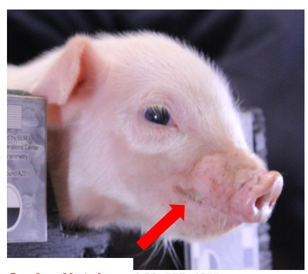
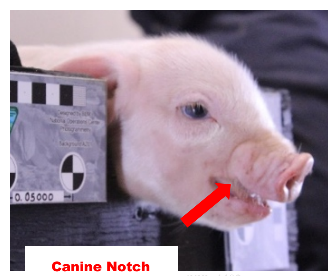
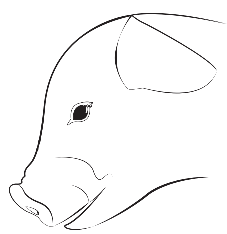
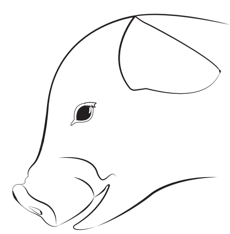
Muscle tightening of this region will give a moderate “wrinkled forehead” appearance, with medium intensity

Temporal tension may affect ear position, as forehead muscles work congruently with ear muscles</td>
<td>Forehead gradient has an obvious change, with a significant increase in wrinkles and tightness

Full tightening of the skin on the forehead and/or above and in-between the eyes (pre-snout)

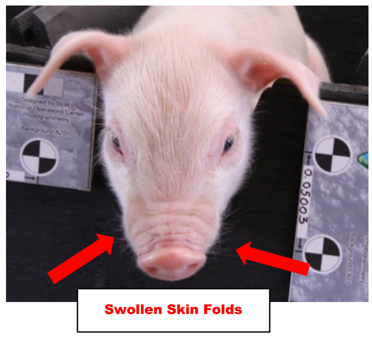
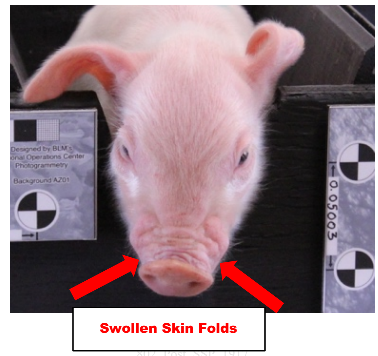
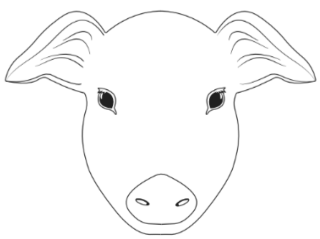
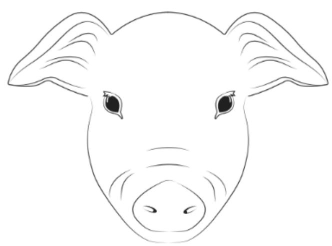
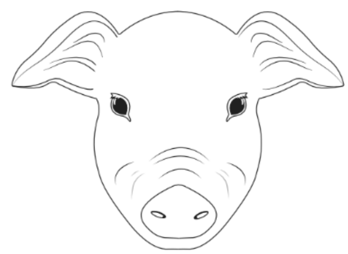
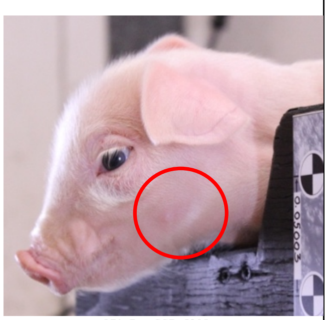
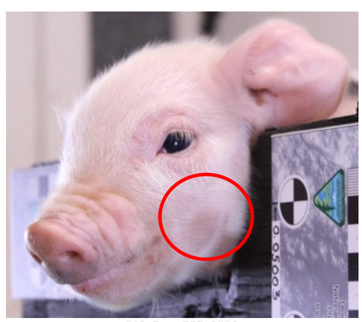
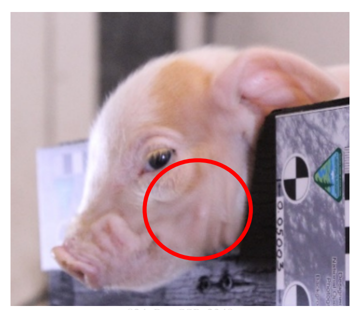
Muscle tightening of this region will give an obvious “wrinkled forehead” appearance, with high intensity

Temporal tension may affect ear position, as forehead muscles work congruently with ear muscles</td>
</tr>
<tr><td colspan="3">Note: A score of 9 can be given if the FAU is not visible or if cradle has interfered.</td></tr>
</table>

Upper Lip Contraction	
Not Present (0)	**Present (1)**
	 Lips begin to pull apart  Lips are pulled apart
	 
 Canine Notch	 Canine Notch  Canine Notch
	 
Baseline Lip line is straight, with no tension or bulging above upper lip Mouth is fully shut closed **Canine notch** is not present	Upper lip begins to slightly or fully contract and pull apart Moderate or high intensity tension and bulging on the upper lip Size of **canine notch** is partly or obviously present Snout plate may move inward, like a pushed in snout

	These features may be seen only or more strongly on 1 side of the face
Note: A score of **9** can be given if the FAU is not visible or if cradle has interfered.	

Nose and Cheek Region

Nose Bulge/Cheek Tension		
Not Present (0)	**Moderately Present (1)**	**Obviously Present (2)**
 914_Hr22_SSP_4391	 863_Hr 22_SSP_3671	
		
 971_Pre_SSP_5308	 863_Post_SSP_2885	 824_Pre_SSP_2349
		

Baseline Cheek gradient is flat and smooth, with relaxed skin. No tightening of the skin on cheeks **Pigs naturally have a few subtle wrinkles/skin folds on sides and top of nose** **Note:** Nose muscles move congruently with cheek muscles	Cheek gradient moderately changes, with an increase in skin tension. Sides of cheeks appear to have slight indentations/wrinkles Nose bulging affects cheek tension, as nose muscles work congruently with cheek muscles In response to a moderate increase of movement/bulging of nose muscles, skin on cheeks have moderate tension Skin on the sides and top of the nose appears partly "**swollen**", with several skin folds Snout plate may move inward, like a pushed in snout **These features may be seen only or more strongly on 1 side of the face and nose**	Cheek gradient has an obvious change, with a significant increase in skin tension. Sides of cheeks appear to have obvious indentations/wrinkles Nose bulging affects cheek tension, as nose muscles work congruently with cheek muscles In response to an obvious increase of movement/bulging of nose muscles, skin on cheeks have obvious tension Skin on the sides and top of the nose appears very "**swollen**", with a significant amount of skin folds Snout plate may move inward, like a pushed in snout **These features may be seen only or more strongly on 1 side of the face and nose**
Note: A score of **9** can be given if the FAU is not visible or if cradle has interfered.		

Table 1. Settings used to process images into textured 3D models in Agisoft Metashape.

Image Alignment	
Accuracy	High
Key Point Limit	75,000
Tie Point Limit	75,000
Adaptive Camera Model Fitting	Yes
Build 3D Model	
Source Data	Depth Maps
Surface Type	Arbitrary
Face Count	250,000
Interpolation	Enabled
Calculate Vertex Colors	Yes
Build Texture	
Mapping Mode	Generic
Blending Mode	Mosaic
Texture Count / Size	1 x 4096 pixels
Hole Filling	Yes
Ghost Filter	Yes

Figure 3. Model Processing in Agisoft Metashape (Litter 10, Pig 3, 4h post-castration): a) set of 11 images input into Metashape; b) set of 9 images successfully aligned in 3D space by Metashape as represented by blue rectangles viewed from the front and above; c) from left to right a sparse point cloud, wireframe, and textured (i.e. with 3D photo overlay) model view of a processed piglet model; d) location of the 16 landmarks, including 4 landmarks for scale on the cradle

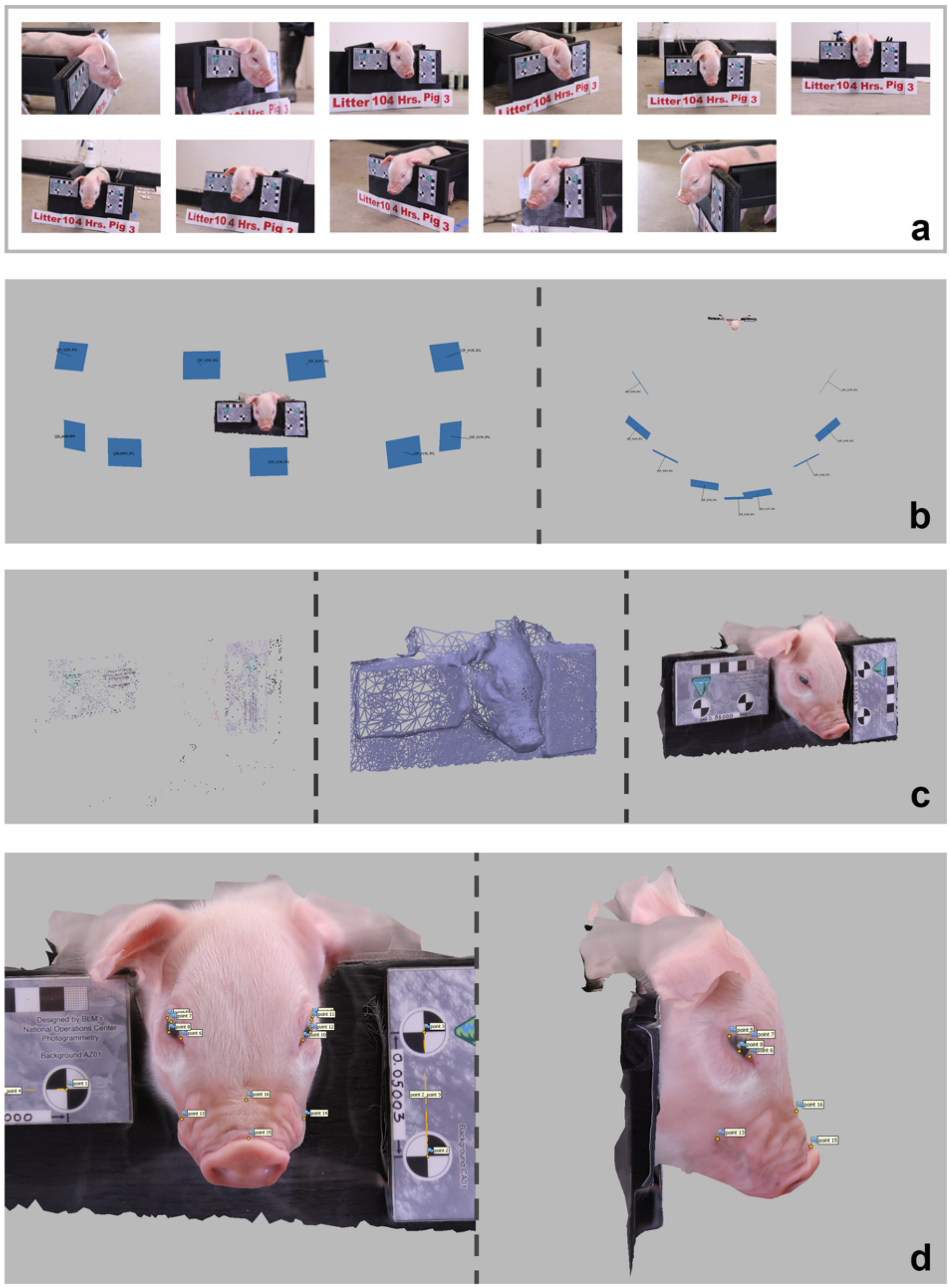


Table 2. Location of landmarks placed on the 3D piglet models.

Marker	Position
1-4	End points of scale bars
5	Outer corner of right eye
6	Inner corner of right eye
7	Top of right eye
8	Bottom of right eye
9	Outer corner of left eye
10	Inner corner of left eye
11	Top of left eye
12	Bottom of left eye
13	Corner of right lip
14	Corner of left lip
15	Tip of the snout (superior point of the end of the snout)
16	Base of the snout (center of proximal nose wrinkle)

Figure 4. Distribution of orbital tightening scores per time point for both treatments (44 piglets/treatment). Photographs of piglets were taken at four time points: baseline (T1 - immediately before castration), post-castration (T2 - immediately after castration), 4 h post-castration (T3), and 22 h post-castration (T4).

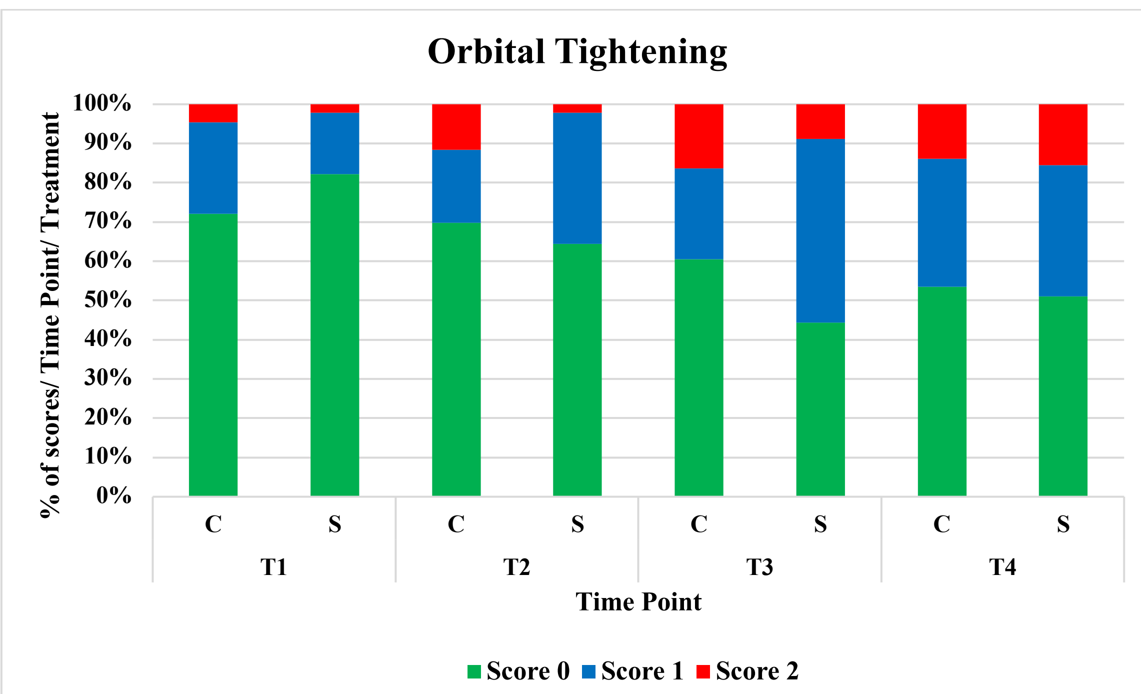


Figure 5. Distribution of ear position scores per time point for both treatments (44 piglets/treatment). Photographs of piglets were taken at four time points: baseline (T1 - immediately before castration), post-castration (T2 - immediately after castration), 4 h post-castration (T3), and 22 h post-castration (T4).

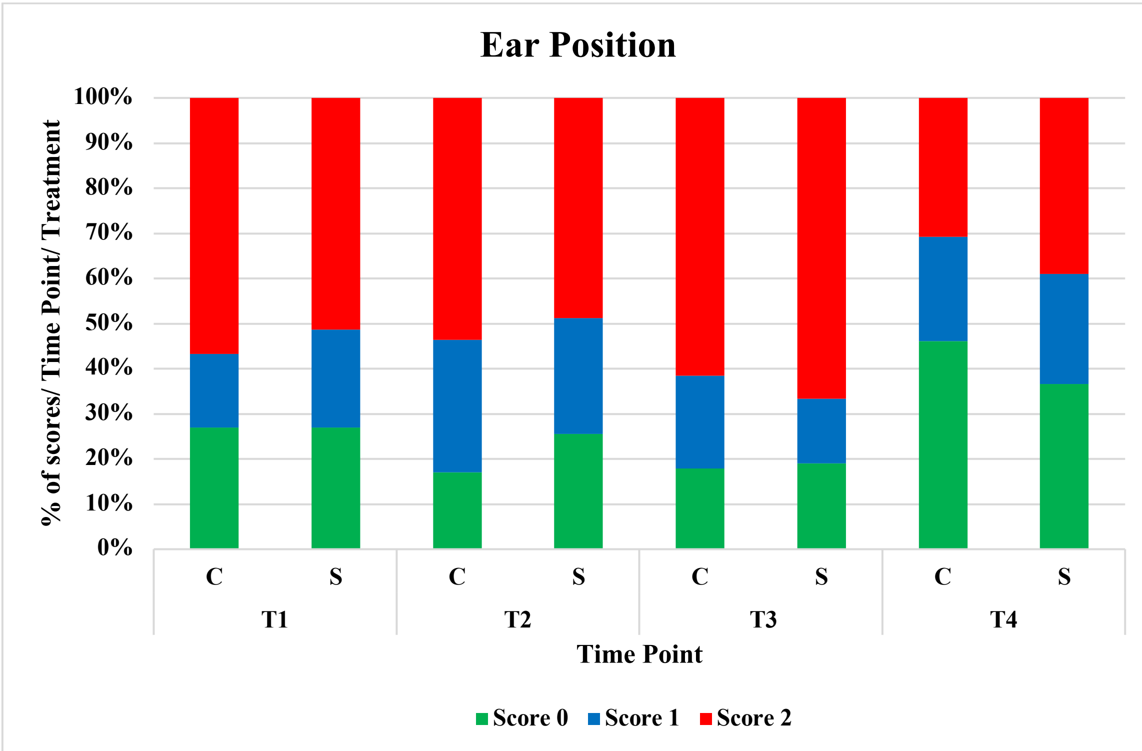


Figure 6. Distribution of temporal tension scores per time point for both treatments (44 piglets/treatment). Photographs of piglets were taken at four time points: baseline (T1 - immediately before castration), post-castration (T2 - immediately after castration), 4 h post-castration (T3) , and 22 h post-castration (T4).

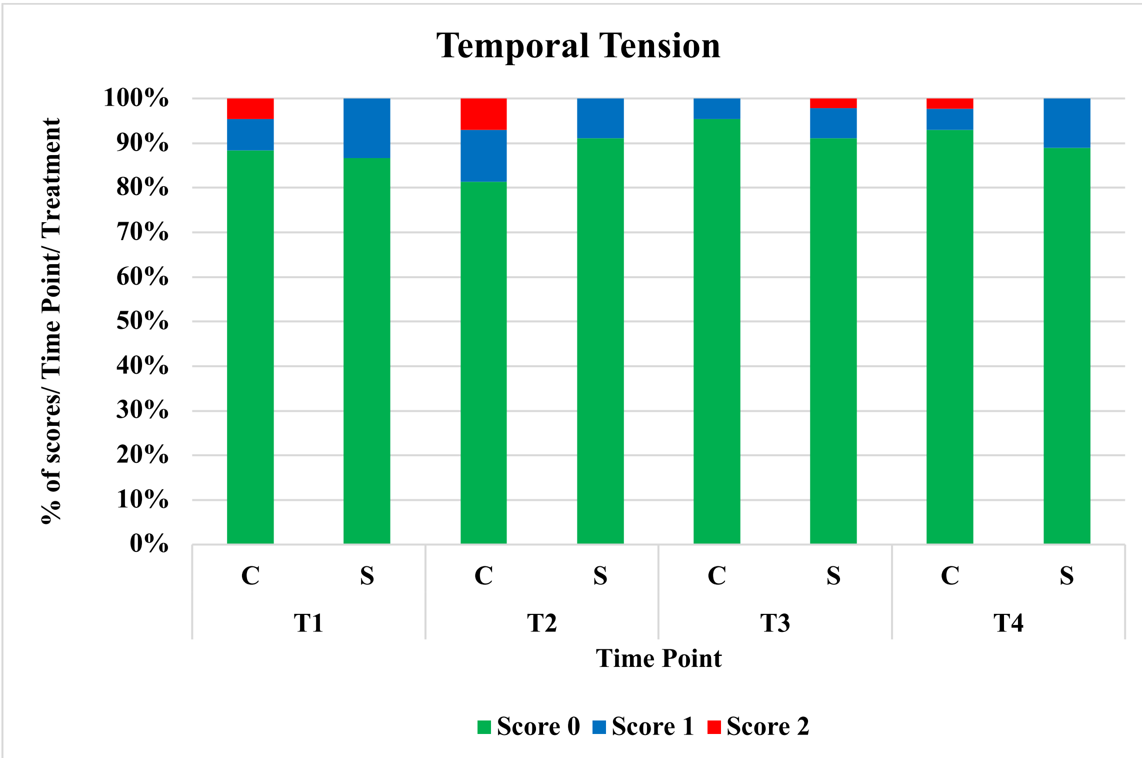


Figure 7. Distribution of lip contraction scores per time point for each both treatments (44 piglets/treatment). Photographs of piglets were taken at four time points: baseline (T1 - immediately before castration), post-castration (T2 - immediately after castration), 4 h post-castration (T3), and 22 h post-castration (T4).

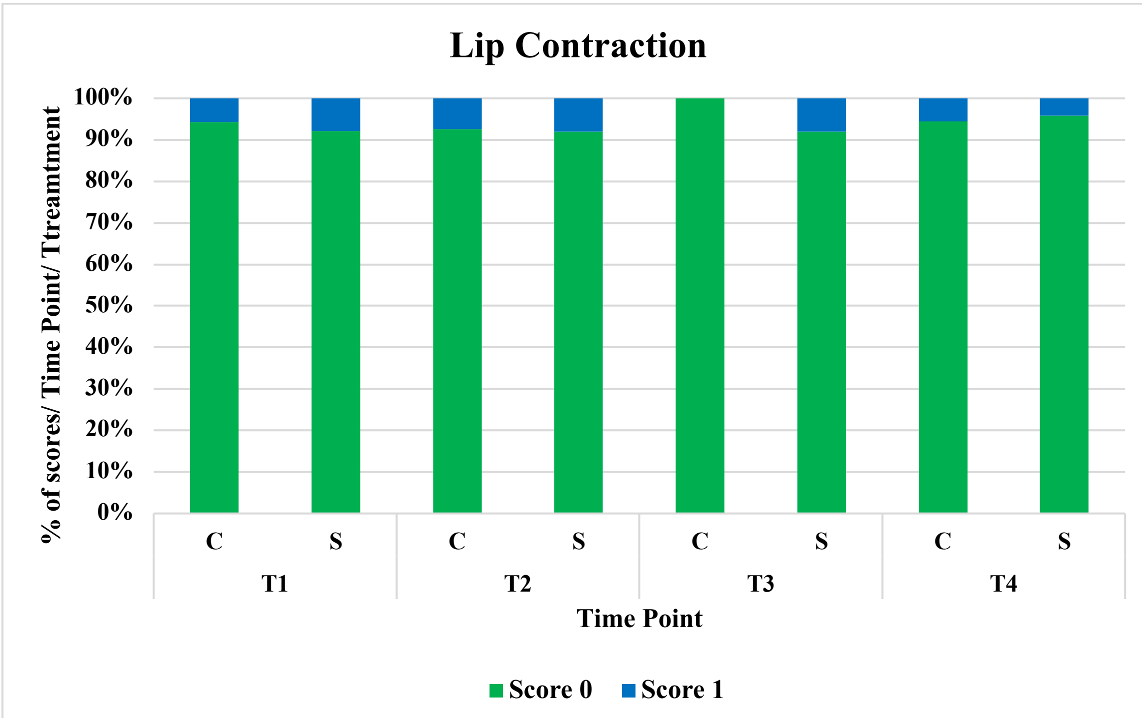


Figure 8. Distribution of nose bulge/cheek tension scores per time point for both treatments (44 piglets/treatment). Photographs of piglets were taken at four time points: baseline (T1 - immediately before castration, post-castration (T2 - immediately after castration), 4 h post-castration (T3), and 22 h post-castration (T4).

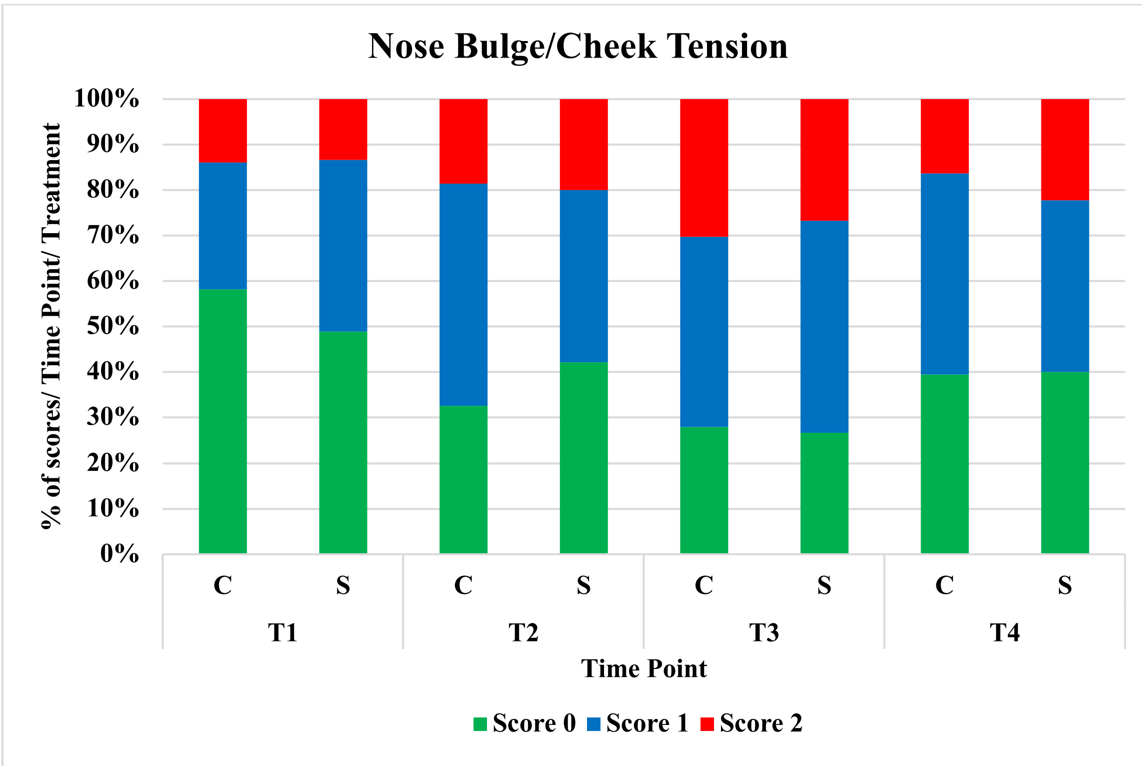


Table 3. Likelihood of castrated piglets scoring in a higher grimace category compared to sham-castrated piglets by Genmod analysis[a]

FAU[b]	Odds Ratio[c]	95% CI_L[d]	*P*-Value for Odds Ratio
Orbital Tightening	0.95	0.50	0.87
Ear Position	0.97	0.50	0.93
Temporal Tension	1.03	0.38	0.95
Lip Contraction	0.66	0.17	0.54
Nose Bulge/Cheek Tension	0.98	0.52	0.95

[a]Proc Genmod – Effect of treatment across all time points (T1-T4). Treatment and time point were fixed effects, with pig as the subject for repeated measures
[b]FAU – facial action units/response variables
[c]Odds ratio – odds of castration scoring in a higher grimace category
[d]Cl_L – lower limit of 95% confidence interval. CI greater than 1 were considered significant differences

Table 4. Cross-validation discriminant analysis [a] for each treatment utilizing all landmarks

Treatment	Castration	Sham-castration	Total
Castration	103 **59.88%** [b]	69 40.12%[c]	172 100.00%
Sham-Castration	84 46.67%[c]	96 **53.33%** [b]	180 100.00%

[a] Cross-validation discriminant analysis – to test for facial action differences across treatment groups
[b] Percent of piglets classified into the correct group classification
[c] Percent of piglets classified into the incorrect group classification

Table 5. Cross-validation discriminant analysis [a] for each time point utilizing all landmarks

Time point	TP 1	TP 2	TP 3	TP 4	Total
TP 1	22	29	22	15	88
	25.00% [b]	32.95% [c]	25.00% [c]	17.05% [c]	100.00%
TP 2	34	16	25	13	88
	38.64% [c]	**18.18%** [b]	28.41% [b]	14.77% [b]	100.00%
TP 3	10	25	29	24	88
	11.36% [c]	28.41% [c]	**32.95%** [b]	27.27% [c]	100.00%
TP 4	16	15	33	24	88
	18.18% [c]	17.05% [c]	37.50% [c]	**27.27%** [b]	100.00%

[a] Cross-validation discriminant analysis – to test for facial action differences across time points
[b] Percent of piglets classified into the correct group classification
[c] Percent of piglets classified into the incorrect group classification

Table 6. Cross-validation discriminant analysis [a] for orbital tightening for each treatment utilizing all orbital tightening landmarks

Treatment	Castration	Sham-castration	Total
Castration	102	70	172
	59.30% [b]	40.70% [c]	100.00%
Sham-Castration	70	110	180
	38.89% [c]	**61.11%** [b]	100.00%

[a] Cross-validation discriminant analysis – to test for orbital tightening differences across treatment groups
[b] Percent of piglets classified into the correct group classification
[c] Percent of piglets classified into the incorrect group classification

Table 7. Cross-validation discriminant analysis [a] for orbital tightening for each time point utilizing all orbital tightening landmarks

Time point	TP 1	TP 2	TP 3	TP 4	Total
TP 1	22	30	20	16	88
	25.00% [b]	34.09% [c]	22.73% [c]	18.18% [c]	100.00%
TP 2	29	18	24	17	88
	32.95% [c]	**20.45%** [b]	27.27% [c]	19.32% [c]	100.00%
TP 3	20	18	24	26	88
	22.73% [c]	20.45% [c]	**27.27%** [b]	29.55% [c]	100.00%
TP 4	14	18	28	28	88
	15.91% [c]	20.45% [c]	31.82% [c]	**31.82%** [b]	100.00%

[a] Cross-validation discriminant analysis – to test for orbital tightening differences across time points

[b] Percent of piglets classified into the correct group classification

[c] Percent of piglets classified into the incorrect group classification

CHAPTER 4: THE DEVELOPMENT OF A GOAT KID GRIMACE SCALE FOLLOWING THERMAL DISBUDDING

Maria Lou[1, 3], Whitney A. Knauer[2], Beth Ventura[1], John Deen[2], and Yuzhi Li[3]

[1]Dept. of Animal Science, University of Minnesota, St. Paul, MN.
[2] Dept. of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN.
[3]West Central Research & Outreach Center, University of Minnesota, Morris, MN.

Synopsis

The objective of this study was to develop a Goat Kid Grimace Scale (GKGS) as a novel method of pain assessment following disbudding. Goat kids (n=42) of mixed sex and breed between the age of 2-15 days were randomly assigned to one of seven treatment groups via block randomization (6 blocks, 7 kids per block): 1) simulated disbudding (SHAM) or thermal disbudding after administration of 2) 0.05 mg/kg IM xylazine (X), 3) 1 mg/kg oral meloxicam (M), 4) 4 mg/kg SQ buffered lidocaine (L), 5). xylazine + buffered lidocaine (XL), 6) xylazine + oral meloxicam (XM), and 7) xylazine + oral meloxicam + lidocaine (XML). All pain agents were administered 20 min prior to disbudding. Photographs of kids' faces were taken at six time points: baseline (T1 – 20 min before administration of pain agents), pre-blood collection (T2), pre-disbudding (T3), post-disbudding (T4), and directly before (T5) and after (T6) mechanical nociceptive testing (4 h post-disbudding). Four trained raters scored 120 kid photographs twice over two sessions using four facial action units: orbital tightening, ear position, lip tightening, and nostril dilation. The intra-class correlation coefficients evaluating inter-rater reliability were as follows: orbital tightening (0.79 & 0.84), ear position (0.67 & 0.61), nostril dilation (0.45 & 0.56), and lip tightening (0.45 & 0.56) for the first and second session, respectively. Prior to disbudding (T1-T3), the odds of X, XL, XM, and XML scoring in a higher grimace category for orbital tightening was 79.1, 164.80, 128.0, and 86.0 to SHAM, respectively (all $P < 0.001$). Across all time points (T1-T6), the odds X, XL, XM, and XML scoring in a higher grimace category for orbital tightening was 43.5, 89.0, 82.8, and 71.2 to SHAM (all $P < 0.001$), respectively. The odds of X, M, L, XL, XM, XML scoring in a higher grimace category for ear position was 23.1, 5.1, 12.2, 23.6, 32.1, and 45.9 to SHAM (all $P < 0.05$), respectively. The odds of X, L, XL, and XML scoring a higher grimace category for lip tightening was 8.3, 12.3, 15.9, and 16.0 to SHAM (all $P < 0.05$), respectively. The odds of XM scoring in a higher grimace category for nostril dilation was 5.89 to SHAM (P = 0.0005), respectively. Results suggest that orbital tightening and ear position may be promising FAUs for a GKGS. Pain agents (X, XL, XM, and XML) alone induced a change in orbital tightening. The combined effect of pain agents (X, L, XL, XM, and XML) and disbudding also caused all FAUs to change.

Key Words: pain assessment, facial expressions, welfare, disbudding

Introduction

In the wild, horns are advantageous to protect goats from predators, aid in thermoregulation, and establish dominance [4]. However, in a dairy production setting horns may present a safety risk to pen mates and caretakers, damage housing facilities, require extra space, and decrease meat quality through carcass bruising [2]. As a result, dairy goat kids are routinely disbudded within the first week of life, preferably between two to seven days of age [4]. Disbudding refers to the method of destroying the corium from which the horn is destined to grow [114]. Goat kids are most commonly disbudded via thermal cautery iron and without the use of an analgesic or anesthetic agent [115]. Clinically, thermal disbudding can cause inflammation, subcutaneous damage, third degree burns, and bacterial infection and induces physiological and behavioral changes in goat kids indicative of acute pain [2][57].

Pain assessment in prey species remains a challenge given their innate instinct to mask pain as an aid in survival [35]. It is more so a challenge with goat kids due to the relative lack of information on pain indicators expressed as a result of disbudding [57]. With little knowledge on reliable pain indicators elicited by goat kids, it is difficult for veterinarians and producers to properly manage and prevent pain caused by disbudding [56][57]. Researchers have looked to the beef and dairy cattle literature as a reference in assessing disbudding pain in goats, but differences in anatomy and pain management procedures add to the challenge [1][8].

Physiological and clinical parameters (e.g. cortisol, glucose, lactate concentrations, respiration rate, heart rate, and body temperature) have been investigated to understand the pain response in goat kids following disbudding [56][116][117]. Behavioral indicators such as high intensity vocalizations, head and tail shaking, rubbing, cage biting, and struggles have also been examined [2][57][118]. However, these methods of pain assessment are limited by certain factors. For some behavioral methods, video and audio recordings are time-consuming, may only provide retrospective assessment, and can require specialized equipment for sensitive measurements [64][84]. Physiological and clinical parameters are costly, require specialized training and technique and are labor intensive and potentially invasive, increasing stress onto the animal and researcher [64][84]. The grimace scale has certain advantages over these methods as it is non-invasive, species-specific, requires no specialized equipment and techniques, and may provide real-time pain assessment [64]. The grimace scale is a method of acute pain assessment that identifies and assess post-procedural changes in facial expression [15]. These pain-induced changes in facial expression are controlled by involuntary muscle movements within the major regions of the face known as facial action units (FAUs), which include orbital tightening, ear position, cheek tension, and nose bulge [84].

The grimace scale has been developed to evaluate pain in a wide variety of species including cats, piglets, rodents, horses, rabbits, and sheep [84]. For instance, the Sheep Pain Facial Expression Scale (SPFES) identified consistent changes in orbital tightening, abnormal ear position, and cheek tightening in sheep diagnosed with painful conditions (footrot and mastitis) sheep [119]. Additionally, diseased sheep had higher scores compared to the controls [119]. The Sheep Grimace Scale (SGS) reported increased scores for sheep recovering from surgery lasting up to day 3 post-surgery [91]. The Lamb Grimace Scale (LGS) also showed differences in mouth features and orbital tightening in tail-docked lambs [92]. To our knowledge, a grimace scale specific to goats has not yet been developed.

Therefore, the objective of this portion of the study was to develop and assess the utility of a Goat Kid Grimace Scale (GKGS) as a non-invasive method to assess pain in disbudded goat kids.

Materials and Methods

This study was part of a larger project to assess acute behavioral and physiological impacts of multimodal pain management strategies associated with disbudding of goat kids. The protocol for the project was approved by the University of Minnesota Institutional of Animal Care and Use Committee (#1808-36275A).

Animals and Facilities

The experiment was conducted in March 2019 at the University of Minnesota, College of Veterinary Medicine's Large Animal Teaching Barn located in St. Paul, MN. Goat kids (n = 42) of mixed sex and breed and between the ages of 2 and 15 days from a commercial dairy goat farm located near St. Paul, MN were enrolled. All kids were returned to their home farm upon completion of the study. All kids were examined by a veterinarian upon arrival and passed a physical examination to ensure good health status. Management of goat kids adhered to standards outlined in the FASS Guide for the Care and Use of Agricultural Animals in Research and Teaching. Kids were housed in six pens (2.1 x 3m; 7 kids per pen) within a temperature-controlled area of the barn. Pens were equipped with sawdust and ab libitum water. Kids were individually bottle-fed pasteurized goat milk 3 times per day at a rate of 20% body weight/day and were weighed daily at 0600 prior to the morning feeding.

Experimental Design and Kid Enrollment

Kids were acclimated upon arrival to the barn for three days prior to study commencement. Kids were randomly assigned to one of seven treatment groups via block randomization utilizing the pen as the block (6 blocks, 7 kids per block) and were fitted with colored collars (Caprine Supply, De Soto, KS, USA) for treatment identification. Breed (Alpine, Toggenburg, and Sannen) and gender were also balanced between treatment groups. The seven treatments were as follows: simulated disbudding (SHAM), thermal disbudding after administration of 0.05 mg/kg IM xylazine (X), 1 mg/kg oral meloxicam (M), 4 mg/kg SQ 2% lidocaine buffered with 8.4% sodium bicarbonate in a 1:9 solution (L), xylazine + buffered lidocaine (XL), xylazine + oral meloxicam (XM), and xylazine + oral meloxicam + buffered lidocaine (XML). All treatments were equally represented within each pen (**Table 1**).

Treatment Procedures

The animal trial was carried out sequentially over a two-day period and kids were divided equally into two groups (n=21 kids/day). Kids in pens 1-3 and 4-6 received treatment on day one and two, respectively. The day prior to treatment, all kids were shorn over their horn buds and both jugular veins using electric clippers (Andis Detachable Plus Model ag, Andis Company, Sturtevant, WI, USA) to facilitate administration of treatment and blood collection on treatment day. Treatment administration and blood collection were conducted by trained technicians. Treatment day began at 0800 h, with X, M, L, XL, XM, and XML kids receiving pain agents 20 min prior to disbudding (**Fig. 1**).

Xylazine (Anased® Injection 20mg/ml, LLOYD Inc. Shenandoah, IA, USA) was administered to X, XL, XM, and XML kids through the semimebrinosis/semitendenosis muscles at a dose of 0.5 mg/kg. Meloxicam (Meloxidyl Oral Suspension 1.5mg/ml, Ceva

Animal Health, LLC, Lenexa, KS, USA) at a dose of 1 mg/kg was administered orally before any other treatments to M, XM, and XML kids. Lidocaine (Lidocaine HCl 2%, MWI Animal Health, Boise, ID, USA) was buffered in a 1:9 dilation with 8.4% sodium bicarbonate (NEOGEN Vet, Lexington, KY, USA), diluted to a 10mg/ml solution, and administered at a dose of 4mg lidocaine per site to block the cornual branch of the infratrochlear and lacrimal nerve of both horn buds. Kids that received multi-modal pain management were always administered oral treatments first, then intramuscular, then lidocaine as appropriate.

Blood samples (3 ml) from the jugular vein were taken at each time interval for all goat kids (**Fig. 1**) for plasma cortisol and prostaglandin E2 analysis under the aims of the larger study. Mechanical nociceptive threshold testing (Wagner Pain Test FPIX Digital Algometer, Wager Instruments, Riverside, CT, USA) was conducted to test pressure sensitivity prior to administration of pain agents (-20 min) and 4 h after disbudding for all goat kids at a single location associated with each horn bud. Blood plasma and sensitivity results are reported elsewhere. Following administration of treatment medication, all kids were restrained manually and thermally disbudded using the Rhinehart X50 disbudding iron (Rhinehart Development, Spencerville, IN, USA). All kids were disbudded by the same trained individual, blinded to treatment. SHAM kids were restrained in the same manner, except that they received simulated disbudding with a cold iron (Rhinehart Development, Spencerville, IN, USA). Both the hot and cold iron were applied to each horn bud for a maximum of 3 seconds per bud at a time until a copper ring of cauterized skin was present. The total disbudding procedure lasted 63 ± 17s (mean ± SD, range: 44-120s) with no overall difference between treatment groups ($P = 0.43$).

GKGS Recording

Photographs of kids were taken at six time points (**Fig. 1**): baseline (T1 – 20 min before administration of pain agents), pre-blood collection (T2 - 1 min before disbudding), pre-disbudding (T3 - immediately before disbudding), post-disbudding (T4 - immediately after disbudding), pre-mechanical nociceptive testing (T5 - 4 h after disbudding), and post-mechanical nociceptive testing (T6) as in previous work [92]. The lead author took photographs using a point-and-shoot camera (Cannon EOS M50, Cannon, Japan), maintaining a distance of 30.5 cm (1ft) from each kid. The flashing setting was turned off to minimize disturbance to the kids.

GKGS Image Processing

A total of four images were taken of each kid per time point, resulting in 1,008 images. From those images, a total of 250 images, one per kid per time point, were selected based on quality, angle, and lighting [119]. Prior to scoring, images were imported into Preview (Version 10.1) (Apple Inc., California, USA) and cropped to exclude the colored collars and other treatment-identifying information [85][92][119]. Images were assigned a random number using a random number generator in Microsoft Excel (Version 16.27) (Microsoft Corporation, Washington, USA) to randomize the order in which they were presented to raters as to prevent identification of time point [119]. From the 250, 120 images were selected for scoring.

GKGS Scoring

Four of the authors from this study served as raters and were blinded to treatment to score the GKGS images. Raters participated in an initial and collaborative training session prior to scoring session 1 and 2, respectively. For both scoring sessions, raters were given a GKGS Pictorial Guide (**Fig. 2**) and score sheets with only a list of random numbers representing the images to input their scores. Raters scored images in one sitting in an effort to maintain consistency in scoring. The GKGS was developed referencing the sheep and lamb grimace scales and adapted for a goat face [91][92][119]. Raters scored images based on four facial action units (FAUs): orbital tightening, ear position, lip tightening, and nostril dilation. Each FAU was scored using a 3-point scale (0 = Not Present, 1= Moderately Present, and 2 = Obviously Present).

Initial training consisted of raters briefly receiving instruction on all FAUs and scoring guidelines. However, as the first round of scores resulted in lower ICCs than anticipated, a second collaborative training session was held. Collaborative training included instruction on all FAUs, scoring guidelines, and a practice scoring session of a set of sample images. For both scoring sessions, raters scored the same set of 120 images for intra-class correlation coefficient (ICC) analysis for inter-rater reliability among the four raters [15]. In addition, the main investigator of the study scored all 250 images twice after both the initial and collaborative training sessions to calculate intra-rater reliability. Furthermore, scores collected from the 250 images by the main investigator after the collaborative training were analyzed to evaluate treatment effect on the GKGS.

Statistical Analysis

All data were analyzed using SAS software (Version 9.4, SAS Inst. Inc., Cary, NC, USA). The ICC was calculated using the Mixed Procedure to determine inter-rater reliability among the four raters as well as the main investigator's intra-rater reliability between the two scoring sessions.

Treatment effect was analyzed using the Proc Genmod Procedure for non-parametric dependent variables (i.e. grimace scores for each FAU). Due to impact of the pain agents alone on FAUs, two separate analyses were conducted. The first analysis was for scores collected prior to disbudding (T1-T3) to test the effect of pain agents alone. The second analysis was for scores collected across all time points (T1-T6) to test the combination effect of pain agents and disbudding. In both models (pre-disbudding and all time points), treatment and time point were fixed effects, with kid as the subject for repeated measures. Sham disbudding (control) and T1 (baseline) were used as references to compare all other treatments and time points in the models. Statistical significance was set at $P \leq 0.05$ for odds ratios. Descriptive data were summarized using the Proc Frequency Procedure.

Results

Inter- and intra-rater reliability

The ICCs among the four raters for scoring sessions 1 and 2 (120 images/session) were as follows: orbital tightening (0.79 and 0.84), ear position (0.67 and 0.61), lip tightening (0.21 and 0.28), and nostril dilation (0.45 and 0.56). The ICCs for the main investigator between the two sessions using all 250 images/session were 0.88 for orbital tightening, 0.73 for ear position, 0.54 for lip tightening, and 0.67 for nostril dilation.

Facial Action Units (FAU) scores for all time points and treatments

The descriptive statistics depicts the percentage of all FAU scores at all time points for each treatment group only for the main investigator (**Fig. 3-6**). Visually, orbital tightening had the second largest scoring variation including all three score types (0, 1, and 2) (**Fig. 3**). After administration of pain agents, scores began to increase at T2 and remained high throughout T4 immediately after disbudding. The majority of scores at T1 and T5-6 prior to administration and at 4 h post-disbudding, respectively were 0. SHAM scored 0 across all time points, except at T3-4, scoring 1. All xylazine treatment groups (X, XL, XM, and XML) had the highest scores compared to all other treatment groups, scoring 2 at T2-4, followed by M at T3.

Visually, ear position had the largest scoring variation compared to all FAUs, with every time point incorporating all three score types (0, 1, and 2) (**Fig. 4**). At baseline, scores were already elevated and continued to increase throughout T4. Scores then declined at T5 and slightly increased again at T6. SHAM scored 0 across time points except T4 and T6 scoring 2 and 1, respectively. Again, xylazine treatment groups had the highest scores, followed by L and then M.

Lip tightening had the least amount of scoring variation with the majority of scores equaling 0 (**Fig. 5**). Scores were also elevated at baseline and increased at T2. However, scores slightly dropped at T3 and rose again at T4. At T5-6 scores greatly decreased. SHAM scored 0 for all time points, except at T4 scoring 1. The highest scores were observed at T2 and T4 by xylazine treatment groups. Meloxicam was the only treatment group to score 2 at T4 and T5. Lidocaine scored 1 at T1-T2 and T4-T5.

Nostril dilation had the third largest scoring variation (**Figure 6**). At baseline, scores were highly elevated and continued through T2. Then, scores greatly decreased at T3-T4 and increased again at T5-T6. Sham scored 0 across all time points, except for TP 2, 3, and 4, scoring 1. As observed with all other FAUs, xylazine treatment groups had the highest scores. Lidocaine and meloxicam scored 2 at T5-T6 and T3, respectively.

Treatment effect on GKGS prior to disbudding

During the pre-disbudding period (T1-T3), the odds of X, XL, XM, and XML scoring in a higher grimace category for orbital tightening was 79.1, 164.80, 128.0, and 86.0 to SHAM, respectively (all $P < 0.001$; **Table 2**). The odds of M to SHAM and L to SHAM for orbital tightening did not differ from 1. The odds of all other treatments to SHAM for nostril dilation did not differ from 1. Estimates for ear position and lip tightening could not be calculated as the scores were unbalanced for T2-3.

Treatment and disbudding effects on GKGS across all time points

During the entire data collection period (T1-T6), the odds X, XL, XM, and XML scoring in a higher grimace category for orbital tightening was 43.5, 89.0, 82.8, and 71.2 to SHAM (all $P < 0.001$; **Table 3**), respectively. The odds of M to SHAM, and L to SHAM for orbital tightening did not differ from 1. The odds of X, M, L, XL, XM, XML scoring in a higher grimace category for ear position was 23.1, 5.1, 12.2, 23.6, 32.1, and 45.9 to SHAM (all $P < 0.05$), respectively. The odds of X, L, XL, and XML scoring a higher grimace category for lip tightening was 8.3, 12.3, 15.9, and 16.0 to SHAM (all $P < 0.05$), respectively. The odds of M to SHAM, and XM to SHAM for lip tightening did not differ from 1. The odds of XM scoring in a higher grimace category for nostril dilation was 5.89 to SHAM ($P = 0.0005$), respectively. The odds of X to SHAM, M to SHAM, L to SHAM, XL to SHAM, and XML to SHAM for nostril dilation did not differ from 1.

Discussion

Evaluating pain in goat kids is a challenge given limited knowledge on their responses elicited by the painful procedure of thermal disbudding. Differences in anatomy and disbudding protocol compared to beef and dairy calves add further challenges to assessing pain in goat kids. This is the first study to evaluate the utility of a kid grimace scoring system to evaluate pain associated with disbudding in goat kids. We observed that inter-rater reliability was best for orbital tightening and ear position. We also found differences in GKGS scores associated with treatment and disbudding compared to SHAM kids. A key difference in our study is the enhancement of the pictorial guide. Unlike other pictorial guides, with only real-time photos and a single written description for all three scores, the GKGS includes a description, real-time photograph, and sketch for each individual score to help raters know what specific features should be observed as the severity of pain increases from 0 to 2.

Inter- and intra-rater reliability

According to Koo and Ki [60], the ICC values for the first scoring session were orbital tightening (0.79), ear position (0.67), lip tightening (0.21), and nostril dilation (0.45) indicative of good, moderate, poor, and poor inter-rater reliability, respectively. The ICC values for the second scoring session did not improve outside of the bracket, except for nostril dilation from poor to moderate (0.56), suggesting that the collaborative training improved inter-rater reliability for nostril dilation, but not for all other FAUs among the four raters. Additionally, the main investigator's ICC values for the both scoring sessions were orbital tightening (0.88), ear position (0.73), lip tightening (0.54), and nostril dilation (0.67) indicative of good, moderate, moderate, and moderate intra-rater reliability, respectively. Collectively, these results suggest orbital tightening was most reliably scored, while lip tightening proved most challenging under the GKGS developed in the current study.

The good to moderate reliability in scoring orbital tightening and ear position observed here coincides with high ICC values for these FAUs among other species, including mice, pigs, cats, horses, rabbits, rats, and sheep [15][16][87][86][88][90][119]. Both the eyes and ears are prominent features of the face essential for communication between conspecifics [84]. Their easy visibility may facilitate scoring as it did in lambs [92]. Tightening of the eyes is an evolutionally conserved facial expression and works as a protective mechanism in protecting sensitive areas of the face during an attack [84]. Ears are not directly on the face, but their movements are controlled by facial muscles [84]. Ears in a forward position suggest attention, while backwards or flattened positions are assumed to be in response to a negative experience [84].

Given their low ICC values, lip tightening and nostril dilation may have been harder to identify by incorporating groups of facial muscles not activated disbudding or reduced as a side-effect of the pain agents(s). Mouth changes (i.e. flattened and tightened lips) in tail-docked lambs and “abnormal lip and jaw profile” and “abnormal nostril and philtrum shape” in diseased sheep had the lowest agreement among raters due to poor angle and image quality [92][119]. The Horse Grimace Scale (HGS), also recorded a low ICC for “strained nostrils and flattening of the profile” attributed to both photo angle (i.e. front vs. profile view) and horses of dark brown or black coats being harder to score than grey and light brown [88]. The Piglet Grimace Scale eliminated lip contraction, nostril dilation, and lower jaw profile from analysis given the large inconsistencies between raters for the same reasons [16].

Similarly, in our study the angle and lighting of photographs possibly made scoring of all FAUs difficult by causing unwanted shadowing and suboptimal quality, which is a limitation considered by many grimace scales [16][92][119][120][121]. Additionally, the variation in goat breeds added to the challenge in scoring lip tightening and nostril dilation. Goats of lighter skin and coat color were easier to score (i.e. white vs. black). Thus, future development of a goat grimace scale should focus on obtaining high quality images, front and profile view angles, and in addition to species-specificity, breed-specificity, ideally using goats of light coat color (e.g. Saanen).

Treatment impacts

Prior to disbudding, kids administered X, XL, XM, and XML had higher odds of scoring in a higher grimace category for orbital tightening than SHAM. A higher category indicates grimace scores representing more pain (i.e. 1 and 2 vs. 0). Xylazine is an alpha-2-adenoreceptor that regulates an animal's level of awareness, arousal, and vigilance in the brainstem [79]. It is commonly used for disbudding kids given its analgesic, sedative, and muscle relaxant effects [80]. Xylazine activates within 10 mins of administration and it may take goat kids up to one hour to reach full recovery [4][81]. Given its rapid sedative effects, xylazine may have caused kids to become drowsy and sleepy inducing eye closure.

Moreover, the clinical and administration effects of lidocaine should be considered. There is evidence to suggest that lidocaine injections take into effect within 5 min of administration, but have an aversive, irritant effect and for this reason lidocaine was buffered for this study [2][4]. Unlike dairy calves, both cornual branches of the lacrimal and infratrochlear nerves must be blocked in goat kids [4]. Although buffered it is possible that lidocaine injections were still slightly aversive considering sensitivity thresholds vary among individuals. Also, nerve structures are relatively near sensitive areas of the face (i.e. eyes and ears) and needle insertion alone may have caused changes, particularly for orbital tightening prior to disbudding.

Treatment and disbudding impacts on FAUs

The combined effect of treatment and disbudding was analyzed across all time points (T1-6). Treatment groups X, XL, XM, and XML had higher odds of scoring in a higher category for orbital tightening than SHAM. This suggest that xylazine treatments and disbudding caused eye tightening. Ear position was the only FAU significantly affected across all time points, with all medicated kids having higher odds of scoring in a higher category for ear position than SHAM, indicating all treatments used in this study and disbudding all induced changes in ear position. Lip tightening and nostril scores were also affected by treatment and by disbudding. Kids administered X, L, XL, and XML had higher odds of scoring in a higher category for lip tightening than SHAM. Lastly, for nostril dilation XM kids had higher odds of scoring in a higher category than SHAM for nostril dilation.

The consideration for the effect of meloxicam is also important. Meloxicam, an non-steroidal anti-inflammatory drug, is commonly used for post-operative pain initiating an effect 30 min after administration if given orally with long-lasting analgesic effects [77][82][83]. Meloxicam does not target acute pain, but rather works as a long-term pain reliever shown to reduce pain for the first 24 h post-disbudding [4][122]. Thus, to some extent the M treatment group represented our positive control at the time of disbudding and the likelihood of X, L, and XL treatment groups scoring in a lower grimace category would be expected. However, these findings indicate that mainly xylazine and potentially lidocaine treatments together with disbudding had more of an effect on goat facial expressions.

It should be noted that this study was also limited by other factors. This study was not specifically designed to capture a GKGS, but to measure other physiological and behavioral parameters (presented elsewhere). In addition to photographs, invasive methods of blood samples and mechanical nociceptive testing was conducted on every kid. Extensive research has shown that any handling and manipulation causes stress and discomfort and alone could cause a change in facial expression [63][92]. An effort was made to minimize handling stress by acclimating kids to the same group of trained technicians. Furthermore, each treatment group had 6 kids per treatment, a small sample size which may have also added to difficulties in calculating odd ratio estimates and evaluating treatment and disbudding effects. Therefore, additional work is needed on a larger scale to help validate FAUs and the effects of treatment and disbudding. Lastly, given that xylazine, lidocaine injections, and potentially meloxicam caused major changes in facial expression in relation to all FAUs prior to disbudding, it is important for future studies to be cautious when separating behavioral responses (i.e. pain-relieving agents vs. disbudding) and using sensitive drugs.

Conclusion

Results suggest that orbital tightening and ear position may be promising FAUs compared to lip tightening and nostril dilation for a GKGS in relation to the pain agents used in this study and thermal disbudding. Finally, estimates determined that xylazine and lidocaine alone and multi-modal approaches using these pain agents caused significant changes in facial expression pre- and post-disbudding.

Table 1. Baseline enrollment characteristics

	Treatment groups									
	SHAM	X	M	L	XL	XM	XLM	Overall		P-Value
Breed										
Alpine	3	3	4	4	4	4	3	25		
Toggenburg	2	3	2	2	1	2	2	14		
Sannen	1	0	0	0	1	0	1	3		
Gender										
Male	2	2	1	1	3	2	2	13		
Female	4	4	5	5	3	4	4	29		
Age (d)	9.7	9.2	8.8	9.2	8.2	11.2	10.7	9.5	(2-15)	0.79
Weight (kg)	3.9	4.2	4	4.2	4.2	4.2	4.6	4.4	(3-5.8)	0.62

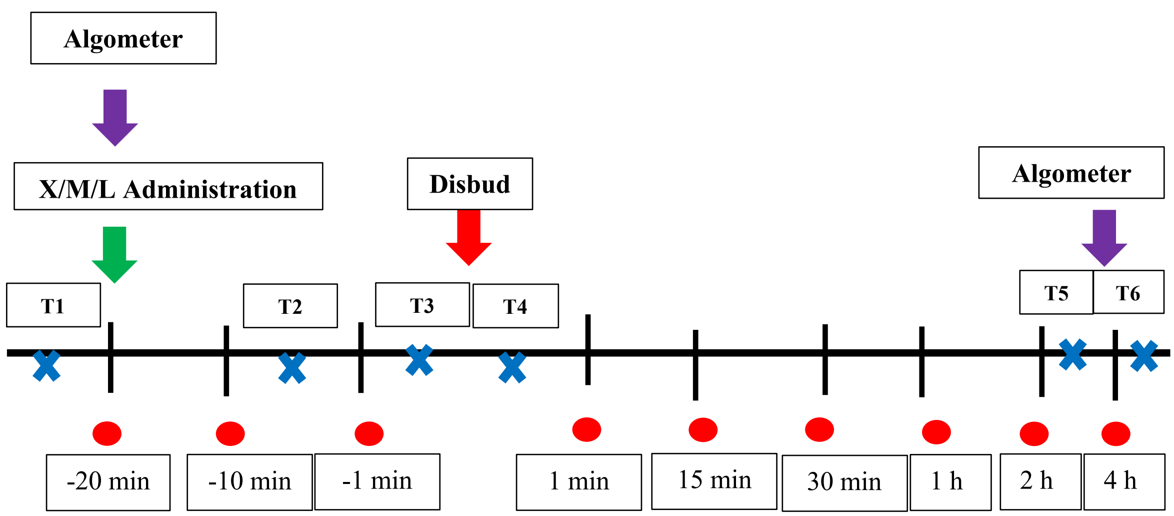
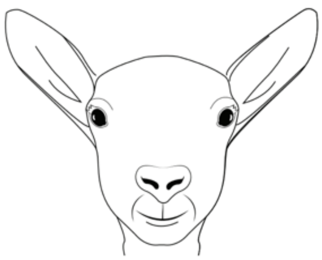
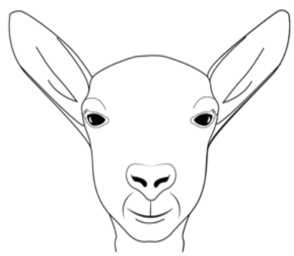


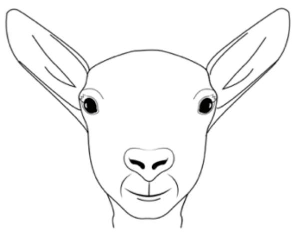
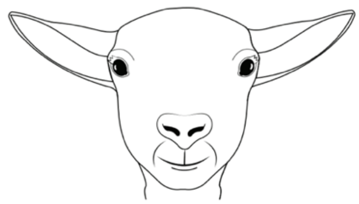
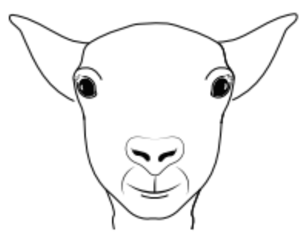
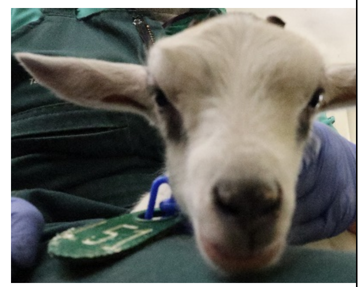
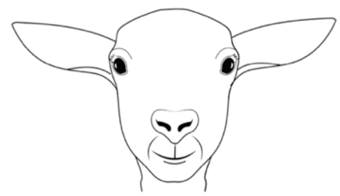
Figure 1. Project Timeline

Blue X – time point of kid photograph (TP 1-6)
Green arrow – X/M/L administration
Purple arrow - mechanical nociceptive testing
Red arrow – sham or thermal disbudding
Red circle – blood collection

Eye

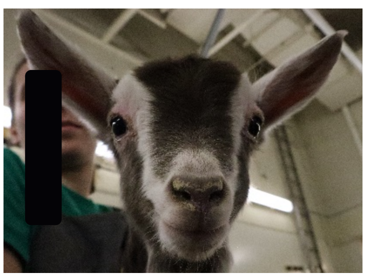
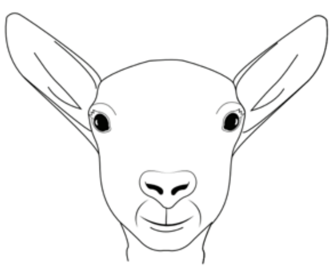
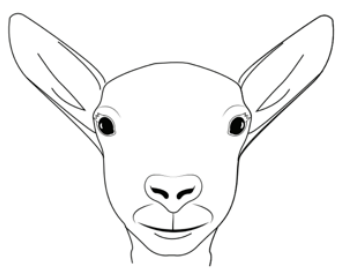
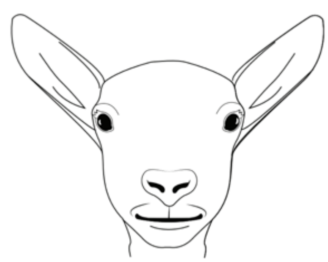
Orbital Tightening		
Not Present (0)	**Moderately Present (1)**	**Obviously Present (2)**
 	 	 
Baseline Eyes are wide open, showing a round circular shape No tightening of or around the eyelids Opening between eyelids is fully open (palpebral fissure) Pupils are not dilated (eye aperture)	Eyes are **half way** closed Partial tightening of or around the eyelids, with medium intensity, described as "eye squeezing" Opening between the eyelids is closed halfway Slight increase in pupil dilation (eye aperture) These features may be seen only, more strongly in 1 eye, **or** both eyes	Eyes are closed **more than half way**, showing almost or full eye closure Eyelid is fully tightened, high intensity "eye squeezing" Opening between the eyelids is more than hallway or fully closed Eyes maybe fully dilated These features may be seen only, more strongly in 1 eye, **or** both eyes

Ear

Ear Position		
Not Present (0)	**Moderately Present (1)**	**Obviously Present (2)**
 	   	   
Baseline Ears are relaxed and fully upright The **inner pinna** of the ear is fully visible The distance between the tips and bases of ears is normal	Ears can point **downwards** or **backwards** from the **baseline** position The **inner pinna** of the ear is **partially not** visible If facing **downwards**: • Ears are halfway ventrally rotated	Ears can point **downwards** or **backwards** from the **baseline** position The **inner pinna** of the ear is **not** visible If facing **downwards**: • Ears are fully parallel to the ground

	• Distance between both the tips and bases of the ears is increasing • Ears appear to be midway between the head If facing **backwards**: • Distance between both the tips and bases of the ears is decreasing halfway These features may be seen only, more strongly in 1 ear, **or** both ears	• Distance between both the tips and the bases of the ears is 180° If facing **backwards**: • Ears are fully parallel to the head • Distance between both the tips and bases of the ears is decreasing more than halfway These features may be seen only, more strongly in 1 ear, **or** both ears

Mouth and Jaw

Lip Tightening		
Not Present (0)	**Moderately Present (1)**	**Obviously Present (2)**
 	 	 
Baseline Lips show a normal, relaxed appearance, like "**smiling**" Lip line to corner of mouth appears smooth and straight, like an **upward** smile	Lips are partially puckered, showing a bolder, fuller appearance Lip line to corner of mouth shows a slight bulging appearance, like a **downward** smile	Lips are fully puckered and compressed Lip line to corner of mouth appears swollen Muscles on jaw profile are fully tightened, with high

Muscles on the jaw profile are relaxed	Muscles on the jaw profile are tightened with low to medium intensity These features may be seen only, more strongly in 1 lip, **or** both lips	intensity and giving a clenched jaw appearance These features may be seen only, more strongly in 1 lip, **or** both lips

Nose and Cheeks

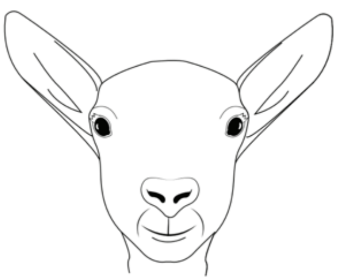
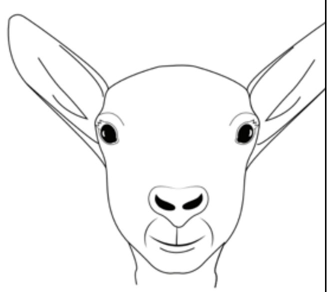
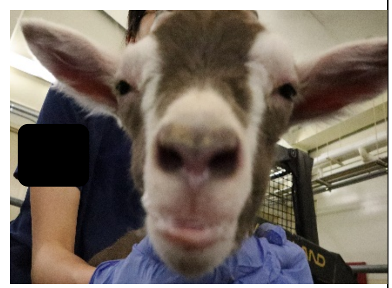
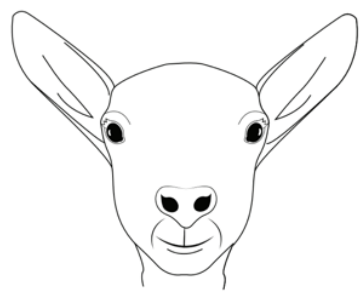
Nostril Dilation		
Not Present (0)	**Moderately Present (1)**	**Obviously Present (2)**
 	 	 
Baseline Opening of nostrils (nares) are of normal size Nare(s) appear to be almost fully closed, like a long narrow line	Opening of nostrils (nares) is increasing, showing a half circle appearance Nare(s) may appear to have tear drop shape These features may be seen only, more strongly in 1 nostril, **or** both nares	Opening of nostrils (nares) are fully open, showing a full circle appearance Nare(s) are fully flared opened These features may be seen only, more strongly in 1 nostril, **or** both nares

Figure 2. Goat Kid Grimace Scale. All FAUs scored using a 3-point scale (0 = Not Present, 1= Moderately Present, and 2 = Obviously Present).

Richard, C. 2020. *Orbital Tightening, Ear Position, Lip Tightening, and Nostril Dilation Digital Illustrations*. Digital Content Library, College of Liberal Arts, University of Minnesota.

Figure 3. Distribution of orbital tightening scores per time point for each treatment (6 kids/treatment). Photographs of kids were taken at six time points: baseline (T1 – 20 min before administration of pain agents), pre-blood collection (T2 - 1 min before disbudding), pre-disbudding (T3 - immediately before disbudding), post-disbudding (T4 - immediately after disbudding), pre-mechanical nociceptive testing (T5 - 4 h after disbudding), and post-mechanical nociceptive testing (T6).

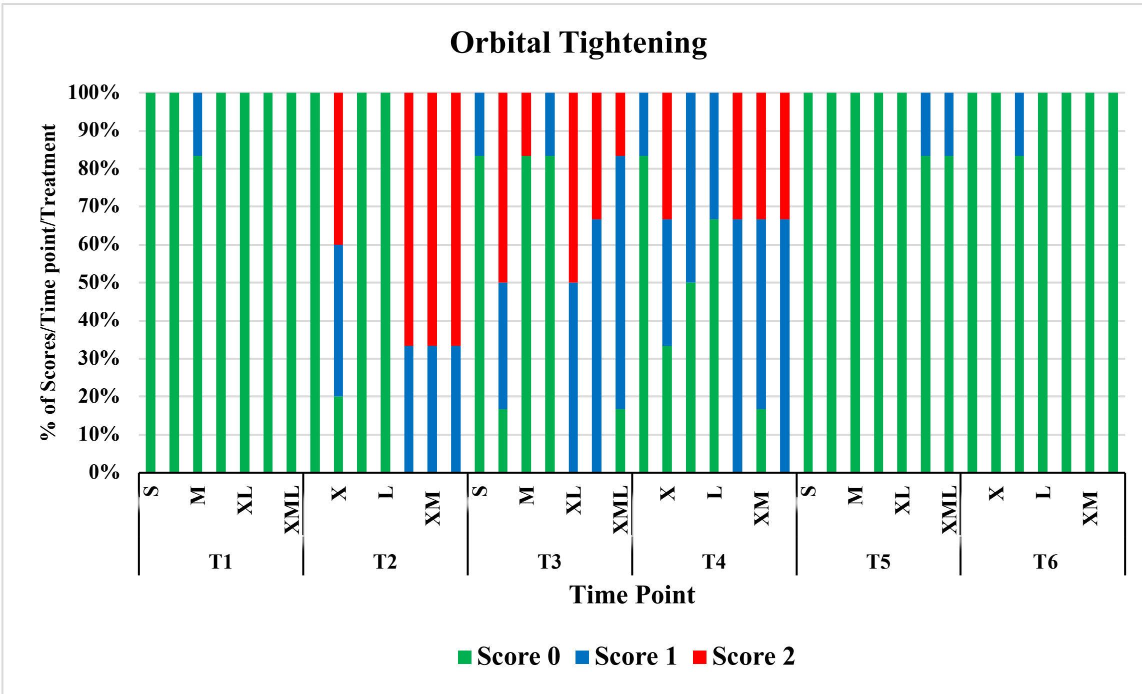


Figure 4. Distribution of ear position scores per time point for each treatment (6 kids/treatment). Photographs of kids were taken at six time points: baseline (T1 – 20 min before administration of pain agents), pre-blood collection (T2 - 1 min before disbudding), pre-disbudding (T3 - immediately before disbudding), post-disbudding (T4 - immediately after disbudding), pre-mechanical nociceptive testing (T5 - 4 h after disbudding), and post-mechanical nociceptive testing (T6).

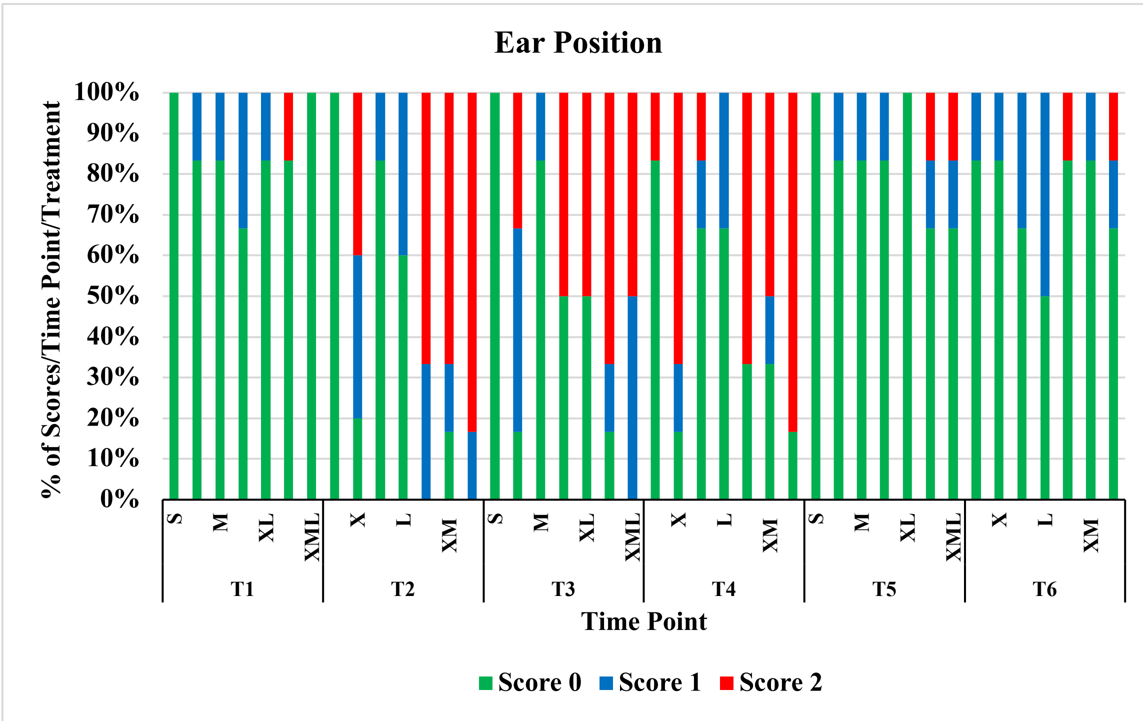


Figure 5. Distribution of lip tightening scores per time point for each treatment (6 kids/treatment). Photographs of kids were taken at six time points: baseline (T1 – 20 min before administration of pain agents), pre-blood collection (T2 - 1 min before disbudding), pre-disbudding (T3 - immediately before disbudding), post-disbudding (T4 - immediately after disbudding), pre-mechanical nociceptive testing (T5 - 4 h after disbudding), and post-mechanical nociceptive testing (T6).

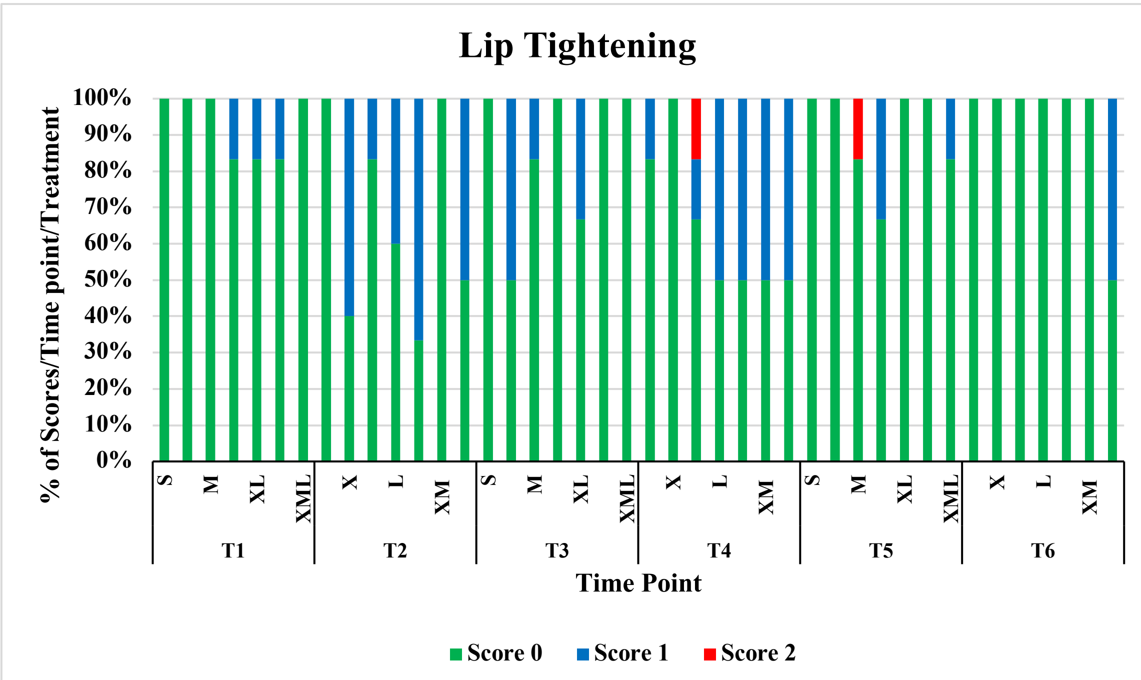


Figure 6. Distribution of ear nostril dilation scores per time point for each treatment (6 kids/treatment). Photographs of kids were taken at six time points: baseline (T1 – 20 min before administration of pain agents), pre-blood collection (T2 - 1 min before disbudding), pre-disbudding (T3 - immediately before disbudding), post-disbudding (T4 - immediately after disbudding), pre-mechanical nociceptive testing (T5 - 4 h after disbudding), and post-mechanical nociceptive testing (T6).

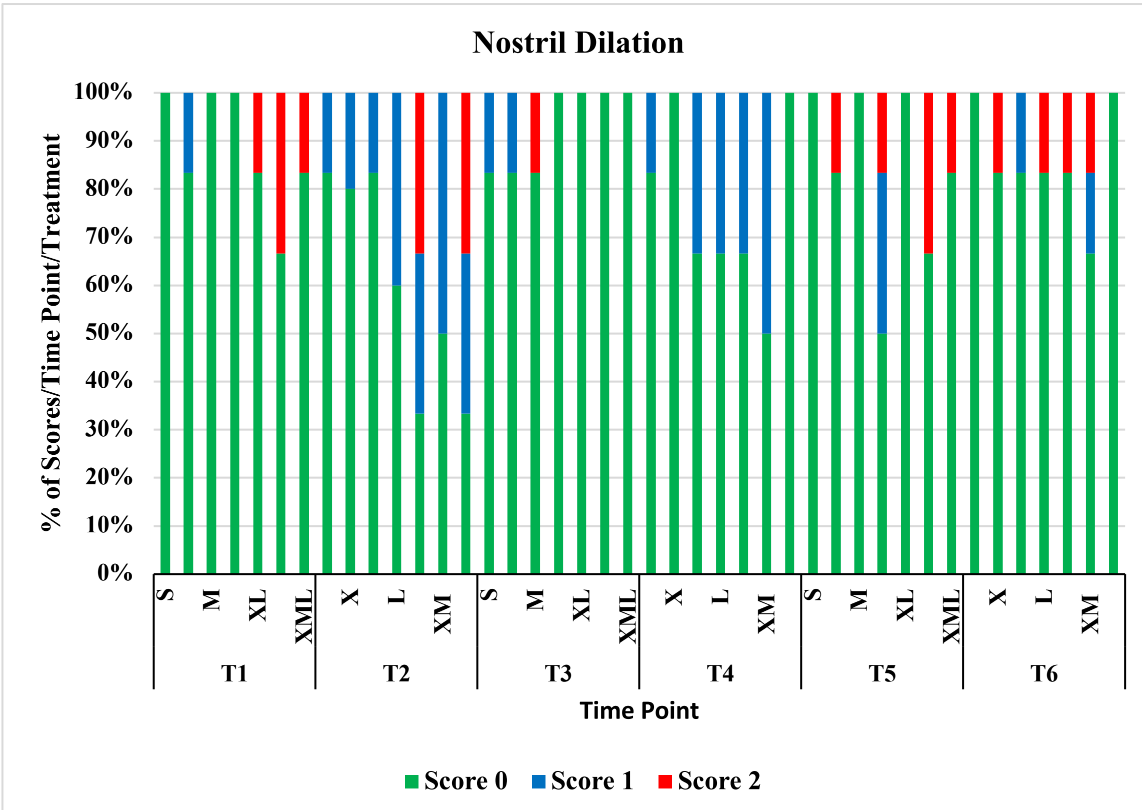


Table 2. Likelihood of kids receiving pain agents scoring in a higher grimace category compared to SHAM by Genmod analysis[a]

FAU[b]	TrT[c]	Odds Ratio[d]	95% CI_L[e]	*P*-Value for Odds Ratio
Orbital Tightening				
	X[f]	79.13	6.12	**0.0008**
	M[g]	2.41	0.13	0.55
	L[h]	1.11	0.06	0.94
	XL[i]	164.80	14.55	**<0.0001**
	XM[j]	127.96	13.48	**<0.0001**
	XML[k]	86.01	9.12	**<0.0001**
Nostril Dilation				
	X[f]	1.91	0.24	0.54
	M[g]	1.02	0.08	0.99
	L[h]	1.11	0.17	0.92
	XL[i]	4.07	0.67	0.13
	XM[j]	3.31	0.61	0.17
	XML[k]	4.07	0.67	0.13

[a]Pre-Disbudding - effect of treatment alone (TP 1-3). Treatment and time point were fixed effects, with kid as the subject for repeated measures
[b]FAU – response variable
[c]TrT – treatment, all treatment groups compared to control (SHAM) and baseline (TP 1)
[d]Odds ratio – odds of all other treatments scoring in a higher grimace category compared to SHAM
[e]CI_L – lower limit of 95% confidence interval. Confidence intervals greater than 1 were considered significant differences
[f]X (0.05 mg/kg IM xylazine)
[g]M (1 mg/kg oral meloxicam)
[h]L (4 mg/kg SQ buffered lidocaine)
[i]XL (xylazine + buffered lidocaine)
[j]XM (xylazine + oral meloxicam)
[k]XML (xylazine + oral meloxicam + buffered lidocaine)

Table 3. Likelihood of disbudded kids receiving pain agents scoring in a higher grimace category compared to SHAM by Genmod analysis[a]

FAU[b]	TrT[c]	Odds Ratio[d]	95% CI_L[e]	*P*-Value for Odds Ratio
Orbital Tightening				
	X[f]	43.5	3.42	**0.0004**
	M[g]	4.18	0.40	0.23
	L[h]	1.71	0.13	0.68
	XL[i]	88.99	9.66	**<0.0001**
	XM[j]	82.81	8.91	**0.0001**
	XML[k]	71.21	7.02	**0.0003**
Ear Position				
	X[f]	23.10	4.70	**0.0001**
	M[g]	5.08	1.15	**0.03**
	L[h]	12.18	2.17	**0.005**
	XL[i]	23.61	4.95	**<0.0001**
	XM[j]	32.12	6.98	**<0.0001**
	XML[k]	45.88	9.54	**<0.0001**
Lip Tightening				
	X[f]	8.30	1.08	**0.04**
	M[g]	6.72	0.76	0.09
	L[h]	12.32	1.22	**0.03**
	XL[i]	15.89	2.13	**0.007**
	XM[j]	4.63	0.63	0.13
	XML[k]	15.97	2.41	**0.004**
Nostril Dilation				
	X[f]	2.04	0.53	0.30
	M[g]	1.79	0.39	0.45
	L[h]	3.45	0.94	0.06
	XL[i]	3.55	0.94	0.06
	XM[j]	5.89	2.19	**0.0005**
	XML[k]	2.54	0.62	0.20

[a]Post-disbudding - combined effect of treatment and disbudding (TP 2-6). Treatment and time point were fixed effects, with kid as the subject for repeated measures
[b]FAU – response variable
[c]TrT – treatment, all treatment groups compared to control (SHAM) and baseline (TP 1)
[d]Odds ratio – odds of all other treatments scoring in a higher grimace category compared to SHAM
[e]CI_L – lower limit of 95% confidence interval. Confidence intervals greater than 1 were considered significant differences
[f]X (0.05 mg/kg IM xylazine)
[g]M (1 mg/kg oral meloxicam)
[h]L (4 mg/kg SQ buffered lidocaine)
[i]XL (xylazine + buffered lidocaine)
[j]XM (xylazine + oral meloxicam)
[k]XML (xylazine + oral meloxicam + buffered lidocaine)

BIBLIOGRAPHY

[1] J.-L. Rault, D. C. Lay, and J. N. Marchant-Forde, “Castration induced pain in pigs and other livestock,” *Appl. Anim. Behav. Sci.*, vol. 135, no. 3, pp. 214–225, Dec. 2011.

[2] L. Alvarez, R. A. Nava, A. Ramírez, E. Ramírez, and J. Gutiérrez, “Physiological and behavioural alterations in disbudded goat kids with and without local anaesthesia,” *Appl. Anim. Behav. Sci.*, vol. 117, no. 3–4, pp. 190–196, Mar. 2009.

[3] National Farm Animal Care Council, *The Code of Practice For the Care and Handlings of Pigs*. Agriculture and Agri-Food Canada, 2014.

[4] J. Matthews and B. Dustan, “Disbudding of goat kids,” *In Pract.*, vol. 41, no. 9, pp. 433–444, Nov. 2019.

[5] D. M. Weary, L. Niel, F. C. Flower, and D. Fraser, “Identifying and preventing pain in animals,” *Appl. Anim. Behav. Sci.*, vol. 100, no. 1–2, pp. 64–76, Oct. 2006.

[6] National Pork Producers Council, “Pork Facts,” 2020. [Online]. Available: https://nppc.org/pork-facts/. [Accessed: 07-May-2020].

[7] AVMA Animal Welfare Division, “Literature review on the welfare implications of swine castration,” 2013.

[8] British Veterinary Association, “BVA and Goat Veterinary Society policy position on goat kid disbudding and analgesia,” 2018.

[9] J. Reid, M. Scott, A. Nolan, and L. Wiseman-Orr, “Pain assessment in animals,” *In Pract.*, vol. 35, no. 2, pp. 51–56, Feb. 2013.

[10] M. Hansson, N. Lundeheim, G. Nyman, and G. Johansson, “Effect of local anaesthesia and/or analgesia on pain responses induced by piglet castration,” *Acta Vet. Scand.*, vol. 53, no. 1, p. 34, Dec. 2011.

[11] L. Anil, S. S. Anil, and J. Deen, “Pain Detection and Amelioration in Animals on the Farm: Issues and Options,” *J. Appl. Anim. Welf. Sci.*, vol. 8, no. 4, pp. 261–278, Oct. 2005.

[12] S. H. Ison, R. E. Clutton, P. Di Giminiani, and K. M. D. Rutherford, “A Review of Pain Assessment in Pigs,” *Front. Vet. Sci.*, vol. 3, no. November, pp. 1–16, Nov. 2016.

[13] G. . Bath, “Management of pain in production animals,” *Appl. Anim. Behav. Sci.*, vol. 59, no. 1–3, pp. 147–156, Aug. 1998.

[14] L. Cobianchi *et al.*, “Pain assessment in animal models: do we need further studies?,” *J. Pain Res.*, vol. 7, p. 227, May 2014.

[15] D. J. Langford *et al.*, “Coding of facial expressions of pain in the laboratory mouse,” *Nat. Methods*, vol. 7, no. 6, pp. 447–449, Jun. 2010.

[16] P. Di Giminiani *et al.*, “The Assessment of Facial Expressions in Piglets Undergoing Tail Docking and Castration: Toward the Development of the Piglet Grimace Scale,” *Front. Vet. Sci.*, vol. 3, no. November, pp. 1–10, Nov. 2016.

[17] D. Fraser, “Animal welfare: translating science into practice,” in *Advances in Agricultural Animal Welfare*, no. 2017, Elsevier Ltd, 2016, pp. 264–273.

[18] OIE, “Animal Welfare,” in *Terrestrial Animal Health Code*, 2019, pp. 1–4.

[19] D. Fraser, “Assessing Animal Welfare at the Farm and Group Level: The Interplay of Science and Values,” *Anim. Welf.*, vol. 12, no. 4, pp. 433–443, Jul. 2003.

[20] F. WEMELSFELDER and S. MULLAN, “Applying ethological and health

indicators to practical animal welfare assessment," *Rev. Sci. Tech. l'OIE*, vol. 33, no. 1, pp. 111–120, Apr. 2014.
[21] I. Zulkifli, "Review of human-animal interactions and their impact on animal productivity and welfare," *J. Anim. Sci. Biotechnol.*, vol. 4, no. 1, p. 25, Dec. 2013.
[22] P. . Hemsworth, "Human–animal interactions in livestock production," *Appl. Anim. Behav. Sci.*, vol. 81, no. 3, pp. 185–198, May 2003.
[23] C. Tallet, S. Brajon, N. Devillers, and J. Lensink, "Pig-human interactions: Creating a positive perception of humans to ensure pig welfare," *Adv. Pig Welf.*, pp. 381–398, 2018.
[24] M. J. Brown and C. Winnicker, "Animal Welfare," in *Laboratory Animal Medicine*, Elsevier, 2015, pp. 1653–1672.
[25] B. N. Fraser, D., Weary, D. M., Pajor, E. A., & Milligan, "A scientific conception of animal welfare that reflects ethical concerns," *Anim. Welf.*, vol. 6, no. 2, pp. 187–205, 1997.
[26] EFSA Panel on Animal Health and Welfare, "Statement on the use of animal-based measures to assess the welfare of animals," *EFSA J.*, vol. 10, no. 6, pp. 1–29, Jun. 2012.
[27] D. Mellor and N. Beausoleil, "Extending the 'Five Domains' model for animal welfare assessment to incorporate positive welfare states," *Anim. Welf.*, vol. 24, no. 3, pp. 241–253, Aug. 2015.
[28] P. Bateson, "Assessment of pain in animals," *Anim. Behav.*, vol. 42, no. 5, pp. 827–839, Nov. 1991.
[29] D. Mellor, "Updating Animal Welfare Thinking: Moving beyond the 'Five Freedoms' towards 'A Life Worth Living,'" *Animals*, vol. 6, no. 3, p. 21, Mar. 2016.
[30] M. J. Guesgen and C. J. Bench, "What can kinematics tell us about the affective states of animals?," *Anim. Welf.*, vol. 26, no. 4, pp. 383–397, 2017.
[31] Broom DM., "The Scientific Assessment of Animal Welfare," *Appl. Anim. Behav. Sci.*, vol. 20, pp. 5–19, 1988.
[32] D. M. Broom, "Animal welfare: concepts and measurement," *J. Anim. Sci.*, vol. 69, no. 10, pp. 4167–4175, 1991.
[33] M. S. Herskin and P. Di Giminiani, "Pain in pigs," in *Advances in Pig Welfare*, no. January, Elsevier, 2018, pp. 325–355.
[34] V. Molony and J. E. Kent, "Assessment of acute pain in farm animals using behavioral and physiological measurements.," *J. Anim. Sci.*, vol. 75, no. 1, p. 266, 1997.
[35] R. Guatteo *et al.*, "Minimising pain in farm animals: the 3S approach – 'Suppress, Substitute, Soothe,'" *Animal*, vol. 6, no. 8, pp. 1261–1274, Aug. 2012.
[36] R. A. Meintjes, "An overview of the physiology of pain for the veterinarian," *Vet. J.*, vol. 193, no. 2, pp. 344–348, 2012.
[37] D. B. Morton and P. H. Griffiths, "Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment.," *Vet. Rec.*, vol. 116, no. 16, pp. 431–436, 1985.
[38] Mathews *et al.*, "Guidelines for Recognition, Assessment and Treatment of Pain," *J. Small Anim. Pract.*, pp. 1–59, 2014.
[39] L. U. Sneddon, R. W. Elwood, S. A. Adamo, and M. C. Leach, "Defining and

assessing animal pain," *Anim. Behav.*, vol. 97, pp. 201–212, Nov. 2014.
[40] V. Bonavita and R. De Simone, "Pain as an evolutionary necessity," *Neurol. Sci.*, vol. 32, no. SUPPL. 1, pp. 61–66, 2011.
[41] G. Illmann, M. Špinka, L. Schrader, and P. Šustr, "Acoustical mother-offspring recognition in pigs (sus scrofa domestica)," *Behaviour*, vol. 139, no. 4, pp. 487–505, 2002.
[42] J. S. Mogil and S. E. Crager, "What should we be measuring in behavioral studies of chronic pain in animals?," *Pain*, vol. 112, no. 1, pp. 12–15, 2004.
[43] J. Barrett, "United States Hog Inventory Up 4 Percent," *United States Department of Agriculture*, 2019. [Online]. Available: https://www.nass.usda.gov/Newsroom/2019/06-27-2019.php. [Accessed: 07-Jul-2019].
[44] P. Edwards, Sandra and Di Giminiani, "Identifying Pain in Pigs and the Theory of Treatment," in *Pig Welfare Symposium*, 2017, pp. 1–27.
[45] B. Fredriksen *et al.*, "Practice on castration of piglets in Europe," *animal*, vol. 3, no. 11, pp. 1480–1487, Nov. 2009.
[46] A. Haper, "Castration of Pigs," *The Pig Site*, 2008. [Online]. Available: https://thepigsite.com/articles/castration-of-pigs. [Accessed: 31-Jul-2020].
[47] The Humane Society of the United States, "The Welfare of Piglets in the Pig Industry," *HSUS Reports*, vol. 27, 2010.
[48] A. A. Taylor and D. M. Weary, "Vocal responses of piglets to castration: identifying procedural sources of pain," *Appl. Anim. Behav. Sci.*, vol. 70, no. 1, pp. 17–26, Nov. 2000.
[49] M. A. Sutherland and C. B. Tucker, "The long and short of it: A review of tail docking in farm animals," *Appl. Anim. Behav. Sci.*, vol. 135, no. 3, pp. 179–191, Dec. 2011.
[50] American Veterinary Medical Association, "Literature Review on the Welfare Implications of Teeth Clipping , Tail Docking and Permanent Identification of Piglets," 2014.
[51] J. N. Marchant-Forde, D. C. Lay, K. A. McMunn, H. W. Cheng, E. A. Pajor, and R. M. Marchant-Forde, "Postnatal piglet husbandry practices and well-being: The effects of alternative techniques delivered separately," *J. Anim. Sci.*, vol. 87, no. 4, pp. 1479–1492, 2009.
[52] E. Leslie, M. Hernández-Jover, R. Newman, and P. Holyoake, "Assessment of acute pain experienced by piglets from ear tagging, ear notching and intraperitoneal injectable transponders," *Appl. Anim. Behav. Sci.*, vol. 127, no. 3–4, pp. 86–95, Nov. 2010.
[53] M. N. Hempstead *et al.*, "Pain sensitivity and injury associated with three methods of disbudding goat kids: Cautery, cryosurgical and caustic paste," *Vet. J.*, vol. 239, pp. 42–47, 2018.
[54] M. N. Hempstead, J. R. Waas, M. Stewart, V. M. Cave, A. R. Turner, and M. A. Sutherland, "The effectiveness of clove oil and two different cautery disbudding methods on preventing horn growth in dairy goat kids," *PLoS One*, vol. 13, no. 11, pp. 1–14, 2018.
[55] M. L. Stock, S. L. Baldridge, D. Griffin, and J. F. Coetzee, "Bovine Dehorning," *Vet. Clin. North Am. Food Anim. Pract.*, vol. 29, no. 1, pp. 103–133, Mar. 2013.

[56] O. N. Nfor, J. P.-W. Chan, M. Kere, and H.-C. Peh, “Disbudding pain: The benefits of disbudding goat kids with dexmedetomidine hydrochloride,” *Small Rumin. Res.*, vol. 139, pp. 60–66, Jun. 2016.
[57] M. N. Hempstead, J. R. Waas, M. Stewart, V. M. Cave, and M. A. Sutherland, “Behavioural response of dairy goat kids to cautery disbudding,” *Appl. Anim. Behav. Sci.*, vol. 194, no. March, pp. 42–47, Sep. 2017.
[58] I. Ajuda, M. Battini, S. Mattiello, C. Arcuri, and G. Stilwell, “Evaluation of Pain Mitigation Strategies in Goat Kids after Cautery Disbudding,” *Animals*, vol. 10, no. 2, p. 277, Feb. 2020.
[59] F. Gottardo *et al.*, “Pain alleviation during castration of piglets: a comparative study of different farm options1,” *J. Anim. Sci.*, vol. 94, no. 12, pp. 5077–5088, Dec. 2016.
[60] T. K. Koo and M. Y. Li, “A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research,” *J. Chiropr. Med.*, vol. 15, no. 2, pp. 155–163, Jun. 2016.
[61] J. A. Moberg, G.P. and Mench, *The biology of animal stress: basic principles and implications for animal welfare*. Wallingford: CABI, 2000.
[62] K. Bayne and A. International, “Reliance on Behavior as a Metric of Animal Welfare,” pp. 461–463.
[63] T. Grandin and C. Shivley, “How Farm Animals React and Perceive Stressful Situations Such As Handling, Restraint, and Transport,” *Animals*, vol. 5, no. 4, pp. 1233–1251, Dec. 2015.
[64] K. McLennan, “Why Pain Is Still a Welfare Issue for Farm Animals, and How Facial Expression Could Be the Answer,” *Agriculture*, vol. 8, no. 8, p. 127, Aug. 2018.
[65] M. A. Sutherland, P. J. Bryer, N. Krebs, and J. J. McGlone, “Tail docking in pigs: acute physiological and behavioural responses,” *Animal*, vol. 2, no. 2, pp. 292–297, 2008.
[66] S. Llamas Moya, L. A. Boyle, P. B. Lynch, and S. Arkins, “Effect of surgical castration on the behavioural and acute phase responses of 5-day-old piglets,” *Appl. Anim. Behav. Sci.*, vol. 111, no. 1–2, pp. 133–145, May 2008.
[67] M. A. Sutherland, B. L. Davis, T. A. Brooks, and J. F. Coetzee, “The physiological and behavioral response of pigs castrated with and without anesthesia or analgesia1,” *J. Anim. Sci.*, vol. 90, no. 7, pp. 2211–2221, Jul. 2012.
[68] B. Walker, N. Jäggin, M. Doherr, and U. Schatzmann, “Inhalation anaesthesia for castration of newborn piglets: Experiences with isoflurane and isoflurane/N2O,” *J. Vet. Med. Ser. A Physiol. Pathol. Clin. Med.*, vol. 51, no. 3, pp. 150–154, 2004.
[69] M. S. Leidig, B. Hertrampf, K. Failing, A. Schumann, and G. Reiner, “Pain and discomfort in male piglets during surgical castration with and without local anaesthesia as determined by vocalisation and defence behaviour,” *Appl. Anim. Behav. Sci.*, vol. 116, no. 2–4, pp. 174–178, 2009.
[70] G. Manteuffel, B. Puppe, and P. C. Schön, “Vocalization of farm animals as a measure of welfare,” *Appl. Anim. Behav. Sci.*, vol. 88, no. 1–2, pp. 163–182, 2004.
[71] B. Puppe, P. C. Schön, A. Tuchscherer, and G. Manteuffel, “Castration-induced vocalisation in domestic piglets, Sus scrofa: Complex and specific alterations of the vocal quality,” *Appl. Anim. Behav. Sci.*, vol. 95, no. 1–2, pp. 67–78, 2005.

[72] N. M. Chapel, J. R. Lucas, S. Radcliffe, K. R. Stewart, and D. C. Lay, "Comparison of vocalization patterns in piglets which were crushed to those which underwent human restraint," *Animals*, vol. 8, no. 8, pp. 1–7, 2018.
[73] G. Marx, T. Horn, J. Thielebein, B. Knubel, and E. von Borell, "Analysis of pain-related vocalization in young pigs," *J. Sound Vib.*, vol. 266, no. 3, pp. 687–698, Sep. 2003.
[74] E. Von Borell, B. Bünger, T. Schmidt, and T. Horn, "Vocal-type classification as a tool to identify stress in piglets under on-farm conditions," *Anim. Welf.*, vol. 18, no. 4, pp. 407–416, 2009.
[75] D. M. Weary, L. A. Braithwaite, and D. Fraser, "Vocal response to pain in piglets," *Appl. Anim. Behav. Sci.*, vol. 56, no. 2–4, pp. 161–172, 1998.
[76] A. A. Taylor, D. M. Weary, M. Lessard, and L. Braithwaite, "Behavioural responses of piglets to castration: The effect of piglet age," *Appl. Anim. Behav. Sci.*, vol. 73, no. 1, pp. 35–43, 2001.
[77] M. Kluivers-Poodt, B. B. Houx, S. R. M. Robben, G. Koop, E. Lambooij, and L. J. Hellebrekers, "Effects of a local anaesthetic and NSAID in castration of piglets, on the acute pain responses, growth and mortality," *Animal*, vol. 6, no. 9, pp. 1469–1475, Sep. 2012.
[78] A. Flecknell, Paul and Waterman-Pearson, *Pain Management in Animals*, 1st ed. London: W.B. Saunders, 2000.
[79] M. D. Sinclair, "A review of the physiological effects of α2-agonists related to the clinical use of medetomidine in small animal practice," *Can. Vet. J.*, vol. 44, no. 11, pp. 885–897, 2003.
[80] U. Celestine Okwudili, E. Chinedu Athanasius, and U. Rita Ijeoma, "Assessment of Common Anaesthetic and Clinical Indices of Multimodal Therapy of Propofol, Xylazine, and Ketamine in Total Intravenous Anaesthesia in West African Dwarf Goat," *J. Vet. Med.*, vol. 2014, pp. 1–6, 2014.
[81] R. Van den Brom, S. Greijdanus-van der Putten, M. Van der Heijden, K. Lievaart-Peterson, P. Vellema, and J. De Grauw, "Thermal disbudding in goat kids in the Netherlands: Current practice, complications and considerations," *Small Rumin. Res.*, vol. 183, no. August 2019, p. 106036, Feb. 2020.
[82] M. C. Burkemper, M. D. Pairis-Garcia, L. E. Moraes, R. M. Park, and S. J. Moeller, "Effects of Oral Meloxicam and Topical Lidocaine on Pain associated Behaviors of Piglets Undergoing Surgical Castration," *J. Appl. Anim. Welf. Sci.*, vol. 23, no. 2, pp. 209–218, 2020.
[83] E. Fraccaro *et al.*, "A study to compare circulating flunixin, meloxicam and gabapentin concentrations with prostaglandin E2 levels in calves undergoing dehorning," *Res. Vet. Sci.*, vol. 95, no. 1, pp. 204–211, Aug. 2013.
[84] K. et al. Descovich, "Facial expression: An under-utilised tool for the assessment of welfare in mammals," *ALTEX*, vol. 34, no. 3, pp. 409–429, 2017.
[85] A. L. Miller and M. C. Leach, "The Mouse Grimace Scale: A Clinically Useful Tool?," *PLoS One*, vol. 10, no. 9, p. e0136000, Sep. 2015.
[86] S. G. Sotocina *et al.*, "The Rat Grimace Scale: A Partially Automated Method for Quantifying Pain in the Laboratory Rat via Facial Expressions," *Mol. Pain*, vol. 7, pp. 1744-8069-7–55, Aug. 2011.
[87] S. C. J. Keating, A. A. Thomas, P. A. Flecknell, and M. C. Leach, "Evaluation of

EMLA Cream for Preventing Pain during Tattooing of Rabbits: Changes in Physiological, Behavioural and Facial Expression Responses," *PLoS One*, vol. 7, no. 9, p. e44437, Sep. 2012.
[88] E. Dalla Costa, M. Minero, D. Lebelt, D. Stucke, E. Canali, and M. C. Leach, "Development of the Horse Grimace Scale (HGS) as a Pain Assessment Tool in Horses Undergoing Routine Castration," *PLoS One*, vol. 9, no. 3, p. e92281, Mar. 2014.
[89] K. B. Gleerup, P. H. Andersen, L. Munksgaard, and B. Forkman, "Pain evaluation in dairy cattle," *Appl. Anim. Behav. Sci.*, vol. 171, no. 2015, pp. 25–32, Oct. 2015.
[90] M. C. Evangelista *et al.*, "Facial expressions of pain in cats: the development and validation of a Feline Grimace Scale," *Sci. Rep.*, vol. 9, no. 1, p. 19128, Dec. 2019.
[91] C. Häger *et al.*, "The Sheep Grimace Scale as an indicator of post-operative distress and pain in laboratory sheep," *PLoS One*, vol. 12, no. 4, p. e0175839, Apr. 2017.
[92] M. J. Guesgen, N. J. Beausoleil, M. Leach, E. O. Minot, M. Stewart, and K. J. Stafford, "Coding and quantification of a facial expression for pain in lambs," *Behav. Processes*, vol. 132, pp. 49–56, Nov. 2016.
[93] A. V. Viscardi, M. Hunniford, P. Lawlis, M. Leach, and P. V. Turner, "Development of a Piglet Grimace Scale to Evaluate Piglet Pain Using Facial Expressions Following Castration and Tail Docking: A Pilot Study," *Front. Vet. Sci.*, vol. 4, Apr. 2017.
[94] L. R. Finka *et al.*, "Geometric morphometrics for the study of facial expressions in non-human animals, using the domestic cat as an exemplar," *Sci. Rep.*, vol. 9, no. 1, p. 9883, Dec. 2019.
[95] D. E. Slice, "Geometric Morphometrics," *Annu. Rev. Anthropol.*, vol. 36, no. 1, pp. 261–281, Sep. 2007.
[96] A. Pezzuolo, V. Milani, D. Zhu, H. Guo, S. Guercini, and F. Marinello, "On-Barn Pig Weight Estimation Based on Body Measurements by Structure-from-Motion (SfM)," *Sensors*, vol. 18, no. 11, p. 3603, Oct. 2018.
[97] H. Guo *et al.*, "3D scanning of live pigs systems and its application in body measurements," *ISPRS - Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci.*, vol. XLII-2/W7, no. 2W7, pp. 211–217, Sep. 2017.
[98] J. Wu, R. Tillett, N. McFarlane, X. Ju, J. P. Siebert, and P. Schofield, "Extracting the three-dimensional shape of live pigs using stereo photogrammetry," *Comput. Electron. Agric.*, vol. 44, no. 3, pp. 203–222, Sep. 2004.
[99] H. Minagawa, "Stereo Photogrammetric Errors in Determining the Surface Area of a Small Pig Model with Non-Metric Cameras.," *J. Agric. Meteorol.*, vol. 51, no. 4, pp. 335–343, 1995.
[100] A. Wongsriworaphon, B. Arnonkijpanich, and S. Pathumnakul, "An approach based on digital image analysis to estimate the live weights of pigs in farm environments," *Comput. Electron. Agric.*, vol. 115, pp. 26–33, Jul. 2015.
[101] J. N. Rault, Jean-Loup., Lay, Donald C. Jr., Marchant-Ford, "Castration-induced pain in pigs and other livestock. USDA Farm Animal Welfare Fact Sheet," 2011.
[102] B. Algers and K. Uvnäs-Moberg, "Maternal behavior in pigs," *Horm. Behav.*, vol. 52, no. 1, pp. 78–85, Jun. 2007.
[103] R. G. White *et al.*, "Vocalization and physiological response of pigs during

castration with or without a local anesthetic," *J. Anim. Sci.*, vol. 73, no. 2, pp. 381–386, Feb. 1995.
[104] J.-L. Rault and D. C. Lay, "Nitrous oxide by itself is insufficient to relieve pain due to castration in piglets12," *J. Anim. Sci.*, vol. 89, no. 10, pp. 3318–3325, Oct. 2011.
[105] J. Vallet, "Myelin Matters to Piglet Movement, Reflexes, and Coordination," *Agriculture Research*, Clay Center, pp. 14–15, Feb-2014.
[106] C. Vanden Hole *et al.*, "How innate is locomotion in precocial animals? A study on the early development of spatio-temporal gait variables and gait symmetry in piglets," *J. Exp. Biol.*, vol. 220, no. 15, pp. 2706–2716, Aug. 2017.
[107] T. Grandin, *Livestock handling and transport*, 4th Editio. Wallingford: CABI, 2014.
[108] J. Wathan, A. M. Burrows, B. M. Waller, and K. McComb, "EquiFACS: The equine facial action coding system," *PLoS One*, vol. 10, no. 8, pp. 1–35, 2015.
[109] K. M. McLennan *et al.*, "Conceptual and methodological issues relating to pain assessment in mammals: The development and utilisation of pain facial expression scales," *Appl. Anim. Behav. Sci.*, vol. 217, no. April, pp. 1–15, Aug. 2019.
[110] A. F. da S. Cordeiro, I. de A. Nääs, M. dos S. Baracho, F. G. Jacob, and D. J. de Moura, "The use of vocalizations signals to estimate the level of pain in piglets," *Eng. Agrícola*, vol. 38, no. 4, pp. 486–490, Aug. 2018.
[111] I. Camerlink, E. Coulange, M. Farish, E. M. Baxter, and S. P. Turner, "Facial expression as a potential measure of both intent and emotion," *Sci. Rep.*, vol. 8, no. 1, p. 17602, Dec. 2018.
[112] D. Smeets, P. Claes, D. Vandermeulen, and J. G. Clement, "Objective 3D face recognition: Evolution, approaches and challenges," *Forensic Sci. Int.*, vol. 201, no. 1–3, pp. 125–132, Sep. 2010.
[113] J. A. Marchant and C. P. Schofield, "Extending the snake image processing algorithm for outlining pigs in scenes," *Comput. Electron. Agric.*, vol. 8, no. 4, pp. 261–275, Jun. 1993.
[114] D. Smith, M., & Sherman, *Goat Medicine*, 2nd ed., vol. 184, no. 2. Ames, Iowa: Wiley-Blackwell, 2010.
[115] M. M. Molaei, A. Mostafavi, R. Kheirandish, O. Azari, and M. Shaddel, "Study of disbudding goat kids following injection of clove oil essence in horn bud region.," *Vet. Res. forum an Int. Q. J.*, vol. 6, no. 1, pp. 17–22, 2015.
[116] L. Alvarez *et al.*, "Cortisol and pain-related behavior in disbudded goat kids with and without cornual nerve block," *Physiol. Behav.*, vol. 138, no. 2015, pp. 58–61, Jan. 2015.
[117] M. N. Hempstead *et al.*, "Effect of isoflurane alone or in combination with meloxicam on the behavior and physiology of goat kids following cautery disbudding," *J. Dairy Sci.*, vol. 101, no. 4, pp. 3193–3204, Apr. 2018.
[118] Chandrahas, A. L. Saini, D. S. Malik, and J. Mohindroo, "Effect of local anaesthetics and non-steroidal analgesics on maintenace behaviour of hot- iron disbudded beetal kids under stall- fed conditions," *Indian J. Anim. Res.*, vol. 47, no. 5, pp. 407–411, 2013.
[119] K. M. McLennan, C. J. B. Rebelo, M. J. Corke, M. A. Holmes, M. C. Leach, and F. Constantino-Casas, "Development of a facial expression scale using footrot and

mastitis as models of pain in sheep," *Appl. Anim. Behav. Sci.*, vol. 176, pp. 19–26, Mar. 2016.
[120] K. McLennan and M. Mahmoud, "Development of an Automated Pain Facial Expression Detection System for Sheep (Ovis Aries)," *Animals*, vol. 9, no. 4, p. 196, Apr. 2019.
[121] K. Finlayson, J. F. Lampe, S. Hintze, H. Würbel, and L. Melotti, "Facial Indicators of Positive Emotions in Rats," *PLoS One*, vol. 11, no. 11, p. e0166446, Nov. 2016.
[122] C. INGVAST-LARSSON, M. HÖGBERG, U. MENGISTU, L. OLSÉN, U. BONDESSON, and K. OLSSON, "Pharmacokinetics of meloxicam in adult goats and its analgesic effect in disbudded kids," *J. Vet. Pharmacol. Ther.*, vol. 34, no. 1, pp. 64–69, Feb. 2011.

EXECUTIVE SUMMARY

Neonatal livestock animals within food production settings routinely undergo painful management procedures during their first week of life. For example, male piglets are surgically castrated to reduce aggression, prevent unwanted breeding, and eliminate the production of boar taint to maintain high meat quality. Cooked pork with boar taint will have an unpleasant smell and taste, which approximately 75% of consumers find objectionable according the AVMA. Dairy goat kids are thermally disbudded for the safety of pen mates and caretakers, prevention of housing damages, and to reduce carcass defects. Both surgical castration and thermal disbudding in the US are commonly performed without an analgesic or anesthetic agent for pain relief.

Extensive research has determined that these procedures are painful on the visceral and somatic level, as they require the removal of sensitive tissue. As a negative affective state, pain is considered an indicator of poor welfare and with adverse implications on the health and well-being of animals. However, pain is a complex issue as it is an individual experience and is influenced by multiple factors such as species, breed, sex, age, and health status. Researchers have focused on certain pain indicators to gain insight on the pain and severity animals might be experiencing as a result of these procedures. Behavioral (e.g. maintenance behavior), physiological (e.g. substance P), and clinical (e.g. skin temperature) indicators have shown that neonates deviate from their normal, healthy responses when experiencing pain.

It is evident that pain is better assessed using multiple indicators; however current assessment methods are limited by certain factors. The grimace scale may offer some advantageous as a method of pain assessment, as it non-invasive, species-specific and requires minimal training and no specialized skills or equipment. However, there are few studies using the grimace scale for piglets and none for kids. Chapter 2 provided clear, descriptive definitions to improve consistent assessment of struggle behaviors and during castration of piglets. Well-established vocalizations (i.e. duration and peak frequency) were also analyzed to evaluate how they aligned with struggle behaviors. Surgical castration induced the frequency of leg kicks, body flailing, and high frequency vocalizations in piglets, compared to sham-handling. These results suggest that struggle behavior and vocalizations may be useful indicators of acute pain in piglets during castration.

The results of Chapter 3 demonstrated that agreement among raters was best for orbital tightening and ear position given high intra-class correlation coefficients (ICC), indicating they may be useful facial action units for a Piglet Grimace Scale (PGS). Photogrammetry and 3D landmark-based geometric morphometrics were successful in providing high resolution images and 3D models of moderate quality. However, neither the PGS, photogrammetry, nor 3D landmark-based geometric morphometric were sensitive enough to discriminate differences in facial expression in castrated piglets compared to sham-handling. Photogrammetry and 3D landmark-based geometric morphometrics may be improved with added cameras covering a wider range of angles and adding more visual appearance (e.g. texture) to the scene to enable more landmark annotation through enhancement of 3D models.

Chapter 4 again sought to establish a Goat Kid Grimace Scale (GKGS) to assess pain in neonatal goat kids following thermal disbudding. The ICC was greater for orbital tightening and ear position indicating that these FAUs may useful for a GKGS. Prior to the disbudding procedure, goats who received treatments that included scored higher grimace scores for orbital tightening, indicating that something associated with handling, drug administration procedures, or drug side-effects associated with xylazine. The analysis of all time points demonstrated that all facial action units (orbital tightening, ear position, lip tightening, and nostril dilation) were affected by disbudding and drug administration. As a sedative activated within 10 min, xylazine seemed to have the strongest effect on the grimace scale as the majority of kids given xylazine alone, in combination with meloxicam, lidocaine, and both had the highest odd ratios (i.e. scoring in a higher grimace category). Following xylazine, lidocaine may have also had a strong effect given its acidic compounds and application procedure requiring four injections near the eyes and ears.

In conclusion, the research conducted for this thesis assessed acute pain in piglets and goat kids through the application and development of behavioral indicators, primarily the grimace scale. Based on the information provided in this thesis, further studies may try to focus on further validating the piglet and goat kid grimace scale through 3D technology, keeping influential factors (e.g. breed, age, sex, and color) and grimace score analysis consistent, and most importantly establishing a robust facial expression baseline.

BIOGRAPHY

Maria Elizabeth Lou was born on January 25th, 1994 to Vilma and Carlos Lou in Miami, Florida. Her love for pigs began with a University of Florida Exchange program in Toulouse, France. As part of the program, she worked as an intern on a swine farm called SCEA de l'Erve in Auvers-le-Hamon, France. Following her return back the states, she wanted to learn more about the swine industry, so she took courses on Swine Production and Livestock Behavior and Welfare. She was also hired to work at the University of Florida Swine Unit.

Maria graduated with honors from the University of Florida with a B.S. in Animal Sciences with a Food Animal Emphasis in 2017. After graduation, she worked for the USDA Animal and Plant Health Inspection Services. In 2018, she was accepted to the University of Minnesota to pursue a M.S. in Animal Science with a research focus on swine behavior and welfare under the supervision of Dr. Yuzhi Li. Maria believes that animals provide us with many resources, therefore, ensuring their well-being is the right thing to do. If possible, procedures that cause the least amount of pain should be used. Her passion for neonatal livestock pain research has driven her to pursue a PhD, so she accepted a PhD position at Kansas State University in the Department of Anatomy and Physiology beginning Fall 2020.