Comparative pain capacity of animals in agriculture: are fish treated fairly?

Author: Byron Jarrett

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Abstract

The capacity for pain is an essential determinant for devising animal welfare standards for the avoidance of suffering. Welfare standards for fish are absent; however, the evidence in favour for fish pain capacity is mounting. This research review compares the potential for the experience of pain in animals commonly used in agriculture, namely pigs, chickens, and fish, with a focus on the treatment of fish compared to the other two species. The review examines neuroanatomy, electrophysiological activity, pharmacological response, and both short-term and long-term behavioural responses to pain in these species, as well as their relative ethical agricultural policies. The aim of the review is to provide a comprehensive overview of the current state of knowledge on pain in these animals, and to explore the implications of these findings for ethical decision-making in the treatment of fish in agriculture. Specifically, the aim is to determine whether the discrepancy in the welfare of fish aligns with the latest pain research. The review highlights evidence for similarities and differences between fish and other animals in their experience of pain and discusses the potential mechanisms underlying these differences. The conclusions of the review have important implications for tackling more effective pain management strategies for fish, and for the broader ethical considerations surrounding the treatment of fish.

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Introduction

Definitions of pain

Defining pain is necessary for comparative research, although it is a challenging and still debated topic. The most accepted definition of pain in research is "[a]n unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" by the International Association for the Study of Pain (IASP; Raja et al., 2020). However, as all animals (except humans) are unable to communicate the subjective emotional component, this definition has been argued as anthropocentric (Elwood, 2019). Some non-human animal pain researchers encourage separation by using definitions of pain which focus on observable features (Elwood, 2019; Sneddon et al., 2014), such as that by Zimmerman (1986) who defined pain as "[a]n aversive sensory experience caused by actual or potential injury that elicits protective and vegetative reactions, results in learned behaviour, and may modify species specific behaviour". Additionally, more criteria and objective observable features of pain, such as rapid learning to avoid noxious stimuli, sustained behavioural changes with protective function, and criteria based on neurobiology, would allow for greater objectivity in metrics for pain-associated behaviour (Sneddon, 2009). Reasonably, the discrepancy in definitions could be due to the IASP definition having clinical benefit for patients, with revisions attempting to improve assessment and management (IASP, 2020). Furthermore, the IASP notes that verbal communication is not a necessity for the experience of pain, including non-human animals as a potential example (Raja et al., 2020), suggesting that both neonates and non-human animals could experience pain as "unpleasant", but lack the ability to express this. Both definitions tie into consciousness, highlighting the significance of non-human animal consciousness in determining pain capacity. Hence, as these definitions are not contradictory, both are considered in this review for their respective strengths.

Significance of this review

Pigs (*Sus domesticus*) and chickens (*Gallus gallus domesticus*) represent the two most slaughtered land animals, pigs are the most slaughtered mammal (Mammalia), and chickens are the most slaughtered bird (Aves), which collectively form ~45% of meat production worldwide (243 million tonnes; Ritchie et al., 2019) with fish (and seafood) representing 37% (200 million tonnes of fish; Ritchie & Roser, 2021). Despite this, and how pigs are used in biomedical sciences (as a well-regarded animal model), pigs are still among the least examined mammals in terms of pain (Herskin & di Giminiani, 2018). Hence, their continued assessment and comparison is important. Furthermore, although chickens are the most researched bird in terms of pain due to their agricultural significance (Sneddon, 2018), birds and fish alike are still understudied, especially in pain research (Prunier et al., 2013). For this review, the term fish describes all animals in the Teleostei taxa, as a far greater variety of fish are used in agriculture as opposed to mammals; there is not enough research on a single species of farmed fish to perform a narrower comparison. Although priority is given to studies using fish of the carp family (Cyprinidae; termed cyprinids) as they represent 4 of the 5 most farmed fish

(Mood & Brooke, 2019). Hence, the three non-human animals investigated in this review represent three unique animal classes (mammals, birds, and fish) and around 82% of all animal products consumed worldwide (shown in Figure 1).

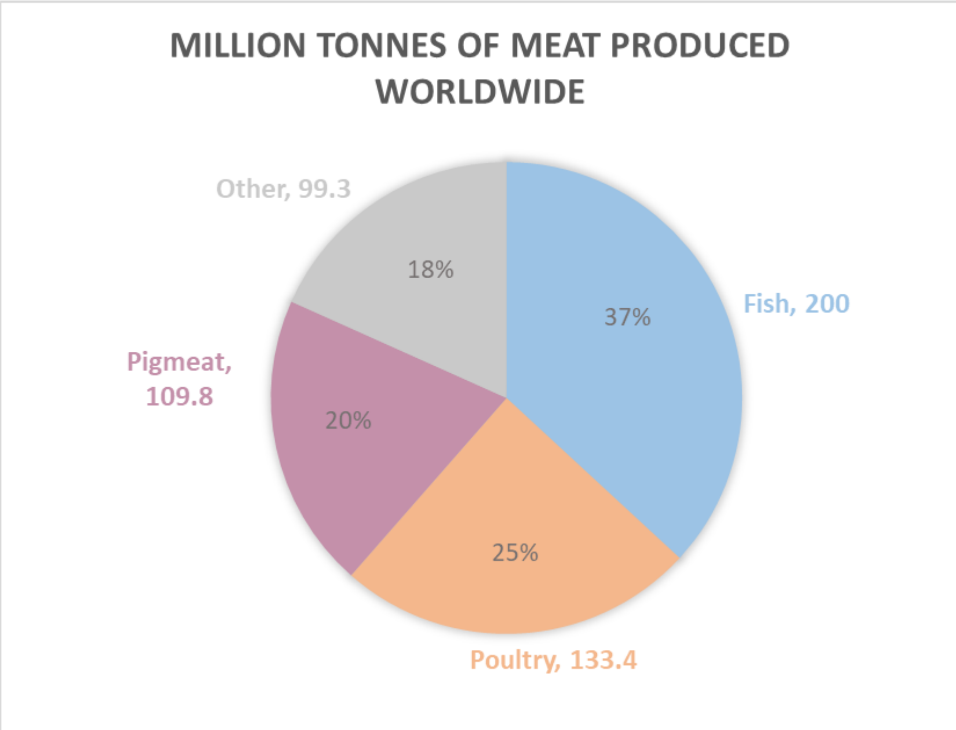


Figure 1. The proportion of meat produced worldwide in million tonnes.

Figure made for the purpose of this review using data from Ritchie et al., 2019; Ritchie & Roser, 2021.

Objective of this review

The main objective considered in this review is to determine whether evidence suggests that fish have a lower pain capacity compared to pigs and chickens. This is important as fish are commonly considered less ethically significant, and welfare in aquaculture is relatively new; consequently, fish receive lower welfare standards in policymaking compared to other animals (Barreto et al., 2022). Furthermore, the annual total for aquaculture and fisheries is over 200 million tonnes of fish (and seafood) (Ritchie & Roser, 2021); hence, vastly exceeds the total count of all land animals used in agriculture, with estimates between 1-2.5 trillion annually (Mood & Brooke, 2018, 2019), a difference is depicted in Figures 2-3.

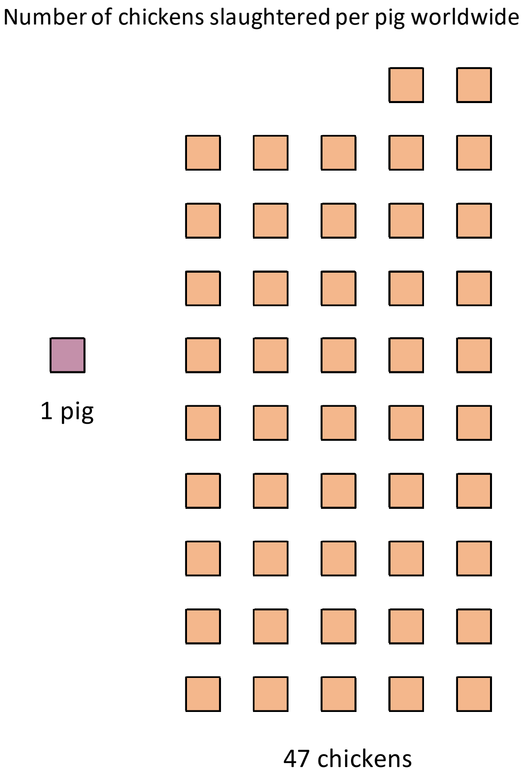


Figure 2. Relative proportions of individually killed animals.
Figure made for the purpose of this review using data

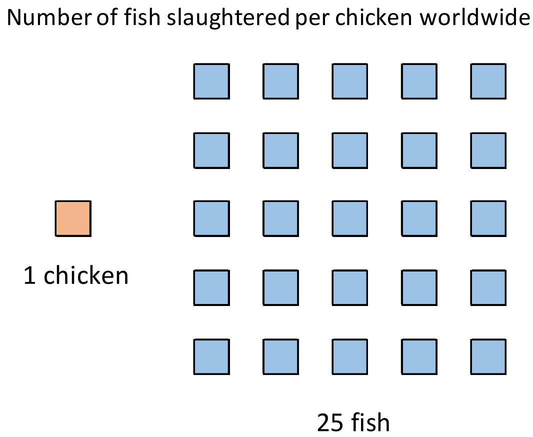
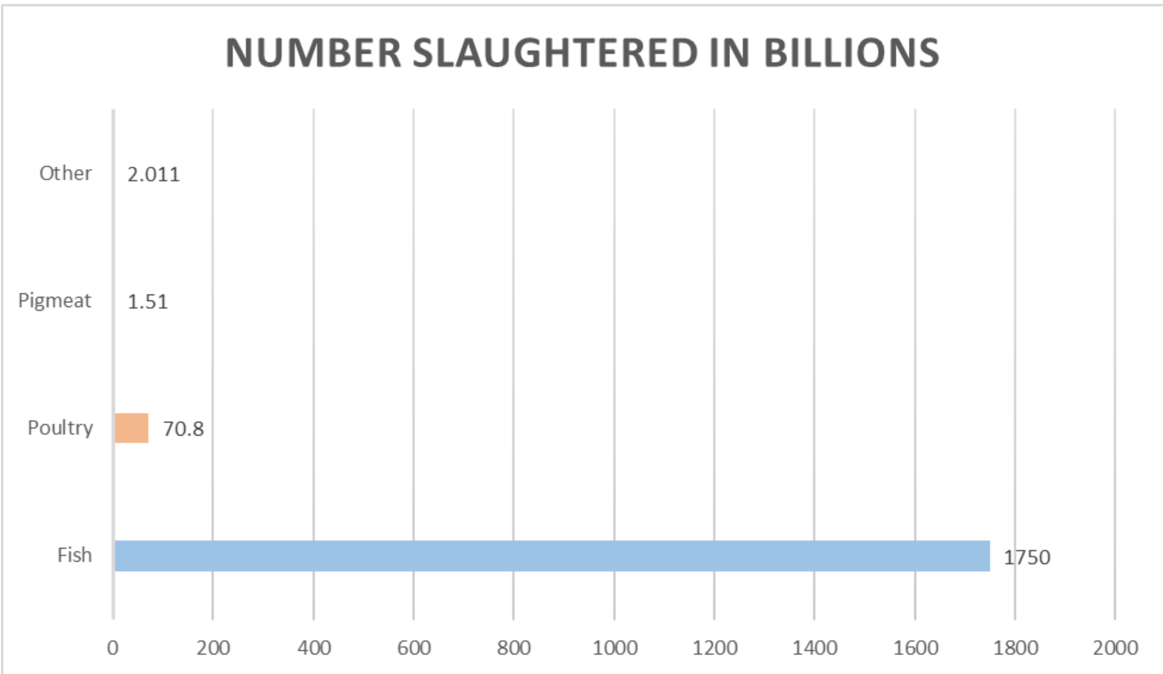


Figure 3. Bar chart with the differences between individual counts of death in agriculture annually.



Addressing the objective

To address the objective, this review will provide a current overview of pain in pigs, chickens, and fish. Although pain is best understood in mammals, it is still difficult to measure, with the best method of measuring pain in humans being self-report; for example, numerical ratings between 0 to 10 (Bendinger & Plunkett, 2016). The subjectivity of assessing pain, behavioural results (especially in fish), and the inability of verbal communication in non-human animals contributes to the challenge of measuring pain capacity across species. Consequently, both biological and behavioural methods are needed to enable a current state comparison of evidence in animal pain capacity. The distinction of pain from nociception is crucial, as pain is not solely inferred from peripheral nerve activity. However, due to the role of nociception, comparing nociceptive systems could also provide insight into certain peripheral aspects of pain capacity. The mammalian nociceptive system can be considered in three sections: distinct neuroanatomic pathways and structures, cellular and molecular components for transducing noxious signals, neural mechanisms for upregulating or downregulating the ability to sense pain stimuli (e.g., antinociceptive and pronociceptive mechanisms) (Malafoglia et al., 2013). Importantly, nociceptive signals need to be transmitted to the brain to be perceived, and integrated with emotional processing to become an experience, and therefore pain (Tracey & Mantyh, 2007). Hence, it is most crucial to identify significant brain areas for high-order activity that may support emotions, conscious experience, and the perception of pain. The assessment of behavioural changes in animals can provide insight into their pain experience by enabling the comparison of pain-associated behaviour, hence, potentially inferring pain capacity and conscious experience. The five main behavioural indicators have been identified as avoidance/defensive behaviours, vocalisations, behaviours focused on the site of painful stimulus, behaviours aiming to reduce pain stimulation, and a change of general activities (such as feeding, social, or regular motor behaviours) (Prunier et al., 2013). These indicators can be used in analgesic studies to assess their efficacy on reducing pain-associated behaviours. Therefore, the objective here is to analyse pain in pigs, chickens, and fish by reviewing behavioural and biological studies. Furthermore, a comparison of the evidence for fish pain compared to other non-human animal pain will be placed in the context of the respective ethical policies in agriculture.

Pigs

Pigs (*Sus domesticus*) are known to have similar nervous system morphology to humans. Most importantly, pigs are commonly understood to have significant cognitive and emotional capacity for the experience of states like pain.

Neuroanatomy and pathways

Mammalian neural pathways have many similarities across species; hence, like humans, pigs possess nociceptive pathways from peripheral areas to the brain, transmitted through the spinal dorsal horn, to reach the somatosensory cortex for pain perception, and further descending modulatory pathways from the brain (Herskin & di Giminiani, 2018). Recent evidence for pain processing has been shown in the somatosensory cortex pigs, with comparable responses to human (Janjua et al., 2021). Early studies showed high correspondence between pig A-delta and C-fibres with humans (Karanth et al., 1991; Lynn et al., 1996) which are crucial for the peripheral aspects of pain perception. Studies investigating pig nerves endings showed similar conduction velocities, axonal excitability, and distribution of sensory nerve endings between pigs and humans (Obreja et al., 2010; Obreja & Schmelz, 2010).

Nociceptors and neurophysiological similarities

Pigs have homologs of a human receptor, transient receptor potential vanilloid 1, (TRPV1) (Ohta et al., 2005), a key ion channel involved in neurotransmission of noxious heat or sensitisation (important for pain response) (Brown et al., 2015). Pigs also have ortholog genes of cyclooxygenase enzymes (COX1 and COX2) for synthesising prostanoids which control inflammation (UniProt, 2022b, 2022a) and supported by the effectivity of NSAIDs. Pigs with spinal compression were shown to have increased levels of substance P (SP) (Cornefjord et al., 1995) a key chemical mediating spinal cord nociception in humans (O'Connor et al., 2004) and implicated in pain perception (Graefe & Mohiuddin, 2022). Irradiation of pig skin causes an inflammatory response similar to human, with physiological response by hyperexcitation of peripheral nociceptors (Rukwied et al., 2008), and behavioural responses (di Giminiani et al., 2014). Studies have determined the use of pig nerves as models (Obreja & Schmelz, 2015) and pig models for pain as beneficial (Gigliuto et al., 2014).

Behaviours

Pain-associated behaviours in pigs are well-assessed and include less activity, and delayed feeding onset (after noxious stimuli) (Malavasi et al., 2006). Furthermore, early studies using noxious stimuli identified typical responses of kicking, twitching, or tail flicking (Herskin & di Giminiani, 2018). One study investigated uncastrated and castrated piglets over 5 days and identified castrated pigs had significantly more pain-associated behaviours (stiffness, trembling, scratching at affected site, huddling up, reduced social cohesion) with the longest changes still significant after 4 days (Hay et al., 2003). Vocalisation is often considered an important feature of pain; pig (and other mammal) pain is often associated with screaming.

Analgesics

Due to highly preserved physiological mechanisms in mammals, most analgesics work effectively in pigs. Opioids like morphine and buprenorphine have been shown to reduce pain-associated behaviours (Malavasi et al., 2006; Meijer et al., 2015). Early pig studies showed that onset of feeding after surgery was sensitive to ketorolac (a non-steroidal anti-inflammatory drug; NSAID) (Andersen et al., 1996). More recently, meloxicam (another NSAID) showed to reduce pain-associated behaviours (increased lying time and potentially agitated movements) (Pairis-Garcia et al., 2015).

Agricultural procedures

Many studies in pig pain assessment focus on injury occurring from procedures in the animal agriculture to develop pain-management strategies. One study examining tail docking (a common agricultural procedure) of 295 piglets identified pain-associated behaviours, with acute responses showing smaller tails significantly increased likelihood of screaming, and postural changes lasting for up to 5 hours (observations ceased after this time), meloxicam was tested but showed very minimal effects on behaviour; however, lidocaine (a local anaesthetic) was also investigated and reduced procedural pain during tail docking, although did not affect post-procedural pain-associated behaviour (Herskin et al., 2016). Interestingly, an early study investigating castration in pigs found that neither aspirin (NSAID) nor butorphanol (opioid) worked to reduce pain-associated behaviours (McGlone et al., 1993). Marx et al. (2003) identified that piglets castrated without anaesthesia screamed more compared to grunting or squealing. Crucially, the intensity of screams was shown to be reduced using lidocaine; hence, this reversibility supports the idea of vocalisation as a pain indicator in pigs.

Complex pain-associated behaviours

More complex behavioural responses to pain have been studied in mammals, although not many in pigs. Early studies have shown that rats will cover electrodes to avoid painful stimulus (Pinel et al., 1989), and self-administer analgesics (Woller et al., 2014). Pigs are widely considered to be a highly intelligent species (Marino & Colvin, 2015); hence, it is unlikely for rodents to exhibit cognitive abilities that are not shared with pigs. A study investigating the navigational ability of castrated piglets identified slower navigation in the castrated group, this was further by the administration of meloxicam which reduced the pain-associated behaviour (Bilsborrow et al., 2016). This reversibility infers a similar role of prostaglandin synthetase in pigs; hence, supporting similar inflammatory pain mechanisms in pigs as humans.

Physiological activity

Physiological indicators of pain in pigs may be measured as sympathetic afferent nerve activity, catecholaminergic activity, or more autonomic changes such as body and skin temperature, blood pressure, heart rate, respiratory rate, pupillary enlargement, or piloerection (Herskin & di Giminiani, 2018). In humans, autonomic changes are used as markers for the assessment of analgesia in unconscious patients (Bantel & Trapp, 2011); hence, they likely function as pain indicators for pigs as well. Many pain studies have used physiological indicators to indicate pain in pigs including a significant increase of adrenocorticotropic hormone (ACTH) and cortisol during surgery, the effect of which was reversed using local anaesthetic (Lykkegaard et al., 2005). Both ACTH and cortisol are known physiological effects of pain on the endocrine system (Tennant, 2013). Furthermore, in agriculture practices, castration is known to immediately increase cortisol levels (Lonardi et al., 2015), and tail docking, iron injections, and ear notching likely all produce similar results (Marchant-Forde et al., 2009; Prunier et al., 2005; Sutherland et al., 2011). These results were further supported by follow-up studies using analgesia to show a reduction in the attenuated cortisol levels in castration (Keita et al., 2010), tail docking and iron injections (Bates et al., 2014). Therefore, both surgery cases and agricultural procedures produce physiological responses that can be reversed using analgesics, further supporting the idea of pain in pigs.

Chickens

Avian pain is often considered analogous to mammals (Kubiak, 2016; Machin, 2005) which is reflected in their growing consideration in both veterinary and agricultural policies.

Neuroanatomy and pathways

The anatomic arrangement of avian spinal cord is overall similarly arranged to mammals, C and A-delta axons bifurcating into peripheral and central branches, connecting to nociceptors and the spinal cord dorsal horn (Necker, 2000). Similar to mammals, birds also have ventral commissural neurons for projecting signals across the spinal cord, which might be multisynaptic neurons transmitting non-localising pain fibres (King & McLelland, 1984). Crucially, despite lacking a distinct cortex, birds have a homologous cerebrum divided into two regions (pallium and subpallium) like mammals (Jarvis et al., 2005), where the forebrain has shown similar connectivity down to the cellular level (Güntürkün & Bugnyar, 2016). Furthermore, showing distinct functional roles alike to the neocortex in mammals (Medina & Reiner, 2000); therefore, these components might help process pain for perception and emotional capacity in birds. Contrastingly, mammal palliums have a unique laminar structure, whereas the avian palliums do not (Reiner et al., 2004). Most importantly, Paul-Murphy et al. (2007) identified activity in areas of avian cerebrum associated with a persistent pain stimulus, therefore suggesting complex pain processing in the bird brain.

Nociceptors and neurophysiological similarities

Early studies identified three classes of nociceptors in birds (Necker & Reiner, 1980), mechanical, thermal, and polymodal nociceptors (which respond to chemical, mechanical, and thermal stimulus) (McKeegan, 2004). Although birds respond to heat, they are notably unaffected by capsaicin due to alterations of vanilloid receptor 1 (VR1), suggested as a mechanism for seed dispersal (Jordt & Julius, 2002). Opioid receptors have been shown to appear as early as 10 days in chicks (Hendrickson & Lin, 1980), with evidence (in pigeons) for μ, δ, and κ receptors being similarly distributed in the forebrain and midbrain as mammals (Reiner et al., 1989). Furthermore, COX1/2 enzymes have also shown wide distribution in chickens (Mathonnet et al., 2001), allowing for NSAID modulation via nonselective inhibition of the enzymes (Lu et al., 1995). Furthermore, SP has been shown to potentially sensitise chicken C-fibre nerve endings (Zhai & Atsumi, 1997).

Behaviours

Behavioural assessment in wild birds is often more difficult, due to their inherent overtness in distress or pain behaviours, as well as masking physiological changes (Whiteside, 2014). Although birds more comfortable in their environment have shown potentially pain-associated behaviours like squinting or limb guarding (Hawkins, 2006). After painful stimulus, birds can often be vocal, show excessive movement, avoidance responses, and potentially aggressive behaviour (Whiteside, 2014). Furthermore, more general behaviours include reduced movement and reduced social cohesion (J. Paul-Murphy & Hawkins, 2012).

Analgesics

The effect of various analgesics on pain-associated behavioural changes have been studied in chickens. Roach & Sufka (2003) injected chickens with a noxious inflammatory stimulus to test the analgesic effects of morphine, dexamethasone (steroidal anti-inflammatory drug), and naproxen (NSAID) showing both NSAIDs to alleviate hyperalgesia and inflammation, with morphine only attenuating to the hyperalgesia. Studies have also tested analgesics using an obstacle course. Carprofen (McGeown et al., 1999), morphine, and butorphanol (Singh et al., 2017) were administered on chickens before using an obstacle course; however, morphine caused sedation, therefore, only the NSAIDs (carprofen and butorphanol) resulted in a faster time for the injured chickens. Similar positive results were also shown in another study using NSAIDs (carprofen and meloxicam) in injured chickens (Caplen et al., 2013). Furthermore, ketorolac was recently shown to safely induce analgesic effects in chicks (Mousa, 2019). Interestingly, birds are particularly sensitive to side effects of local anaesthetics (Malik & Valentine, 2018). For this reason, although lidocaine and bupivacaine appear to have analgesic effects (Khamisabadi et al., 2021), birds must be unconscious for their delivery, and care must be taken for toxicity.

Complex pain-associated behaviours

More complex pain behaviours have been observed in chickens in several studies. Studies investigating motivational changes have injected a noxious stimulus into a leg of a chickens and identified that their pain-associated behaviours (one-legged standing or sitting) could be reduced by both feeding-induced analgesia with starved chickens (Wylie & Gentle, 1998), and attention-induced by placing them in a novel environment (Gentle & Tilston, 1999). These studies provide evidence for descending pain modulation in chickens. Importantly, in the case of feeding-induced analgesia, the effect was reversible with naloxone (reverses effects of opioids), therefore showing that the analgesic effect was likely opioid mediated (Wylie & Gentle, 1998). Furthermore, injured chickens have been shown to selectively choose to eat more analgesic-dosed (carprofen) food than healthy chickens in a potentially severity dependent manner (Danbury et al., 2000). Although interestingly another study showed beak-trimming (a common agricultural procedure) chickens to not consume more analgesic-dosed food; however, the trimmed chickens pecking force correlated to their amount of analgesic-dosed food eaten, which was noted as potential sign of pain-associated behaviour (Freire et al., 2008). Although, the trim in this case was considered light compared to typical chickens in agriculture. Hence overall, using self-administration provides a less subjective overall assessment of the chickens state, and supports the idea that chicken injury is painful.

Physiological activity

Physiological changes associated with pain include corticosteroids, although it can also be associated with other stressful events (Hawkins, 2006), raised blood pressure (often identified after surgeries) is potentially more reliable (Gentle & Hunter, 1991). A veterinary review lists tachycardia and tachyphemia as further physiological parameters of acute pain in birds (Malik & Valentine, 2018).

Fish

The fact that fish morphology differs from that in mammals is the main historical reason for failing to consider their capacity to experience pain. Whether fish feel pain or not is still debated but research in the area is strengthening the view that they do (Lambert et al., 2022; Schroeder et al., 2021).

Neuroanatomy and pathways

Although fish lack a neocortex, their nervous system can be shown as analogous to both mammals and birds, as they possess nociceptive pathways from peripheral areas to the brain, similarly organised to the spinothalamic and trigeminal tracts in mammals (Sneddon, 2004), which are very interconnected to the zebrafishes cortical areas such as the thalamus, dorsal telencephalon (analogous to the pallium), and ventral telencephalon (analogous to the subpallium) (Rink & Wullimann, 2004). Additionally, as in mammals, evidence for descending modulatory pathways exists (Lopez-Luna et al., 2017b; Maximino, 2011). Fish nociception was initially considered as solely reflexive (Rose, 2002); however, this view changed due to pivotal studies which identified activity in the forebrain (the dorsal telencephalon) of fish during pain stimulation (Dunlop & Laming, 2005; Nordgreen et al., 2007). Furthermore, Reilly et al. (2008) showed increased gene expression activity in both midbrain and forebrain (dorsal and ventral telencephalon) areas during noxious stimulation. These studies potentially provide evidence of complex pain processing in the fish brain.

Nociceptors and neurophysiological similarities

Fish would be expected to have very different sensory systems due to the distinct evolutionary pressures found in an aquatic environment. The main expected differences in nociception are the lower risk of noxious chemicals and extreme temperature changes (both due to dilution/dispersion in the aquatic environment) which might have influenced their nociceptive development (Sneddon, 2018). Rainbow trout (*Oncorhynchus mykiss*) have been shown to have C and A-delta fibres and three classes of nociceptor, mechanochemical, mechanothermal and polymodal nociceptors capable of hypersensitisation (alike to both mammals and birds) (Ashley et al., 2007; Sneddon, 2002). Thermal thresholds for trout nociceptors were notably different from mammals (not responding below 4°C and activating above 33°C) likely a result of the environments the species live in (Ashley et al., 2007). This is further supported by the thresholds in zebrafish having a less varied and warmer range which can be explained by their tropical climate (Sneddon, 2019). Furthermore, the mechanical and heat nociceptors were found to have higher sensitivity than mammal cutaneous nociceptors, which could be due to the more fragile nature of fish skin (Sneddon, 2003a). SP has also been shown in early studies to be distributed in fish nervous systems (Sharma et al., 1989).

Zebrafish

The most researched fish is the zebrafish (*Danio rerio*; a cyprinid), it is the third most used animal model in research, behind mice and rats, and second most used model for genetic alterations, only behind mice (European Commission, 2022). This is a recent development with their continued use increasing over the last few years, aligning with the growing interest in fish awareness. Zebrafish have shown to have homologs of human receptors TRPV1 and TRPA1, which are used in response to mechanical pressure, pH, or temperature (Ohnesorge et al., 2021). They have also been shown to have opioid receptors (mu) like mammals (de Velasco et al., 2009). Furthermore, zebrafish have also been shown to have ortholog genes for COX-1 and COX-2 (Grosser et al., 2002; Pini et al., 2005). Recent studies have shown how zebrafish hypothalamic oxytocin neurons are strongly activated by noxious stimuli, including activation of TRPA1 receptors known for damage-sensing (Wee et al., 2019). Overall, these studies infer capacity for the peripheral aspects of pain in fish.

Behaviours

Behaviours in fish are more difficult to interpret than in mammals or birds, partly due to the lack of vocalisation. Pain-associated behaviours assessed in fish include tail beating in zebrafish when acid is administered near the tail (Maximino, 2011), rocking motions by rainbow trout (Sneddon et al., 2003) and common carp (Reilly et al., 2008b) injected with noxious chemicals, and rubbing motions by rainbow trout (Sneddon et al., 2003) and goldfish (Newby et al., 2009) on the injection site.

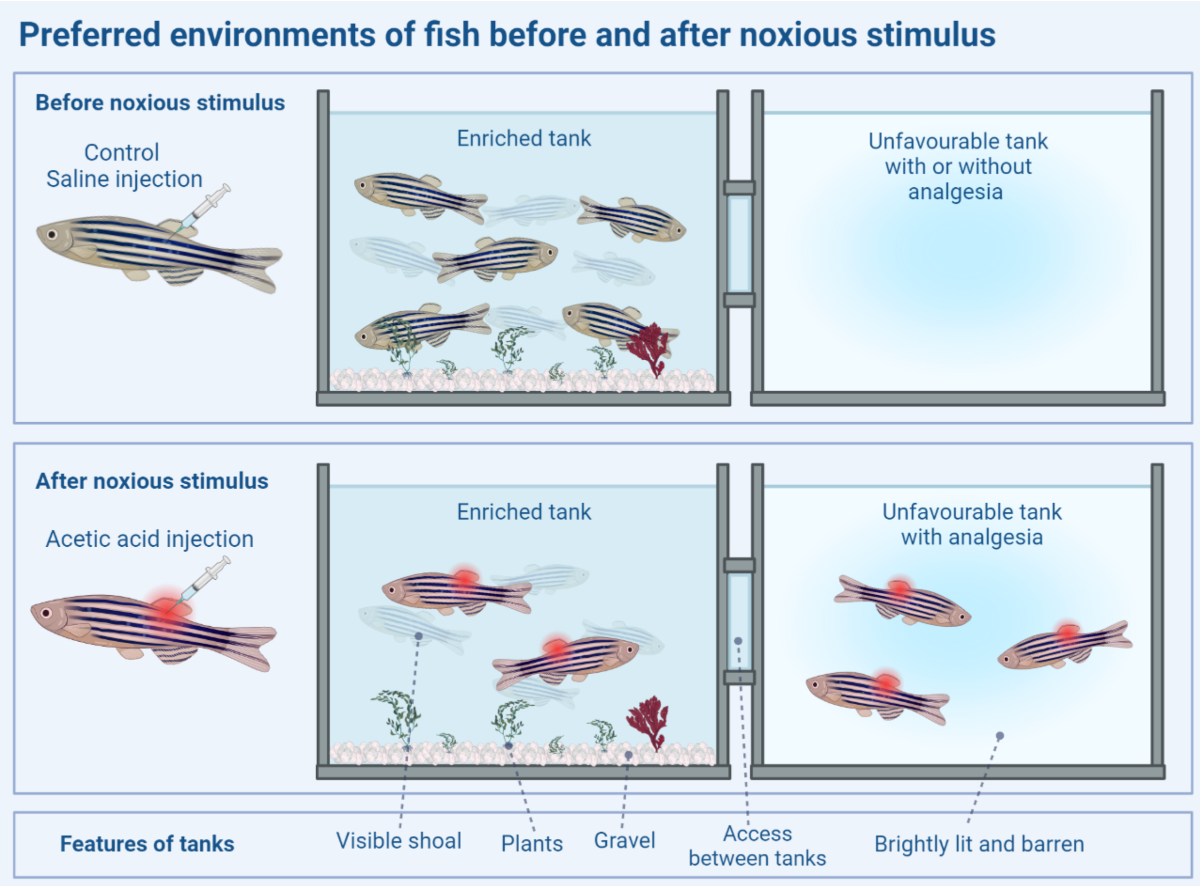
Analgesics

Many studies have shown behavioural evidence of analgesic drugs working in fish. Opioids are the most successfully tested drugs in fish so far, morphine (an opioid analgesic) has been tested in six different fish species and has shown to predominantly ameliorate pain-associated behaviours (such as rocking and rubbing motions) with very few side effects reported (Chadzinska et al., 2009; Lopez-Luna et al., 2017a; Newby et al., 2006, 2007, 2009; Nordgreen et al., 2013; Sneddon, 2003b). Buprenorphine has shown analgesic effects in zebrafish larvae by reducing behavioural and physiological changes (reversing heat hypersensitivity), this was furthered by the effects being reversible using naloxone (Curtright et al., 2015). Furthermore opioids, tramadol, dermorphine, and β-casomorphin were tested and shown to be beneficial in raising nociceptive threshold in carp, cod, and rainbow trout respectively (Chervova & Lapshin, 1997). Butorphanol has had mixed results, although this is likely evidence of poor uptake through immersion techniques (Schroeder & Sneddon, 2017) since it has shown analgesic effects via injection (Baker et al., 2013). Contrastingly, NSAIDs ketoprofen, ketorolac, and flunixin have had limited success in rainbow trout but were shown to be safe (Rizzo et al., 2017). Although aspirin has shown to reduce behaviour induced by acetic acid in zebrafish larvae (but not high temperatures) (Lopez-Luna et al., 2017a) and potential analgesic effects in adult zebrafish after fin clipping (Schroeder & Sneddon, 2017). Local anaesthetics are the least tested analgesic in fish, but lidocaine has shown to significantly reduce pain-associated behaviours in adult zebrafish (Schroeder & Sneddon, 2017) and showing similar potential effects in zebrafish larvae as well (Lopez-Luna et al., 2017a). Furthermore, cases of stress-induced analgesia have also been investigated in both adult zebrafish and zebrafish larvae, providing evidence for descending modulatory control (alike to mammals) (Lopez-Luna et al., 2017b; Maximino, 2011).

Complex pain-associated behaviours

Furthermore, more complex and sustained behavioural responses have also been studied. Both goldfish and rainbow trout were shown to avoid areas where they once received noxious stimulus (Dunlop et al., 2006), although these avoidance behaviours were inhibited once the fish are starved, providing evidence for a balance of these states (Millsopp & Laming, 2008). Fish refrain from eating after being given noxious stimuli, making self-administration experiments (such as those performed in mammals and birds) more difficult (Sneddon et al., 2014). However, a novel approach showed how zebrafish given a noxious stimulus will prioritise environments with analgesia present (even if they are less preferred), suggesting fish are willing to trade-off more preferred environments to potentially reduce pain (Figure 4; Sneddon, 2013). Other studies investigated the effect of noxious stimulus on selective attention, showing that trout will ignore fears of a new object when after stimulus (Sneddon et al., 2003), and appear to ignore predators (Ashley et al., 2009). Lastly, predatory stimulus has shown to potentially modulate pain by reducing impact of treatment on pain-associated behaviours (Alves et al., 2013). Overall, these studies suggest pain as a potentially important stimulus with the capacity to take priority over other stimuli and provide evidence for multiple mechanisms (such as descending modulatory control) for reducing pain in fish.

Figure 4. Zebrafish choosing less preferred environment for analgesia after noxious stimulus.
The control fish are shown to favour the enriched tank, whereas after injecting acetic acid, fish are shown to favour the tank with analgesia present. Figure made for the purpose of this review using BioRender with data from Sneddon (2013).



Physiological activity

There are many physiological factors associated with pain; however, like behaviour, these are especially difficult to differentiate with other stressors as triggers in fish. Therefore, not many studies assessing pain have used these as indicators. These include increased ventilation, heart rate, and endocrine changes like levels of catecholamines and corticosteroids (such as cortisol) (Barton, 2002).

Agricultural treatment

Welfare for fish is absent compared to both pigs and chickens. Although fish are included in general protection acts, there are no detailed protections for fish. This review has conducted a search of laws in the EU and UK (using their respective government databases) which protect pigs, chickens, and fish, these have been summarised in Table 1. Crucially, unlike pigs and chickens (which have specific rules around their space, transport, slaughter, etc); there are currently no specific legal requirements in agriculture for disease prevention among fish or for how fish are captured, kept, fed, transported, or slaughtered. Effectively, there is no evidence for pain management strategies in fish in actual legislation.

Table 1. UK and EU Laws in Agriculture Protecting or Providing Rights to Pigs, Chicken, and Fish. *Table created for the purpose of this review.*

Legislation		Pig	Chicken	Fish
General acts	To prevent unnecessary suffering and provide basic requirements	UK Animal Welfare Act, 2006 EU Council Directive 98/58/EC, 1998		
	Acknowledged as a sentient being	UK Animal Welfare (Sentience) Act, 2022		
Specific legal requirements for treatment	Disease protection and prevention	EU, Council Directive (EC) 2002/60 UK Regulation No 1894/2014	EU, Commission Delegated Regulation 2020/687 England Regulation 2006 No. 2701	None
	Feeding requirements	EU, Council Directive (EC) 2008/120	EU, Council Directives (EC) 2007/43 and 1999/74	None
	Space requirements	EU, Council Regulation (EC) No 1099/2009 EU, Council Directive 2008/120/EC	EU, Council Regulation (EC) No 1099/2009 EU, Council Directive (EC) 1999/74	None
	Transport condition requirements	EU, Council Regulations (EC) No 1/2005 and No 1099/2009	EU, Council Regulations (EC) No 1/2005 and No 1099/2009	None
	Slaughter condition requirements	EU, Council Regulation (EC) No 1099/2009	EU, Council Regulation (EC) No 1099/2009 EU Commission Implementing Regulation, 2018/723	None

Discussion

Limitations of pain indicators

Before addressing the objective, it is important to understand there are many limitations of behavioural indicators in pain, which form a significant portion of our understanding of non-human animal pain. The subjectivity of defining parameters to determine what behaviour ties to which state is an inherent issue in the external assessment of animals. Secondly, stress and illness are common in scientific and agricultural experimental conditions, since many indicators of pain can overlap with stress or illness (e.g., social isolation) it is difficult to assure the behaviours are pain-associated. Furthermore, behavioural studies are performed in experimental settings; hence, comparing their behaviour to agricultural or wild settings may not be valid. Other potential issues were mentioned by Prunier et al. (2013) which include lack of impartiality (potentially including anthropomorphism) and insufficient training and experience in observers. To combat these issues, more training could be done with observers of these studies and the use of blind protocols. Hence, reproducibility and validity should be an important factor of these studies. However, although these disadvantages should be considered, behavioural studies have many benefits. It is non-invasive, which could enable more studies to be conducted in less experimental conditions. Behavioural indicators often appear immediately in cases of (acute) pain. Furthermore, pain-associated behaviours can be specific and help to identify where the pain occurs (facilitating treatment). There are also limitations to physiological indicators in pain, as with behavioural, stress and illness can both cause similar indicators, making interpretation difficult. It is very common for handling of non-human animals to raise their heart rate, increase blood pressure, or other stress-reactions (Moberg, 2000). This has been specifically shown in pigs under constraint which have significantly increased cortisol and ACTH within minutes (Merlot et al., 2011). Similar effects have been shown in chickens with their heart rates (Glatz & Lunam, 1994). For these reasons, handling should be avoided where possible with the usage of remote techniques. Furthermore, physiological measurements require more equipment or potential surgery, which is more invasive and expensive, and might result in stress-response for the animals involved. This might make it more difficult to perform in agricultural or wild settings. However, physiological factors are very useful as indicators for providing a more objective measurement than behavioural, and for their use in testing the effects of analgesics. Lastly, analgesia experiments across species vary the indicators monitored (and analgesics used), making comparison less compelling. Overall, the main difficulty in assessments of pain in non-humans animals is that there is no way to validate that the physiological or observational parameters which are distinctively defined by each study are measuring pain capacity.

Limitations of this review

The main difficulty in the comparative assessment of pain capacity across species is the variety of research methods used in the studies. This makes it challenging to compare results when the same studies have not been performed with each animal. For instance, behavioural studies varied significantly in their assessment and setup between species. Furthermore, only certain analgesics had been tested in multiple of the animals. Additionally, fish and chickens both lacked studies using physiological indicators for pain compared to pigs. In pigs, much of the complex behaviours regarding pain is inferred from rodent studies. Furthermore, the understanding of mammalian pathways is also primarily determined through rodent studies; hence, there is a chance some similarities may not be applicable across mammalian species and that these differences have yet to be discovered.

Avoiding the issue of determining consciousness

It is difficult to compare non-human animals with humans in order to determine their pain capacity; establishing consciousness is complex with many pitfalls (Mason & Lavery, 2022). However, in this review, the objective avoids this issue by focusing on the current state of research in non-human animals, and their relative treatment in agriculture. Like fish, it is still impossible to ascertain whether a pig or chicken feels pain; however, the difference in ethical consideration is substantial (Table 1). This further emphasises the difference for fish welfare with no strict guidelines to-date; hence, fish are likely to be actively subjected to worse conditions and practices. The objective enables determining whether this discrepancy is warranted according to current pain research.

Overview of the results

The overview of potential pain factors across the three non-human animals showed a lot of similarity over both biological and behavioural studies (summarised in Table 2) and analgesics (Table 3). The main discrepancy in biological evidence is the lack of cerebral cortex in chicken and fish; however, there is no reason as to why mammalian neocortices would be essential for conscious experience or pain processing (hence, the inclusion of "Homologous … circuits" rather than "Neocortex" in Table 2). This view was supported by a group of prominent neuroscientists who signed a declaration to "unequivocally" state the neocortex is not essential for consciousness (The Cambridge Declaration of Consciousness, 2012). More importantly, this view is backed up by the scientific (and societal) consensus on birds experiencing pain (without a neocortex; Figure 5 shows a comparison of the brains). However, fish are still far behind in welfare policies despite substantial evidence for the potential of fish pain and the fact that fish brains are used as a model for human brains far more than bird brains.

Table 2. Simple overview of pain assessment in pigs, chickens, and fish.
✔ = Evidence from non-human animal studies supports this, ✖ = No evidence supports this. Table created for the purpose of this review.

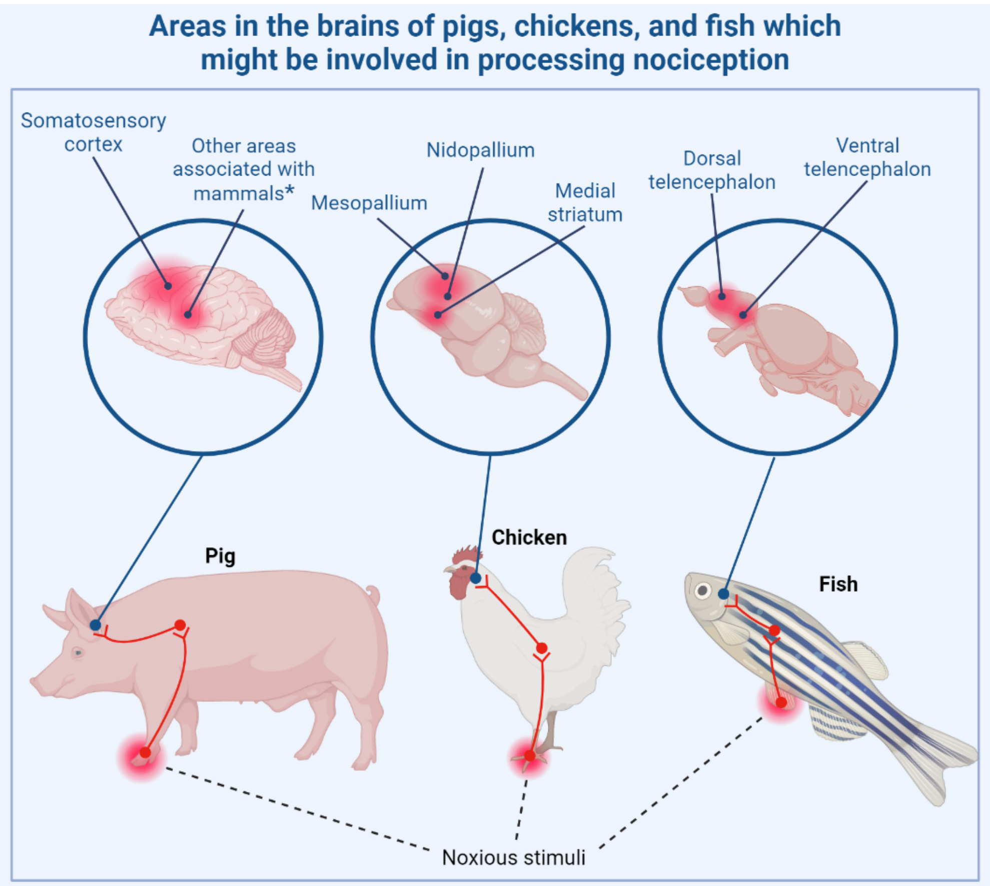
Potential factors which contribute to pain			Pigs	Chickens	Fish
Biological evidence associated with the processing of pain	Neuroanatomic pathways and structures.	Brain to periphery pathway	✔	✔	✔
		Analogous subcortical brain circuits	✔	✔	✔
		C and A-fibres	✔	✔	✔
	Molecular components for transducing noxious signals.	Three classes of nociceptor.	✔	✔	✔
		Opioid receptors.	✔	✔	✔
		COX1/2 enzymes.	✔	✔	✔
		TRP Channels	✔	✔	✔
		Substance P	✔	✔	✔
	Up/downregulating chemical components.	Hypersensitisation	✔	✔	✔
		Self-induced analgesia.	✔	✔	✔
	Integrated for emotional processing.	Higher activity during pain stimulation	✔	✔	✔
Identified behaviours in response to noxious stimulus	Avoidance/defensive behaviours.		✔	✔	✔
	Vocalisations.		✔	✔	✖
	Behaviours focused on the site of painful stimulus.		✔	✔	✔
	Behaviours aiming to reduce pain stimulation.		✔	✔	✔
	General change of activity (effect on feeding, etc).		✔	✔	✔

Table 3. Analgesics tested in pigs, chickens, and fish in pain assessment studies with comparable results. *✔ = Evidence exists to support this,* ✖ *= Evidence suggests otherwise,* **-** *= No experimental evidence. Table created for the purpose of this review.*

Analgesics shown to reduce pain-associated behaviours from a noxious stimulus		Pigs	Chickens	Fish
Opioids	Morphine	✔	✔	✔
	Buprenorphine	✔	-	✔
	Butorphanol	✖	✔	✔
NSAIDs	Aspirin	✖	-	✔
	Ketorolac	✔	✔	✖
	Meloxicam	✔	✔	✖
Local anaesthetics	Lidocaine	✔	✔*	✔

** Must be carefully administered, can be toxic.*

Figure 5. Brain areas potentially associated with pain perception in pigs, chicken, and fish.
Figure made for the purpose of this review using BioRender with results from imaging studies of pigs: Janjua et al. (2021); chickens: Douglas et al. (2018), Paul-Murphy et al. (2005); fish: Dunlop & Laming (2005), Nordgreen et al. (2007), Reilly et al. (2008).



** Studies identifying brain areas associated with a persistent noxious stimulus have not been properly performed in pigs (but have in other mammals).*

Addressing the objective: is fish welfare justified?

The identification of increased activity and gene expression in the forebrain/midbrain of fish during noxious stimulation (Figure 5) could potentially be evidence for the perception of pain. This evidence (amounted with the rest) has often been heralded for the precautionary principle, which would urge caution in areas lacking scientific understanding, as the burden of proof should fall on those taking action to prevent potential harm. The view that fish may possibly feel pain is substantial in the literature, with only a minority arguing strictly against the possibility. Hence, the welfare difference may highlight the slow process from science to policy, with pain research in fish being more recently published than that in mammals or birds. The same process occurred for birds, when increasing research and awareness grew for chickens, and so the policies eventually moved with them. Nevertheless, this shift is especially slow in fish, as emphasised by the European Commission statement in 2009 that "[t]here is now sufficient scientific evidence indicating that fish are sentient beings and that they are subject to pain and suffering notably when they are killed.". Furthermore, the American Veterinary Medicine Association (AVMA) regards the evidence as sufficient for fish feeling pain (AVMA, 2020). The recognition of fish sentience was further advanced by the UK's Animal Welfare (Sentience) Act 2022, which became the first government body to acknowledge it. However, although meaningful, these acknowledgements do not provide any protections for fish. The evidence discussed here highlights the discrepancy of welfare across the species and asks the question, first posed by fish pain researcher Victoria Braithwaite in 2006, "if not fish, why birds?".

"No cortex, no cry" and why fish might not feel pain

The primary argument against fish feeling pain is due to their brain differences (depicted in Figure 5), specifically, their lack of cerebral cortex (Key, 2015). However, aside from being contested by multiple realisability (the fact that there is more than one way for a biological system to be realised through evolution), birds also lack a cerebral cortex. Hence, if cerebral cortices are a requirement for pain, this still does not answer why there is a welfare difference between fish and chickens. This issue arising for either side highlights the need for further investigation in the welfare discrepancy between non-human animals. Additionally, as discussed in the introduction, nociception, and any detection/reaction to noxious stimuli is not sufficient to prove pain as this can be performed without central processing (e.g., in bacteria; Damasio, 2022). Therefore, linking higher activity and cognitive processing to noxious stimuli (Figure 5) is likely the best current evidence for indicating the possibility of pain perception. This idea holds equally true for pigs, fish, and chickens; although only fish and chickens have studies dedicated to this.

Future research opportunities for pigs and chickens

Challenges and Opportunities for Research in Pigs and Chickens

There is a lack of studies using motivational tasks for investigating pain in pigs. Hence, although it is likely they have complex pain mechanisms (as shown in rodents), studies would be beneficial. For example, studies testing the effect of analgesics on pigs shown to avoid locations associated with painful stimuli, or investigating highly motivated behaviour and the effect of analgesics like whether feeding-induced analgesia is possible in pigs (and reversible with naloxone). There are already examples of studies developing setups based on rodent studies for pigs (e.g., plantar stimulator; Sandercock et al., 2009). Compared to pigs, there is a lack of research in chickens measuring physiological parameters in pain studies which might help to further assess pain. Additionally, the majority of review literature assessing bird pain originates from exotic pet medicine, rather than agricultural studies; there are potentially differences in pain assessment between pet and factory farmed chickens which may need further exploring.

Opportunities for Pain Management Strategies in Factory Farms

Although pigs and chickens are far more ethically considered than fish, there are still many agricultural welfare issues that have yet to be investigated for pain management strategies. For instance, the abnormal behaviour of cannibalism is common in agriculture amongst both pigs (Henry et al., 2021) and chickens (Michel et al., 2022); although prevention strategies are investigated, investigations of this behaviour are underrepresented in the literature. Specifically, tail-docking and beak-trimming respectively are the most common procedures to combat this. Despite being banned, surveys still show an average of 77% of pigs being routinely tail-docked across the EU (de Briyne et al., 2018). Whereas beak-trimming is widely legal and common across Europe; hence, it is likely performed on the vast majority of chickens in farms. Although many aforementioned studies have been conducted for the pain assessment of these procedures, both are commonly performed without the use of analgesia; hence, this could be further investigated for both research and policies. Furthermore, it is common for a build-up of ammonia gas (from faeces) to cause lesions in chicken corneas or burn them (Kristensen & Wathes, 2000); however, although studies have demonstrated that chicken nociceptors respond to ammonia (McKeegan, 2004), no research has investigated potential analgesics for ammonia burns in chickens and other pain management strategies are still sparse. Hence, despite the more detailed policies dictating the legal treatment for both pigs and chickens, these issues still arise and raise ethical concerns for the welfare of factory farms and provide numerous areas for continued research.

Future research opportunities for fish

Defining Pain Indicators in Fish

Defining correct indicators for assessing pain in fish is a distinctive challenge due to their many physiological differences. More research is needed to fully understand the complexity of potential fish pain and how it compares to pain in other animals. A potentially more objective method in assessing pain via behavioural indicators in fish is to use machine learning models (as done with other behaviours like hunger; Iqbal et al., 2022).

Future Policies for Fish

The welfare of fish would benefit strongly with the inclusion of strict guidelines for their treatment and a focus on pain management strategies. Promisingly, the first symposium by the Fish Veterinary

Society in collaboration with Laboratory Animal Science Association brought aquaculture and fish practice experts together to discuss anaesthesia (Schroeder et al., 2021). Symposium stakeholders had general agreement for the addition of analgesia protocols for fish, and specifically, the provision of lidocaine to fin-clipped zebrafish was proposed (Schroeder et al., 2021). However, more discussions and awareness are likely needed for these ideas to be introduced into policy.

Conclusion

Summary

Overall, the current state of pain research in pigs, chickens, and fish was assessed, and their relative ethical treatment was considered. While there are many limitations to behavioural and physiological pain indicators, the studies provided important insights into the pain capacity of non-human animals. The biological and behavioural evidence across all three species showed significant similarities inferring a potential shared capacity for pain. Despite this, the ethical treatment of fish is lacking in agriculture.

Future outlook

Although the overall current outlook for ethical treatment of fish is concerning, awareness is growing, and policies should be expected (and urged) to improve over the coming years. However, more discussion and momentum for stricter welfare guidelines for fish is essential.

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