
Quantifying the Pain due to Keel Bone Fractures in Laying Hens

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Bone fractures are among the most serious welfare problems affecting laying hens in modern egg production [1–4]. Although many are the risk factors increasing the likelihood of fractures [4], decades of genetic selection for early sexual maturity, elevated and extended egg production have contributed to increased bone fragility due to the high calcium requirements for eggshell formation and the progressive loss of bone mass [2,4–7]. It has been estimated that a modern layer needs 30 times more calcium for the production of eggs than the calcium available in her body reserves [8]. Accordingly, fractures have become commonplace in the egg production industry, with most hens experiencing multiple fractures over their lives [9,10]. In severe cases, bone fragility can lead to the collapse of the spinal bone and paralysis [11].

In a natural environment, bone health is essential for survival and reproduction. Accordingly, osteoporosis and bone fractures are rare in wild birds [8]. In captivity, however, to the extent that they do not affect growth and productivity, ailments and fragilities are allowed to accumulate until the slaughter age. For example, by limiting mobility, the implementation of cage systems in the 1940s has also prevented the expression of behaviors and movements (e.g., jumps, short flights) that increase the likelihood of fractures in individuals with weakened skeletons. When fractures do occur, the resulting reduction in mobility does not prevent caged hens from eating, drinking, or laying eggs (nearly no displacement is required to perform these activities). Also, many fractures are sustained at a time when egg production is no longer affected: when caged layers are caught and handled during depopulation, transport (Chapter 7 [12]) and in the process of shackling directly prior to slaughter [5]. Because egg productivity is not compromised by fractures at these stages, there has been little financial incentive for the improvement of skeleton integrity in modern layer lines [13].

The keel bone (the prominent ridge that serves as the point of attachment for the wing muscles) is particularly prone to damage due to its anatomical position (Figure 3.1) and the low muscle mass in the breast typical of modern hybrid layer lines [14]. Additionally, modern hybrids begin laying before ossification of the keel bone is complete, when the caudal part (the last part to be ossified) is still fragile. Moreover, the large amounts of calcium required for eggshell production once the laying cycle begins exacerbate this fragility [2,15]. As a result, keel bone lesions (Figure 3.1) are observed in 50 to 99% of hens in commercial flocks [16–20].

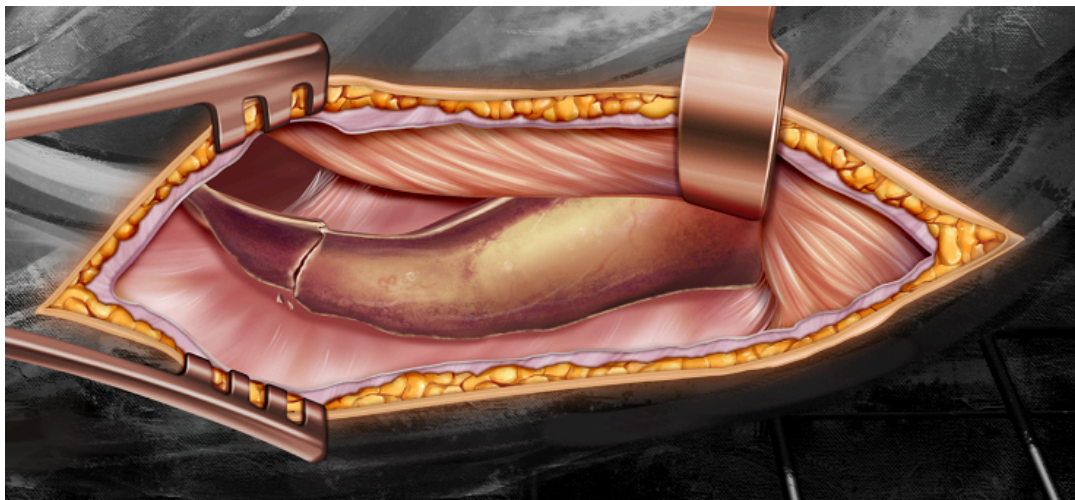


Figure 3.1. Illustration of a typical fracture of the keel bone in a laying hen. The fractured site is located in the caudal third of the bone, the most frequently affected region. For reference, the recreation of a surgery with access to the fracture site is shown in color. Illustration: Olga Kurkina.

Keel bone fractures compromise the welfare of laying hens in multiple ways. Solid evidence for the presence of pain in hens with fractures is available, as based on reduced mobility in fractured individuals, reversal of such reduced mobility with the administration of analgesics, and bird

preference for locations where the effects of analgesics had been previously experienced [21–25]. The pain and discomfort from fractures, in turn, may also inhibit the expression of behaviors that laying hens are strongly motivated to perform. There is even evidence to suggest that fractures induce a depressive-like state typical of situations of chronic stress [10].

In this chapter, we review existing evidence and propose a tentative scheme for the temporal evolution of the pain associated with keel bone fractures in commercial layers using the analytical framework described in Chapter 1 [26].

Evolution of keel bone fracture-associated pain

Damages to the keel bone include both fractures and deviations (i.e. the unnatural bending that may take place in response to long-term pressure on the keel bone, such as the pressure exerted during roosting [27]). Here we focus on the former, to the extent to which deviations also cause pain is not yet well established [2].

The pain of a fracture serves a useful function: by limiting movement, it protects the body from further damage and facilitates healing [28]. The fracture healing process consists of several phases. Initially, an inflammatory response is triggered, resulting in the formation of a hematoma and granulation tissue. Next, a soft callus is formed on the fracture site, consisting of cartilaginous or chondroid tissue. Then, ossification of the soft callus takes place to form a hard or bony callus [29], consisting primarily of woven bone tissue, which is then gradually replaced by lamellar bone tissue during the remodelling phase [27,30]. It is important to note, however, that there is not necessarily a direct correspondence between a specific healing phase and the intensity or duration of the pain felt. Within each of the healing phases, different levels of pain intensity may manifest. Likewise, the time an individual spends in pain may not necessarily correspond to the duration of the healing phase. For example, pain may cease well before or after the healing process is complete (e.g., chronic pain may persist long after the bone tissue has healed).

In the next section we propose a tentative scheme for the temporal partitioning of keel bone fracture-associated pain, where each time segment is characterized by a unique pain intensity pattern, as based on the categories of pain intensity defined in Chapter 1 [26]. Pain intensity and the duration of the healing phases depend on the characteristics of the fracture, including its location (e.g., tip, dorsal, caudal), type (e.g., transversal, comminuted) and factors aiding or impairing the healing processes (e.g., the degree of motion on the fracture site). However, existing evidence of fracture-related pain is not reported separately for fractures of different types, location or severity, or for animals with different mobility patterns. Likewise, there seems to be no data to date that enables distinguishing whether the healing process and corresponding pain patterns differ between laying hens housed in caged and cage-free systems. The proposed scheme represents, therefore, the pain experienced by a bird sustaining what is possibly an average fracture. The possible variation in pain intensity and duration is embedded in the uncertainty ranges proposed in each Pain-Track. Given the multifactorial nature of fracture-associated pain and the scarcity of studies on the subject, the simplifying assumptions made should be revised as knowledge advances.

Pain-Track representation: single fracture

There are three main documented outcomes associated with the clinical development of keel bone fractures in commercial laying hens (Figure 3.2). In most cases, the keel bone seemingly heals

completely some weeks following the fracture [9]. In the case of mild or moderate fractures, there is often neither evidence of substantial displacement of the bone nor major callus formation, with healing occurring by first (primary) intention (outcome A in Figure 3.2). In more severe cases (outcome B), substantial displacement (dislocation and/or angulation) of the bone can occur and healing will be indirect, or by secondary intention [21]. Finally, there is a proportion of fractures (15-20%, outcome C) for which healing is not observed due to non-union or delayed union of the bone, with a fracture gap still visible [9,21].

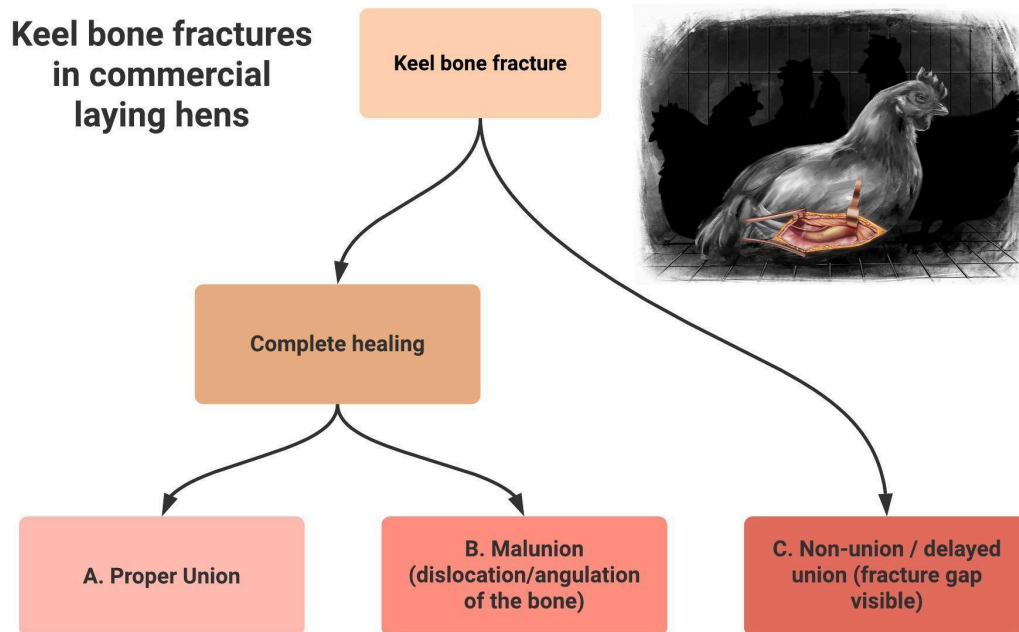


Figure 3.2. Documented outcomes associated with the clinical development of keel bone fractures in commercial laying hens housed in single-tier aviary systems.

In this section, we discuss each of these outcomes, initially focusing on the possible temporal evolution of the pain as a result of a single fracture that heals completely and properly (Pain-Track 3.1), to then represent fractures characterized by malunion, non-union/delayed union and the more realistic case of a laying hen that endures multiple fractures over her lifetime. The pain intensity probabilities and duration ranges depicted in the Pain-Tracks are justified in their corresponding sections. The probabilities associated with each category of pain intensity can be interpreted as the likelihood that pain is experienced at each category of intensity. They may also be interpreted (Chapter 1 [26]) as the proportion of the time experiencing pain at each level or, at the population level, the proportion of individuals experiencing pain at different levels (given expected differences in the severity and type of lesions). Duration ranges represent the uncertainty around estimates of duration (the interval where the mean values of duration are expected to lie). Since the Pain-Track is an attempt at organizing existing knowledge and fostering targeted research, the authors invite readers and specialists to join them in this effort by criticizing or refining the proposals.

Pain in Fractures with Proper Union

Pain-Track 3.1. Hypothesis for the temporal evolution of the pain associated with a keel bone fracture with complete and proper healing (outcome A in Fig.2) (for the definition of intensity categories, see Box 1.2 in Chapter 1 [26]).

	Acute phase: point of fracture	Acute inflammatory response			Subacute phase: callus formation
		i	ii	iii	
Excruciating					
Disabling	100%	80%	50%	30%	
Hurtful		20%	50%	70%	60%
Annoying					40%
	0.5 - 2 hours	1 - 2 days	1 - 2 days	2 - 3 days	2 - 12 weeks

Pain at the point of fracture and during the inflammatory response

The immediate pain following a fracture is mainly attributed to the activation of mechanosensitive nociceptors in the affected bone and surrounding tissues. In humans, this translates into a sharp, piercing pain as described by patients at the time of injury [31]. The intensity is most likely of a disabling nature, as sufferers are unable to perform other activities and strong analgesia is required. In humans, the time to the onset of pain is usually of five minutes [32].

In mammals, the periosteum has an extremely dense sensory and sympathetic innervation, in which most of the mechanosensory fibers activated immediately after fracture are believed to be located [33]. This pain-generating process can be reactivated by movements or mechanical distortions of the broken bone, which supports the mechanosensitivity role of the nerve fibers innervating the bone [34]. After the injury, an influx of inflammatory cells to the fracture site results in the release of inflammatory mediators that will cause the swelling of the site (hence further pain through activation of pressor receptors) and activate undamaged nociceptors [21,22,35]. Sensitization of the peripheral and central nervous systems may also occur, manifesting as hyperalgesia (increased sensitivity to pain) due to inflammation [22,35]. During this initial inflammatory phase, which in humans typically peaks within the first 24 to 48 h [29] and lasts approximately seven days, bone fractures are reported as acutely painful.

In young laying hens with fractured keel bones, the expression of inflammatory factors has been shown to be increased in the brain, liver, and abdominal muscle [36]. As in mammals, it is adaptive for birds to experience intense pain during the initial, inflammatory phase of a severe fracture: it ensures the reduction of movement needed to facilitate healing and protect the individual from further injury. Furthermore, decreased movement and increased rest time can help positively counterbalance the increased metabolic demand on the body [37–39]. Given the similarities between avian and mammalian nociceptors [40–42] we suggest that laying hens experience acute pain

immediately following a fracture, followed shortly by pain associated with hyperalgesia due to inflammation.

Although we are not aware of data indicative of the duration (and intensity) of pain in poultry in the inflammatory period immediately following a fracture, evidence from a model of post-fracture pain in pigeons indicates that this initial phase is severely painful in other avian species [35]. In this study, postoperative pain was evaluated for four days following the osteotomy of the femur (which was immobilized by intramedullary pins). Pigeons with no post-surgery analgesia spent significantly less time perching on the first postoperative day and marginally less on the second day compared with baseline. They also spent greater time lying on the floor on day 1, and marginally more on day 2. The time spent exploring was reduced, and resting time was higher, across all four days. Alertness also decreased following the surgery, with some animals showing little reaction to the presence of an observer. Analysis of limb position and subjective scores of overall pain suggested that birds that received 2.0 mg of meloxicam/kg were more comfortable than those with no analgesia or only 0.5 meloxicam/kg. Additionally, the daily position repertoire of birds in the high-dose group was similar to that before surgery, whereas the repertoire of birds with no or low dose analgesics differed from baseline during the first three postoperative days. The substantial changes in behavior and alertness of most birds relative to baseline on the first day (with some changes observed even in birds receiving higher doses of analgesia) are compatible with the definition of the disabling category (definitions in Box 1.2, Chapter 1 [26]), with the intensity of pain then gradually subsiding in the following days.

Because in farming conditions fracture sites are not immobilized, fracture-associated pain in laying hens is likely to last longer at more intense levels than those described for the pigeons. This is particularly true for keel bone fractures: because the keel bone anchors the wing muscles, further disruption of keel fractures (hence reactivation of mechanosensory fibers) can occur during activities such as flapping or perching, or even due to basic movements involved in respiratory motion [2].

In the time segments of Pain-Tracks 3.1 corresponding to the point of fracture and inflammatory response we provide a first attempt at translating these expectations into a hypothesis on the intensity and duration of fracture-associated pain at these stages. Since the healing process in birds is generally believed to be similar to mammals [43], we assume that the inflammatory period lasts from 4 to 7 days before initiation of soft callus formation. During this period, the duration of pain at the Disabling level is assumed to last longer than that estimated based on the experimental observations of pigeons, for the reasons previously explained (though the uncertainty range associated with the duration contemplates the possibility of a shorter period). However, because it would not be adaptive to endure pain at a Disabling level for too long (as it prevents animals from conducting activities critical to survival, such as eating), we restrict the time range where a Disabling pain is more likely to one to four days. Afterwards, pain is more likely to be of a Hurtful nature (still disrupts behavior, but not substantially). The hypothesis put forward is compatible with the observations of a longitudinal study that took weekly measurements of keel bone fracture status along with observations of behavior [44]. In that study, hens stayed in the nest for a longer time in the 10 days following a fracture, possibly as a result of the increased pain during oviposition and/or the propensity to hide in the nests to avoid locomotion or interaction with other hens.

Callus formation and remodelling

As inflammation decreases, symptoms of severe pain and functional impairment are expected to subside as the formation of a callus starts. In humans and animal models, the formation of a soft callus is carried out by chondrocytes and fibroblasts. Such cells are stimulated by the coordinated

expression of several growth factors, such as transforming growth factor- β (TGF- β 1 and β 2), platelet-derived growth factor (PDGF), fibroblasts growth factor-1 (FGF-1) and insulin-like growth factor (IGF), along with other elements that promote cell proliferation and cartilage formation [45]. These elements also participate in the activation of the painful path associated with the fracture [34], indicating that the molecular mechanisms involved in the early stages of bone repair are also involved in pain signaling.

Pain is thus likely to occur during hard callus formation and bone remodeling. To activate the systems that lead to new bone formation and remodeling, some biochemical mediators are required. Specifically, members of the Bone Morphogenetic Protein (BMP) family are involved, some of which have the ability to activate some pain pathways, as observed in animal models (rodents) of bone cancer [46]. The expression of BMPs at the injury site, as osteoinduction mediators, is often linked with persistent pain experienced by human patients with fractures, even when the fracture is already considered stable. However, in the final stages of bone remodeling, fractured individuals report nearly no pain and can perform their normal activities, including those involving weight or loads.

Evidence on the intensity of pain endured during the healing process in poultry, or birds in general, is more scarce. Considering the similarities in the fracture healing process in higher vertebrates, we tentatively suggest that pain during healing follows a pattern similar to that in mammals such that pain is most intense during the inflammatory phase. Subsequently, pain likely subsides gradually until the final stages of bone remodeling in cases in which healing is proper and complete and chronic pain does not develop (outcome A in Figure 3.2). We expect that once a soft callus starts forming (approximately 1 week after a fracture) the healing process will last on average 7 weeks. This estimate is based on a recent longitudinal study of keel bone damage in 150 laying hens using an improved method of radiographic evaluation [9], which reported that 85% of keel bone fractures took an average of 7 weeks to heal. Although healing may take considerably longer in some individuals, in this study less than 11% of complete healed fractures required between 12 and 32 weeks. We, therefore, assume that a uniform range of 2 to 12 weeks should describe the healing period appropriately for most fractures. The minimum estimate of 2 weeks is based on a study that used radiography to monitor the healing process of naturally occurring keel bone fractures in hens [21]. The wide uncertainty interval allows for variability across individuals, as well as the uncertainty due to the inference being drawn from two studies only. These duration patterns are proposed for the callus formation and remodeling segments of Pain-Tracks 3.1 to 3.3, and apply to outcomes A and B (Figure 3.2), in which the keel bone seemingly heals completely, either with proper union of the bone (outcome A) or where substantial displacement is present (outcome B).

Pain in Fractures with Malunion

In bones that are properly healed, with no major displacement of the bone, no further pain is expected and the bone should function as efficiently as prior to breaking. With effective bone healing, nerve growth factor levels decrease and the new local nerve fibers that had sprouted as a result of the fracture are "pruned back", resulting in normal bone innervation and non-sensitized nociceptors [47].

However, in those cases characterized by important displacements of the keel bone (typically classified as severe [21]), there is some evidence to suggest that pain might still be present after a fracture line is no longer visible (outcome B in Figure 3.2; Pain-Track 3.2). In a series of studies, Nars and colleagues [22–25] provide compelling evidence for the presence of pain in hens with suspected healed keel fractures characterized as severe [25]. In their experiments, fractures were diagnosed by

the detection of an already formed callus (hence after the inflammatory phase) and behavioral observations were collected five weeks after callus detection. Although foraging was not affected in fractured hens (hence indicating that pain was unlikely of a disabling intensity), hens with fractured bones took greater time to complete a runway test, had greater latencies to fly down from perches, spent more time sleeping on the floor and resting on the nest, being less active overall [25]. Reduced mobility in hens with severe fractures was partially mitigated by treatment with the non-steroidal anti-inflammatory, meloxicam, and, more effectively, with the opioid butorphanol [23] (an indication that pain was more intense than a simple annoyance). There was also a conditioned preference for a location associated with the administration of butorphanol in hens with healed keel fractures [24], suggesting that this location was associated with a positive affective state induced by the analgesic drug. Where the results were disaggregated according to the severity of the fracture [25], hens with more severe fractures were less likely to access a perch at 50cm, took greater time to reach an obstacle, as well as a greater time to fly from the ground to a perch and to land from a perch to the ground. In a more recent experiment conducted in a commercial setting (multi-tier aviary), Lohmann Brown (LB) hens with more severe fractures were observed to spend more time in the top tier and less time in the litter zone and lower tier of the aviary (potentially due to the avoidance of proximity to conspecifics and agonistic interactions) [48]. However, no association between fracture severity and behavior was observed for Lohmann Selected Leghorns, LSL [48], though fracture severity was in general lower for LSL than LB hens. Mobility patterns, as measured by the number of transitions between aviary zones, were not affected by fracture severity for any of the breeds.

Pain-Track 3.2. Hypothesis for the temporal evolution of the pain associated with a keel bone fracture where healing is complete but with substantial displacement (mal-union) of the bone (outcome B in Figure 3.2) (for the definition of intensity categories, see Box 1.2 in Chapter 1 [26]).

	Acute: point of fracture	Acute: inflammation			Subacute: callus formation & remodeling	Chronic phase
Excruciating						
Disabling	100%	80%	50%	30%		
Hurtful		20%	50%	70%	60%	30%
Annoying					40%	70%
	0.5 - 2 hours	1 - 2 days	1 - 2 days	2 - 3 days	2 - 12 weeks	8 - 42 weeks

The behavioral changes observed in the studies described are compatible with a proportion of the hens experiencing pain at the hurtful level in the chronic phase (definitions in Box 1.2, Chapter 1 [26]), as based on the presence of disruption of some (but not all) of the behaviors observed, which in turn was mitigated with analgesic drugs. However, we find it unlikely that pain was sustained at the hurtful level most of the time, or by most of the hens (hence the <50% likelihood of chronic pain at the hurtful level in the chronic phase in Pain-Track 3.2), since there was no significant difference in the frequency of behaviours that require movement (e.g., foraging and walking) between hens with and without keel bone fractures [25]. Additionally, no major changes in mobility pattern were observed in other commercial settings [48], an observation compatible with prior studies showing that the mean number of aviary-housed hens in the open litter area was not different at the end of lay (at a time likely associated with a higher proportion of hens, sustaining a higher number of fractures) compared to mid or peak lay [49]. Where present, the behavioral changes were observed for several weeks, thus

chronic pain may be present until the end of the production cycle. Considering that healing takes an average of 8 weeks (1 week in the inflammatory phase and an average of 7 for callus formation; Pain-Track 3.1), that the average onset of fractures lies somewhere between 30 and 45 weeks of age (a wide range to account for uncertainty across individuals, breeds and settings) [9,44,50], and a laying cycle of 60 to 80 weeks, hens sustaining fractures with this outcome can experience chronic pain anywhere from 8 to 42 weeks.

In a detailed longitudinal study of keel bone fractures using radiographic evaluation [9], spontaneous fracture healing led to malunion of the keel bone in most cases, with 35% of fractures showing dislocation, 53% displaying angulation and 24% possessing both. This outcome was expected due to improper stabilization and the widespread prevalence of osteoporosis in layer flocks. If these findings can be generalized, an expected 50-60% of fractures will be associated with malunion (outcome B in Figure 3.2), increasing the likelihood of chronic pain.

Pain in Fractures with no evidence of healing (non-union/delayed union)

When the fracture heals properly, pain decreases with soft callus formation and the bone bridge disappears with bone repair [51]. However, fracture healing is incomplete (or slower) in some cases and a clear fracture gap remains visible. In a study that used radiography to monitor the healing process of naturally occurring keel bone fractures in hens [21], 20% of the ten birds diagnosed with new fractures did not achieve complete healing by 42 days after fracture diagnosis. Baur and collaborators [9] also observed that 16% of fractures did not show evidence of healing for several months, until the end of the production cycle. The observed failure of fractures to heal in some birds may be due to the severity of damage (e.g., too large fracture gap due to extensive fragment dislocation) and persisting motion that is typical in commercial settings. This circumstance can lead to a delayed union or an atrophic, or hypertrophic, non-union of the bone fragments [9]. Additionally, most laying hens are affected by osteoporosis [5,52], a condition that involves loss of structural bone. This not only increases the incidence of fractures, but also interferes with bone healing.

Cases of nonunion or delayed union of a bone (outcome C in Figure 3.2) perpetuate the inflammatory process [53]. In such cases, callus formation is not complete (Pain-Track 3.3), and pain is likely to persist due to the constant mobility of the fractured site and the chronic nature of the inflammatory process. In mammals, ectopic and exuberant sprouting of sympathetic and sensory nerve fibers and the formation of neuroma-like structures are involved in the establishment of persistent pain in bones with impaired healing [54], a process that may find parallels in birds. In Pain-Track 3.3 we represent the proposed temporal evolution of pain in such cases. Instead of considering a chronic phase of pain once callus formation is complete (as in Pain-Track 3.2), we assume that the process of callus formation is perpetuated chronically. In such cases, the greater lack of stability and persisting damage to the periosteum suggest that the pain endured is likely greater than in cases of malunion [55]. The length of time during which the hen experiences pain is thus given by the subtraction of the age at depopulation from the onset of the fracture. For layers depopulated at 60-80 weeks, this corresponds to approximately 7-42 weeks, considering the onset of the fracture at approximately 30-45 weeks of age.

Pain-Track 3.3. Hypothesis for the evolution of the pain associated with a keel fracture with incomplete healing (non-union/delayed union) and visible fracture gap (outcome C in Figure 3.2)

	Acute: point of fracture	Acute: inflammation			Subacute: callus formation
Excruciating					
Disabling	100%	80%	50%	30%	
Hurtful		20%	50%	70%	60%
Annoying					40%
	0.5 - 2 hours	1 - 2 days	1 - 2 days	2 - 3 days	7 - 42 weeks

A more realistic picture: multiple fractures

Previous research suggests that hens sustain multiple keel bone fractures over the laying cycle, with an average of three fractures per keel bone [9,56]. Therefore, to depict more realistically the cumulative pain experienced by the average laying hen as a result of keel bone fractures, it is necessary to consider successive fracture events over her lifetime. Here, we consider the case of the average hen, sustaining three fractures over the laying period, and adapt Pain-Tracks 3.1-3.3 to the case of successive fractures in the same bone.

Four scenarios are considered, depicted in Figure 3.3:

- Scenario I represents the possibility that the intensity of the pain experienced in the keel bone is not affected by subsequent fractures, nor is the pain associated with new fracture sites added to the pre-existing pain from the prior fractures. Here, because all fractures are sustained in the same bone (and often in the same region), birds would not be able to differentiate three sources of pain, but instead would experience only one painful sensation emanating from the keel bone. For example, in the case of a bird with chronic pain following a first fracture, the pain emerging from a second fracture would simply ‘replace’ the pre-existing chronic pain.
- Scenario II postulates that the perception of pain is independent for each new fracture, with the pain at each fracture site adding to the pre-existing pain (as if they were felt in different anatomical locations), but not potentiated by it.
- Scenario III is similar to Scenario I in that individuals do not differentiate multiple sources of pain emerging from the keel bone, but instead experience one single painful sensation. However, it considers that, with each new fracture, the perception of pain increases due to sensitization caused by prior injuries.
- Scenario IV similarly considers that pain perception increases with each new fracture due to the sensitization caused by prior injuries, but in this case the pain from each fracture site is perceived as an independent source of pain.

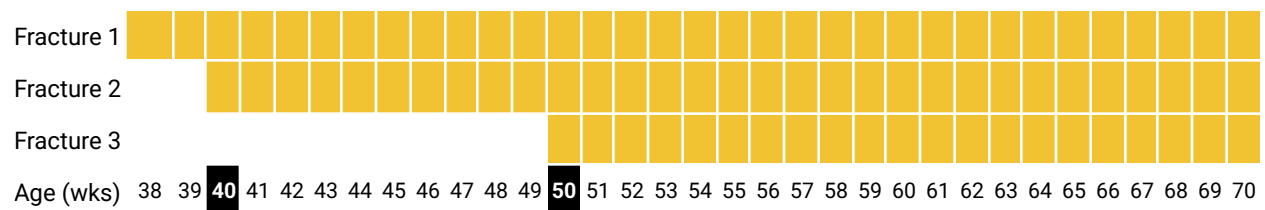
In all cases, we make the simplifying assumption that the first fracture is endured at 30 weeks of age, with 10 weeks in between each fracture. This assumption is in line with evidence suggesting that the likelihood of fractures is reduced after 45 weeks of age [57,58]. Following the first fracture at 30 weeks of age (average onset of fractures [9]), cases of chronic pain first develop on average 8 weeks later (the duration of healing; Pain-Tracks 3.1-3.3).

Figure 3.3. Scenarios considered for the possible evolution of pain endured by a laying hen with three successive keel bone fractures, sustained at 30, 40 and 50 weeks of age. For simplicity, the laying cycle is shown from week 38 onwards (as following the first fracture at 30 weeks of age, cases of chronic pain will develop on average 8 weeks later). Darker colors indicate higher pain intensity (though no correspondence with the discrete intensity categories defined in Box 1.2 of Chapter 1 [26] is attempted here). Depopulation is assumed to occur at 70 weeks of age.

Scenario I. The perception of pain intensity is not changed by subsequent fractures on the same bone. Chronic pain continues unaltered until depopulation at 70 weeks.



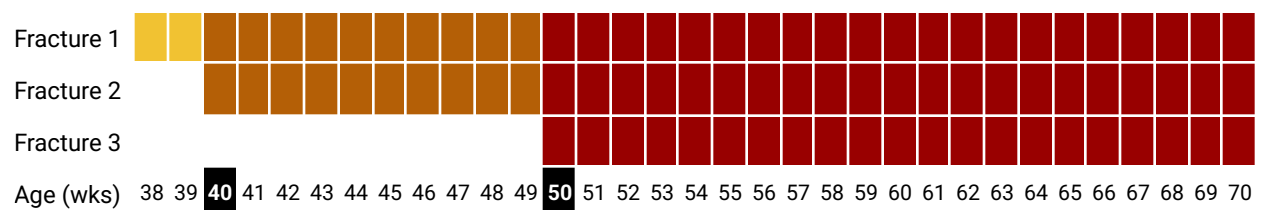
Scenario II. The perception of pain is independent for each new fracture, even if all fractures are sustained in the same bone. Chronic pain persists in all cases until depopulation.



Scenario III. With each new fracture, the perception of pain increases due to sensitization caused by prior injuries. However, since all fractures are sustained in the same bone, the resulting pain is not felt separately for each fracture.



Scenario IV. With each new fracture, the perception of pain increases due to the sensitization caused by prior injuries. Sensitization affects all fracture sites. Pain from each fracture is perceived independently.



Research in humans and animal models of fracture suggests that prior injuries at a certain site associated with chronic pain can sensitize the area and lead to more intense pain with subsequent trauma. This happens through a number of mechanisms. As discussed, malunion, non-union or delayed union of a bone can perpetuate the inflammatory process, thus perpetuating inflammatory-associated pain. The cellular and humoral processes typical of any fracture are prolonged when there is no stabilization of the fracture and the activity of the osteoclasts is

maintained over time, which contributes to inflammation and pain [59]. Additionally, there is also evidence to suggest that when an individual develops chronic pain due to a poorly or improperly healed fracture, the classic nociceptive component is no longer the only factor involved in the genesis of pain. Furthermore, the neuropathic factor appears due to the ectopic sprouting of sympathetic and sensory nerve fibers mediated by nerve growth factor [47]. These nerve fiber sprouts appear to be in part responsible for the peripheral sensitization that occurs after tissue injury and can lead to the appearance of hyperalgesia and allodynia (when non-noxious stimulation of the bone is perceived as noxious). In general, the density of sensory innervation of the bone is correlated to the severity of pain [54], so if a new fracture occurs in a previously broken and poorly healed bone that developed chronic pain, the pain will most likely be worse.

Besides peripheral sensitization and the sprouting of new local nerve fibers, central sensitization may also occur and exacerbate the pain by activating painful pathways that do not normally function as such. While peripheral sensitization occurs in a local environment of inflammation, central sensitization no longer requires the local inflammatory process to generate pain, even at a distance (secondary hyperalgesia) [60]. Central sensitization can cause hypersensitization of areas adjacent to the injury [61], as well as pain independent of the presence or intensity of peripheral stimulus [62], and tissues that are not injured (referred pain [63]). Clinical trials in animals and humans have shown that certain mechanical and thermal stimuli that are usually tolerable can be painful in individuals with prior injuries, even when applied far from the injury site [64,65].

In light of this evidence, the perception of pain is likely intensified in subsequent fractures due to the peripheral sensitization and central sensitization of the bone (a possibility in line with Scenarios III and IV), even if the clinical evolution of the inflammatory process (in terms of duration) is unaffected. However, because all fractures are sustained in the same bone (often in the same region - the caudal third), we assume that Scenario III matches more closely the perception of pain associated with keel bone fractures. The extent to which these assumptions apply to laying hens in commercial settings remains to be determined. We translate Scenario III into a Pain-Track model of three fractures (Pain-Track 3.4).

Pain-Track 3.4. Hypothesis for the temporal evolution of the pain associated with three successive keel bone fractures (average number of keel fractures endured by hens in cage-free production). The Pain-Track is based on Scenario III (Figure 3.3). The duration of the acute phases are fixed (based on Pain-Tracks 1-3), whereas the duration of the subacute and chronic phases reflects the remaining time until a fracture occurs or until depopulation.

	Fracture 1 (at 30 weeks)						Fracture 2 (at 40 weeks)						Fracture 3 (at 50 weeks)					
	i.	ii.	iii.	iv.			i.	ii.	iii.	iv.			i.	ii.	iii.	iv.		
	Acute: fracture	Acute: Inflammatory Response	Sub-acute	Chronic	fracture	e	Acute: fracture	Acute: Inflammatory Response	Sub-acute	Chronic	fracture	e	Acute: fracture	Acute: Inflammatory Response	Sub-acute	Chronic		
Excruciating																		
Disabling	100%	80%	50%	30%			100%	90%	60%	50%			100%	90%	60%	50%		
Hurtful		20%	50%	70%	60%	25%		10%	40%	50%	70%	33%		10%	40%	50%	80%	36%
Annoying				40%	45%						30%	58%					20%	61%
	.5- 2 hrs	1-2 d	1-2 d	2-3 d	2-9 wks	0-7 wks	.5- 2 hrs	1-2 d	1-2 d	2-3 d	2-9 wks	0-7 wks	.5- 2 hrs	1-2 d	1-2 d	2-3 d	2-12 wks	7-17 wks

Based on the findings of Baur and colleagues [9], mild or moderate fractures that heal completely, with no dislocation or angulation (hence no chronic pain once healing is complete) should represent approximately 25-35% of all keel fractures (outcome A, Figure 3.2)¹. Thus, for every fracture we consider a 30% probability that chronic pain does not develop. Similarly, we assume a 55% probability of mal-union (outcome B, Figure 3.2) and a 15% probability that healing is incomplete (outcome C, Figure 3.2). These probabilities are justified in the previous section. Therefore, the probability that chronic pain does not develop after 1, 2 and 3 fractures (0.3^1 , 0.3^2 , 0.3^3) is respectively 30%, 9% and 3% (thus, in Pain-Track 3.4 the sum of probabilities in the chronic phase does not add up to 100%, under the assumption that 30%, 9% and 3% of the birds with respectively 1, 2, and 3 fractures will have no chronic pain). The remaining percentages (70%, 91% and 97%) represent chronic pain either as a result of malunion (outcome B) or delayed/non-union (outcome C). To determine the intensity of pain in the first chronic phase, we calculate the average of the intensity of chronic pain for outcomes B and C, weighted by their corresponding prevalence². The duration of the chronic phases represent the remaining number of weeks in chronic pain until a new fracture occurs, or until depopulation. Finally, the intensity of the pain in subsequent fractures is assumed to increase, but only to an extent compatible with the behavioral evidence discussed previously. For example, although pain is still assumed to reach a disabling level immediately after the fracture event, such an intensity is unlikely to be present in the subacute and chronic phases, as based on observations that hens continue foraging and exploring the environment until the end of the production cycle.

However, evidence on the average number of fractures endured by hens in caged systems, their severity and healing times is extremely limited to date. To our knowledge, there has been only one report on the number of fractures endured by hens in furnished cages, where the author observed up to eight to ten fractures in one bird [15] - a number of occurrences compatible with that reported for non-cage systems [9]. Similarly, evidence is still lacking on whether the healing process and corresponding pain patterns differ between hens housed in caged and cage-free settings. If on the one hand hens are less mobile in cages (reducing the likelihood of traumatic injuries and further damage during healing), on the other bone strength is impoverished, a risk factor for longer and deficitary healing. Additionally, it is possible that in cage-free environments, where multiple and competing incentives are available, the salience of pain - and possibly the duration of the healing process itself - is lower relative to barren conditions (Chapter 9 [66] and references therein). Further research is critically needed to address these possibilities.

¹ In this study, 50-60% were associated with malunion (35% of fractures showed dislocation, 53% angulation and 24% both), and 16% with nonunion/delayed union, for a total of approximately 65-75% with improper healing (hence the 25-35% estimate of complete and effective healing).

² For example, to determine the likelihood that pain is hurtful in the chronic phase following the first fracture the following calculation is performed: $(30\% \times 55\%) + (70\% \times 15\%) = 25\%$. After the second fracture, an additional 21% of the individuals are assumed to develop chronic pain, of which 16% as a result of malunion, and 5% as a result of non-union/delayed union. Here, the likelihood of pain being hurtful is: $25\% + (30\% \times 16\%) + (70\% \times 5\%) = 33\%$. Finally, after the third fracture, an additional 6% of birds will develop chronic pain, of which ~4% due to malunion and ~2% due to delayed union. Hence, Here, the likelihood of pain being hurtful at the chronic phase after 3 fractures is: $33\% + (30\% \times 4\%) + (70\% \times 2\%) = 36\%$.

Burden of Pain in Affected Individuals

In this section we quantify the burden of pain (as measured by the time spent at each level of pain intensity) endured by laying hens as a result of keel bone fractures. The results concern the duration of pain experienced by a fractured individual (independently of how many individuals are fractured). The assumption here is that the pain of a severe keel fracture is the same regardless of whether it is endured in a cage or cage-free environment (but see Box 9.4 in Chapter 9 [66] for a discussion of the possible analgesic effects of the distraction effects provided by cage-free environments).

Only hours spent awake (16 hours per day) are considered (e.g., if pain is estimated to last 1-2 days, this translates into 16-32 hours of pain). Pain-Track 3.4 is used as the baseline scenario, reflecting the most likely estimates for the time endured at each category of pain intensity, as discussed throughout this Chapter. The results presented in Figure 3.4 consider the uncertainty regarding intensity of pain and duration of each healing phase, as depicted in Pain-Track 3.4. However, if the reader wants to change any of the assumptions regarding the durations and intensity of the pain depicted in this Pain-Track, an interactive web application can be used for this purpose, available at <https://pain-track.org/hens>. In the web application, changes in any of the parameters will immediately update the estimates on the time in pain endured as a result of the fractures.

Overall, fractured layers are expected to experience an average of approximately 159 [143-334] hours of disabling pain, 2,248 [1617-2879] hours in hurtful pain and 1,812 [1312-2312] hours in discomfort (annoying pain) as a result of three keel fractures. These times correspond to a substantial fraction of the laying cycle (5,600 hours, considering an average cycle of 70 weeks, the onset of lay at 20 weeks, and 16 active hours per day). As we discuss in this book, such a burden makes keel fractures the greatest source of physical pain that laying hens endure during their lives.

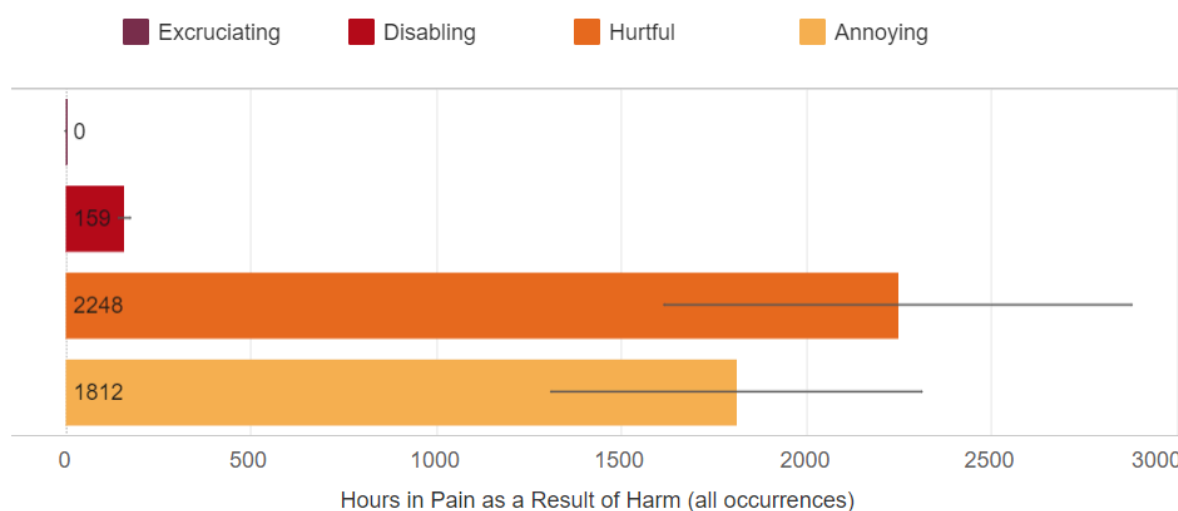


Figure 3.4. Time endured at each level of pain intensity (daylight hours) as a result of 3 fractures of the keel bone. Error bars represent 95% confidence intervals.

Research Gaps

- ❑ Evidence on the duration and intensity of the pain endured as a result of a fracture in poultry, or birds in general, is scarce. In laying hens, research on indicators of fracture-associated pain has been restricted predominantly to hens with old, healed, fractures in cage-free settings. There is currently no direct data on the duration (and intensity) of pain in poultry in the acute phase following a fracture, or how the severity, location and number of fractures affect pain intensity and the healing process [2,21,67].
- ❑ There is also no data that enables distinguishing whether the healing process and corresponding pain patterns differ between hens housed in caged and cage-free settings. Existing evidence is predominantly restricted to cage-free environments, though several factors associated with healing (e.g., mobility, bone strength, perch use) differ between cage and cage-free birds. Importantly, it is possible that in more complex environments, where multiple and competing incentives are available, the salience of pain - and possibly the duration of the healing process itself - is reduced relative to that experienced in barren conditions (Chapter 9 [66]). This hypothesis has not yet been formally tested.
- ❑ To our knowledge, there is no recent data on the average number of keel fractures endured by hens in conventional cages in commercial settings, their nature and timing.
- ❑ It is possible that damage to the keel bone may have detrimental effects on the metabolic and thermoregulatory capacities of hens [2], since it is used as a site of muscle attachment for respiratory motions, which may be impaired due to pain or restricted mobility. Necropsy studies of random samples of (seemingly healthy) hens at the end of lay should help establish the association between keel fractures and lesions indicative of other morbid conditions.

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