There was a need to develop accurate and effective methods for assessing pain in birds in general and in chickens in particular, as chicks are laboratory animals that are easily raised and dealt with, as well as having neurological components to interact and respond to pain the same as in mammals. Birds have physiological and/or behavioral signs of pain. The physiological signs of pain include the change in heart rate, respiratory rate, and blood pressure, and the flapping of the wing. The behavioral signs of pain that extend from the withdrawal response, calling, and the flapping of the wing. This article focused on the methods of pain assessment in birds in the published literature that varying according to the type of stimulus. However, this review offers information on pain assessment approaches and associated behavior, which can enhance specialists' understanding of the pain causes and, thus, we can evaluate the analgesic drugs for pain in chicks.

Keywords: Chicks, Pain assessment, Behavior, Analgesia.
to when publishing in vivo models in high-quality journals [13].

The basis for these requirements and guidelines is based on the 3Rs (replacement, reduction, refinement) concept. In accordance with the replacement theory, the use of live animals should be substituted, where possible, by in vitro or computational methods and, if applicable, the use of inanimate objects or less reactive animals should be favored. Nonetheless, due to the complexity of the behavioral tests, replacement or substitution of animals for non-sensitive items is difficult in a pain study. The importance is therefore also on the number of animals needed to collect data, and optimization of the method with the aim of reducing the amount of pain caused to the animal. A variety of methods can be used to do this. For example, improving the homogeneity of data and improving statistical power[14], which result in fewer animals needing to attain the necessary level of confidence[15]. Likewise, steps to enhance the reliability of data, including adequate randomization and blinding methods, are crucial to assuring the accuracy of the study obtained. Therefore, care must be taken in designing experiments that reduce pain and suffering. It involves reducing model duration, replacing painful model substances with those that cause shorter lasting noxious stimuli, or lowering the doses of drugs administered. In order to minimize the experimental repetition and excessive utilization of animals, particular consideration should also go to the timely publication of data, whether positive or negative results.

Pain tracks
A harmful trigger is the one that will injure the tissue, and a nociceptor is a receptor responsive to an unpleasant or effectively noxious stimulus.

The pathways of pain involve dual processes [16]:
- A peripheral pathway including recognition and conduction of evidence about possible tissue hurt
- A central pathway leading the brain reacts to this evidence.

Peripheral Nervous System
There are 3 kinds of pain receptors that have been recognized in avian species[17]

High-threshold mechanothermal nociceptors
Nociceptors are receptors for polymodal hurt since they have now over one task [18]. They react to heat above 40°C and mechanical stimulus and have been recognized in doves[19], ducks[20], and poultry[18]. Impulse conduction is very sluggish and comparable in these nociceptors to that of mammalian unmyelinated C fibers, which originate from pain-transmitting skin free-nerve endings. It raises the number of responses by increasing the magnitude of the stimulus. Some fibers display a continuous response up to the maximum temperature measured (56°C), whereas other fibers show a clear peak response at a lower temperature, and a decreased response results in an increased stimulus strength above that temperature [21].

Mechanical nociceptors
Mechanical nociceptors respond to extra compression or mechanical twist. They also respond to cuts that disruption the skin. It resembles the myelinated A δ nerve fiber and unmyelinated C nerve fibers derived as of free-nerve cutaneous terminals in mammalian species; It has been documented in hens [22] and waterfowl [20]. These mechanical nociceptors frequently have polymodal characteristics, Consequently it is likely that various of the transducers for thermal stimuli are the similar for mechanical stimuli[17-21].

Thermal nociceptors
Thermal nociceptors are stimulated by harmful heat or coldish at the degree of the different temperatures. These receptors stimulated without mechanical reaction in a way like polymodal nociceptors [23]. Thermal nociceptors may be Aδ or C nerve fibers and have been reported in pigeons [19] and hens [22]. These receptors seem to be less responsive to cool than the similar receptors in mammalian species, but the threshold for hotness receptors in bird species appears to be higher than in mammalian species. This isn’t unexpected since the body and cutaneous temperatures in chicks are greater (41 ° C to 42 ° C)[19]; After all, when compared nociceptor physiological responses in hens with those in mammalian species , The properties of the discharge and the scale of the feature vector are quite equivalent[22].Damage to the tissue and severe soreness may affect mammalian central and peripheral nervous systems and change response to external stimulus[17].

Pain sensitization
Central sensitization
A mechanism called wind-up makes central sensitization develops, leaving the affected portion of the brain in a high reactivity state.
This high reactivity decreases the threshold for what generates pain and contributes to pain retention, frequently after the initial injury has improved [16].

Peripheral sensitization

Occurs after tissue injury induces a pH fall and the discharge of C autacoids that are responsive to minor unmyelinated C nerve fibers. The pain receptors react to a broader variety of stimuli or reply unusually to some kind of persistent stimuli[16] Prostaglandins (PGs) may further alter C fiber activity in mammalian and avian kinds by reducing starting point at the damage to the tissue and inflammation[24]. Furthermore, eicosanoids and prostaglandin E1 and E2 sensitize thermal and mechanical stimulation of the small-diameter sensory nerve fibers. Substance P tends to be an essential mediator of inflammation Components, and its discharge leads to vasodilatation in the circulatory system and additional excitation of the C fiber peripherally [25]. Exposure to prostaglandin E2 in chick sensory nerves causes a dose-dependent rise in the production of substance P over inward Ca influx [24].

Central Nervous System

Bird soreness signals are conveyed from receptors via numerous ascending spinal pathways to many regions of the midbrain and forebrain. In mammals, the dorsal horn of the spinal cord has 10 layers. Both cell size and spread can be distinguished, while hens possess 6 layers[22]. The pain signal is conveyed to the dorsal horn’s layer I and external layer II through primary afferent nerve fibers Aδ and C[26]. In birds, the neuron distribution is similar to that in monkeys and cats of nociceptive spinothalamic tract cells[26], nerves get feedback from substance P – having nerve terminals, which also tend to achieve a major part in bird pain pathways [25]. One study found melatonin can also be involved in the transmission of sensory signals, like pain neurotransmission [26]. Like mammalian species, the nociception of α2-preganglionic receptors can likewise be essential. These receptor positions have been studied in hens, and parallels findings shown in mammalian species [27]. The correlations between birds and mammals pain receptors additional backing the theory which claims chicks can feel an ache. The brain and spinal cord play a significant part in whole harmful material being handled. Like with primates, encephalin and endorphin processes in poultry tend to modulate central processing [28].

Among mammals, some endogenous opioids, like endorphin and enkephaline, work to reduce pain by acting as an agonist on the μ, k and δ opioid receptors. Fowls possess opioid receptors that are closer to human beings receptors [29]. These receptors (opioid receptors) have been found in vivo in chicken embryos since the age of ten days and are located in sections thought to have accomplished important tasks in the processing and memory of sensory input[30]. The distribution in birds telencephalon of b-endorphin and enkephalin-like immunoreactivity is close to that in mammals telencephalon[28] Which suggests the ligands are close. Data shows that the encephalin and endorphin mechanism achieved a crucial role in the control of pain, since roosters with painful joint illness moved slower later administration of encephalin [30].

Pain behaviours

The study of bird pain is limited, but much of it arises from welfare research with consideration for everyday farming techniques. If a bird suffers from pain, one or more of the normal behaviors typically shift or are absent [7, 11].

For effective analgesic choice and treatment of suffering and pain, identification of pain and discomfort in animal species is important. Birds display a link between nociceptor stimulation and physiological and/or behavioral signs of pain [31, 32, 33]. Acute pain, ranging between seconds to some days, is responsive to avoid or minimize tissue injury by offering a defensive function [33], and is complemented by increased blood pressure, heart beat and respiration[21]. Chronic pain is abnormal because it induces a rule- of distress and anxiety because pain persists past the normal time to recover [34]. Poultry appears in reaction to acute pain, represented by fight or flight reactions, escape and distress call behavior, or may not lack ability to move and escape attempts. The sustainability-withdrawal response is called immobility in reaction to a painful stimulus, and many of the harmful dermal thermal stimulation causing hunched or lack of mobility (hunched status with the head bent into the chest and the eyelid shut) has been studied in chickens [35]. Behavioral changes can be attributed to acquired fear as from fight-or - flight reaction to inactivity, weight loss and lack of appetite are unspecific symptoms of pain[36]. Piloerection or a “Fluffed up” look can be attributed to disease or soreness. Grooming activities could be reduced [32], but over-grooming, feather destructive, and
self-resection are also grasped in chronic pain conditions [34]. In order to examine acute pain responses in gallinaceous birds, electrical [37], mechanical, or thermal [38] harmful stimuli were utilized. Aware of African gray parrots in reaction to an electrical voltage, looked at their legs or pecked on the cables, but significant differences in behavior prohibited significant quantitative evaluation of the temperature threshold[39].

**Methods**

**Mechanical stimuli [7]**

**Comb pinch [40]**

Pinch comb.

This test is based on the principle of applying pressure using the index finger and thumb of the researcher on the hen’s mane, and in general, this test is used to check the severity of anesthesia before performing surgical operations, and it results in the bird’s head shaking in birds that have not entered the anesthesia phase. A disadvantage of this test is that the mechanical stimulus resulting from pressure is not equal in intensity between one researcher and another. The duration of the test is two seconds to provide the necessary pain that can be evaluated.

**Behavioral responses**

One of the most important behaviors that may appear on chickens pressed to their comb is the flapping of the wings, widening of the eye opening and a distress call as a result of the induced pain in addition to an increase in the movement of the head and it becomes more aggressive and these behaviors are examined and recorded during the comb squeeze, which does not exceed 15 seconds.

**Physiological responses**

A squeeze comb sensation has had a strong influence on heart activity and peripheral resistance. These stimulation triggered a fast increase in blood pressure of around 12 mmHg within 1-2 sec. followed by declined to 8 mmHg under the before stimuli point going back to the pre-stimulus value within 20-30sec. After stimulation, the heart rate increased quickly to about 22% above the before the stimulus values, with the rate slowly getting back to typical above the next 35-45 seconds. This period span needed to coming back to typical is influenced by the bird’s anxiety condition and activity during excitement.

**Foot pressure test (FPT)**

This experiment used the Randall /Selitto technique [41] and a Paw-Pressure Analgesy Meter specifically modified for the foot of the chicks. This device consists of a tool which provides a pressure (from 0 to 750 gram) which raises at a steady rate (16 gram / second). Such pressure was applied to the feet of the chick that was placed in the middle of the fixed plinth and a pusher plinth. Chicks were free to remove the tool from their feet. A pointer traveling around a linear scale was constantly watching the pressure. The technician ceased the rise in pressure once the chicks struggled and removed its foot from the plinth, and read on the scale the force that the chick reacted (pain threshold). A control test was conducted prior to and after the experiment to determine that the reaction was due to the pressure exerted on the foot and not to the containment in the machine. The control experiment consisted of exposing the animal to the device for up to 15 seconds without even any forces exerted. Outcomes were omitted first from a statistical study of chicks who withdrew their feet from the device prior 15 s in the blank survey. The stressed foot has been identified following each experiment to ensure that no damage was caused by impact with that of the pusher.

**Heat stimuli**

**Thermal foot withdrawal [42]**

The thermal foot withdrawal test is a novel test first described recently by Sanchez-Migallon et al. [42]. A test box fitted with a research perch was used to obtain threshold measurements on all animals. The test perch was created to achieve a heat stimulus to a bird’s left plantar foot surface by using thermal microchips to rapidly change the perch temperature. By raising the foot, the birds could avoid the short noxious heat stimulus and the foot could then be put back on the perch within 2 to 3 seconds of the withdrawal response because the heat decreased rapidly. The test box had dark sides to prevent birds from seeing their surroundings, such as the observer, and a clear front that allowed the observer to track behavioral responses with a remote video camera in real time. Every bird had been adapted to the test chamber prior to the test, mimicking a full day observation of the test. The test box had dark sides to prevent birds from seeing their surroundings, such as the observer, and a clear front that allowed the observer to track behavioral responses with a remote video camera in real time. Every bird had been adapted to the test chamber prior to the test, mimicking a full day observation of the test. The heat stimulus produced by thermoelectric modules ranged from 29° to 70 °C and triggered a rapid increase in perch temperature and subsequent decrease (temperature increase and decrease rate, 0.3 °C / s). The cutoff temperature was 70°C to avoid tissue...
damage. A thermal reaction to the withdrawal threshold was defined as the temperature of the perch that was simultaneous with a response to foot withdrawal. For each cycle, a separate baseline thermal withdrawal threshold was reported by a single measurement obtained 1 hour before administration of the analgesic or control solution.

Jumping test

The jumping test first describes by Hughes[43]. This test is resemble to the hotplate test in the mice[44]. The apparatus of this test comprised of a 63 x 20 x 0.3 centimeter. A plate of copper filled by lead shot, with six (1.5 centimeter diameter) tubes of copper. The tube was 0.9 centimeter center to Centre spaced then connected near the middle part of the plate of copper. Plate of copper stayed maintained via a 63 x 19 x 7 base made of wood through a 23 x 7 centimeter hole in the anterior of it to allow a hot plate to be placed. Fillings were put beneath the legs of the hot plate to ensure close touching among the heating part and the plate of copper. A 17 x 16 x 31 centimeter Plexiglas compartment was placed over the tubes with a hinged lid. The Entirely bare copper part exterior to the compartment was shielded with rigid foam insulation of 2.5 centimeter wide that clasped with duct tape to the equipment base. An electronic timer registered response latencies to the nearest 0.1 sec (Lafayette Industries, model 54030).

The chick was put on heated (61.0±0.5 °C) grid for this test, and onset was reported to do champing reaction with two foot. If the chicks have no jump reaction in 90 seconds, a latency score of 90 sec was removed and allocated.

Hot water test [45]

The hot water measure consists of a Beaker filled with water on the hot plate warmer or using a water bath. The temperature of the water was regulated by setting the temperature of the hot plate at 55 °C. The technique consists of keeping the bird in one hand bent on the leg and the other leg submerged in a water-filled beaker at a 55 °C (±0.3 C). Then calculating time (in a sec) of the bird acquired to remove feet from the hot water. The bird was unrestricted to take his leg out of the beaker or water bath. The onset was calculated in seconds using a stopwatch. Bird fails to respond in 20 seconds after immersion, the leg was removed from the beaker, and after each test the leg examined was submerged in a room temperature water beaker for 15 s to rapidly reduce the temperature of the skin and was then monitored for burning or hyperemia.

Electrical stimuli

There are two procedures in this test that depend on the behavioral response to electrical stimuli

The procedure depending on the behavior of the vocalization

This is based on a test for the generation of acute electrical pain with an electro stimulation device on the skin. Analgesia was tested using an electric stimulator to raise a pain threshold later set frequency about 50 hertz, the width at five ms and the amplitude of the pulse about ten volts. The stimulator Positive and negative poles were subcutaneously placed beneath wing at the upper chest area, moistened by water. After electrical stimulation chick’s reaction to pain was seen as a pain behavior of flapping and/or calling. The birds had the smallest voltage which triggered effective pain reaction previous injection of the analgesic drug and then fifteen minute next injection. For increasing community the up or down in voltage causing pain reaction was determined. Usually, The threshold for successful analgesic reaction became apparent within 2s ust after electrical stimulation[1,46].

The procedure depending on the withdrawal behaviour

Chicks were fitted with an electrode on the right leg’s medial metatarsal area, put in a test box and enabled to habituate. An electrical stimulation (range, 0.0 to 1.46 mA) was transmitted via an aluminum perch to each bird’s foot. A removal response was recorded once the bird lifted its leg from the perch or actively flinched its wings. The baseline level was set to a noxious electrical stimulus [39].

Chemical stimuli

Formalin test [47]

Based on this test, pain is caused by a chemical irritant at a certain concentration in the sole of the foot and, as a result, we can induce pain and inflammation in the region injected[46-48]. The injection of 0.05 ml of 0.1 % formalin solution into the right foot sole part. Left foot planter was injected 15 minutes before the injection of formalin with a physiological saline solution as a control test or with analgesic medicine. The latency to raise the correct foot, longest foot lift time, and Repetition to raise the injected foot in reaction to irritant (formalin) injection were
recorded within 3 minutes immediately after the formalin injection [44].

Intra-articular sodium urate injection test [49,50]

This test depends on the injected microcrystalline sodium urate in the joint as a result; the pain and arthritis was induced. Chicks take an intra-articular injection into the right hock joint of 0.2 ml normal saline or else analgesic medicine. After analgesic administration, the hen was administered by six milligram sodium urate in 0.2 ml of normal saline to confirm the best time of analgesia matched with the full influence of the irritant (sodium urate ). Immediately injected from the plantar part of the joint into the hock’s intra-articular space by a suitable gauge needle. A microcrystal of sodium urate was formulated by utilizing the technique of [51]. A birds’ behavior was documented for one hour starting one hour next administration.

Monitoring is done using a digital camera, every five minutes, the activity of the hens were documented as stand-up, mobility, eating, water consumption, cleaning, sitting awake, sedated with eyelid closed, bathing and dust bathing while resting. Stand-up behaviors were distinguished from dual-legged movement wherever the inserted leg was raised or where the untreated limb was lifted. Standing behaviors in which the injected leg was raised varied from two-legged operation or in which the untreated limb was lifted. The No of walking and standup activity observations (standing behaviour); the No of feeding, drinking and pecking observations (mouth behaviour); and the No of birds found to be latent and quiet (latent behaviour) [50,51,52].

Conclusion

This article offers a brief overview of methods for evaluating chick pain. It addresses the value of understanding a chick’s pain, but recognizes that there is difficulty in doing so. One of the most important metrics for the measurement of analgesic medications is the pain threshold. Pain has various aspect and characteristics depending on receptor and stimulus type.

Authors’ Contributions
All authors worked equally and approved the final manuscript.

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Competing Interests
The researchers claim that they do not have competing interests.

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طرق تقييم الألم في نموذج أفراد الدجاج

أحمد صلاح ناصر و حامد أحمد شعبان
فرع الفسيولوجيا والكيمياء الحياتية والدوائية - كلية الطب البيطري - جامعة الموصل - الموصل - العراق.

كانت هناك حاجة ملحة لتطوير طرق دقيقة وفعالة لتقييم الألم عند الطيور بشكل عام و في أفراد الدجاج بشكل خاص. إن أفراد الدجاج حيوانات مختبرية مفيدة بسهولة التعامل وتربية بالإضافة إلى أن المكونات العصبية لتفاعل الاجداث والملاذات والإلتقاء والاستجابة للألم مشابهة لذين في الثدييات. أفراد الدجاج يستجيبون للألم سللياً وسلوكياً، وتفاوت درجات الاستجابة للألم السلوكية في أفراد الدجاج من الانسحاب إلى الصياح إلى رفرفة الجناح. تم في هذا البحث جمع طرق تقييم الألم في الطيور من البحوث المنشورة والتي تختلف حسب حافز الألم المستخدم وتوفر هذه المراجعة معلومات مهمة عن سلوكيات الألم في أفراد الدجاج التي يمكن أن تعزز فهم المختصين بدراسة الألم. وتاريخنا يمكننا من تقييم الأدوية المسكنة للألم في أفراد الدجاج.

الكلمات المفتاحية: أفراد الدجاج، تقييم الألم، السلوك، تسكين الألم.