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The effect of incubation temperature on the development of the locomotory system and welfare in broiler chickens: A review

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HIGHLIGHTS

- Embryonic muscle responds to higher temperatures by proliferation.
- The timing of temperature manipulation is crucial for the resulting changes.
- Incubation temperature influences leg bone growth, mineralisation and breaking strength.
- The reflection of changes in the muscles and bones on walking ability needs more study.
- Future studies of temperature manipulation should include welfare assessments.

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ABSTRACT

Commercial rearing of broiler chickens can be coupled with compromised animal welfare. Selection for optimal productivity has led to decreased walking ability and associated welfare issues like contact dermatitis. The incubation temperature has previously been shown to affect the development of the locomotory system and can thus be seen as a candidate tool to influence walking ability. This review paper aims to provide an overview on effects of incubation temperature changes in early (week 1), mid-term (week 2) and late (week 3) embryogenesis on broiler muscle and bone development, and subsequent locomotory ability.

A novelty in this paper is the discussion of the possible effect of incubation temperature manipulation on the welfare of broilers. Muscle tissue responds to increased temperatures during embryogenesis by proliferation, but this effect depends on the timing, as embryonic muscle development relies on the expression of regulatory factors and cell lines occurring at specific time points. Furthermore, breast and leg muscles respond differently, especially when different timings of temperature manipulation are compared. Leg bone growth seems to be promoted by increased incubation temperature, but the effect cannot clearly be separated from overall embryo growth. Data on the influence of the incubation profile on bone strength and mineralisation is limited and suggests a positive effect of higher temperature in mid-term embryogenesis, but not when applied over extensive periods. The reflection of the changes in bone and muscle development on walking ability has not been widely studied but a beneficial effect is possible due to the effect on muscle and overall body growth. Concluding, further studies to establish proper timing and temperature enabling beneficial changes in the muscle fibres and bones for improved walking ability are needed. This can be a way to make chicken meat production more sustainable and profitable due to fewer production losses and better animal welfare.

1. Introduction

Through selective breeding, broiler chickens have become one of the most effective domestic animals in terms of growth, feed conversion and yield, especially of the coveted breast meat. At 35 days of age, a modern commercial broiler weighs over 2 kg and has reached a body composition ready for slaughter with around 25% of the eviscerated carcass weight being composed of breast muscle without skin and bones (Aviagen, 2022a). Compared to layer strains (Mueller et al., 2018), the rapid body growth of broilers can lead to welfare issues, with impaired

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walking ability and bone strength being amongst the most important welfare issues in broiler chickens. It has been discerned that, around slaughter age, 19% to 27.6% of the broilers show moderate to severe gait impairment (Knowles et al., 2008; Kittelsen et al., 2017; Granquist et al., 2019) which is associated with pain (McGeown et al., 1999; Caplen et al., 2013; Riber et al., 2021). Broilers that have trouble walking, spend more time sitting (Bessei, 2006) and can have difficulties with dust bathing and reaching the feed and drinking equipment (Vestergaard and Sanotra, 1999; Weeks et al., 2000). This, along with contact dermatitis, adds an economic interest to the ethical aspects of broiler welfare. Contact dermatitis itself is associated with gait score (Granquist et al., 2019) and increased time sitting on litter can exacerbate it (Bessei, 2006), while litter quality and related factors (e.g. stocking density, feeding, litter material, ventilation) are the main promotors of contact dermatitis (Shepherd and Fairchild, 2010). Furthermore, legs affected with dermatitis must be discarded at the slaughter line, leading to economic losses (Shepherd and Fairchild, 2010; Bassler et al., 2013). For these reasons, the improvement of locomotory ability in broilers is beneficial for both animal welfare and the economy of broiler meat production.

Muscles related to walking ability have not been targeted by the selective breeding in the same way as the pectoral muscle has been (Al-Musawi et al., 2011), possibly leading to decreased locomotory ability. The large breast muscle shifts the centre of motion (COM) forward, which disbalances the bird (Paxton et al., 2010). Broilers and layers differ in their gait dynamics, indicating different stress on the locomotory system brought on by selective breeding (Duggan et al., 2016). Moreover, an association between tibia parameters and gait score has been observed (Kittelsen et al., 2017), making this bone a possible target to improve walking ability. The tibia can further be affected by diseases such as tibial dischondroplasia which leads to impaired walking ability (Rath and Durairaj, 2022).

Commercial incubation of chicken eggs takes 21 days. The gold standard is to incubate chicken eggs under 37.8 °C with relative humidity in the range of 55 to 65%. The incubation conditions affect the development of the locomotory system as one part of the organism. Previous studies suggested that temperature manipulation can modulate embryonic muscle and bone development. Therefore, temperature changes during chicken egg incubation can be regarded as a possible tool to improve bone strength (Guz et al., 2020), muscle proliferation (Piestun et al., 2009; Al-Zghoul and El-Bahr, 2019) and the ratio between breast muscle and postural muscles (Oksbjerg et al., 2019) up to slaughter age. Additionally, an effect on muscle enzymes has been found (Krischek et al., 2016), pointing towards possible alterations in the muscle fibre composition, which indicate functional changes.

Incubation is a crucial and increasingly large part of a broiler's life. This topic has been the subject of regular review with a focus on; thermal manipulation as a tool to improve thermotolerance (Loyau et al., 2015; Costa et al., 2020); possibilities to influence satellite cell growth in chickens using manipulation of the temperature pre- and post-hatch (Halevy, 2020); incubation conditions as an influence on the onset of myopathies in poultry (Oviedo-Rondon et al., 2020); muscle development and meat quality (Wang et al., 2022) with temperature being one aspect amongst others; the influence of temperature in connection with light on chick development (Yalcin et al., 2022b); and the effect on chick quality and hatchability (Tainika, 2022). The economic aspects have thus been covered extensively and will not be addressed in the present work. The work presented here reviews incubation temperature as a possible tool to target muscle and bone development in a way that the locomotory abilities and subsequent welfare of broiler chickens can be improved. This aspect of the incubation temperature is relevant in the light of recent sustainability and animal welfare demands of the consumers.

2. Methodology

The literature search was conducted on different reference search engines (Web of Science, PubMed, Google Scholar) and included articles in English language only. The main literature search was conducted in the second half of 2021 and was updated with new publications in the beginning of 2023.

The search strings included (broiler OR chicken) AND (incubation temperature) AND (muscle OR bone) AND development; (broiler OR chicken) AND (thermal OR temperature) AND (manipulation).

Additionally, the reference lists from the publications found in this way were screened for further suitable literature. Studies published earlier than 2000 were not considered. The studies were screened for eligibility based on title, abstract and keywords. Only studies that investigated aspects of the locomotory system, at least muscle weights, were included, excluding studies that only focussed on hatchability, performance aspects, or thermoregulation. Studies conducted in layer chickens were included when information in broiler chickens was lacking. One study in turkeys was included to provide more information on the effects of lower temperature. This led to 35 studies being included in this review. The studies were organised by the timing of the temperature manipulation and divided into intervention in early (week 1), mid-term (week 2), and late (week 3) embryogenesis.

No statistical analysis or meta-analysis was conducted in the review; thus the objective and balanced conclusions are based on the authors' evaluation of the available literature.

3. Myogenesis

Muscles are the "motor" of locomotion. Their mass is determined by the number of muscle fibres, which is settled during embryogenesis or early post-hatch, by the cross-sectional area (CSA) of muscle fibres, and the fibre type. A muscle fibre is formed through the fusion of myoblasts (Stockdale and Holtzer, 1961; Abmayr and Pavlath, 2012), starting in early embryogenesis, when the somites of the paraxial mesoderm differentiate into the sclerotome and the dermatomyotome (Stockdale, 1992). Four myogenic cell types originate from the dermatomyotome. These do not differentiate from each other. They rather proliferate and fuse into myotubes individually and are associated with certain time windows during embryonic and foetal development (Miller and Stockdale, 1987; Biressi et al., 2007). In the order of their occurrence, they are called myotomal cells, embryonic myoblasts, foetal myoblasts, and adult myoblasts. The adult myoblasts are also known under the name of "satellite cells". These are the only cells available to increase muscle fibre size after hatch (Morgan and Partridge, 2003; Jankowski et al., 2020). This process is accompanied by the expression of several regulatory factors, transcription factors and growth factors, as reviewed by Velleman (2007), Braun and Gautel (2011) and Mok et al. (2015). Notably, the myogenic regulatory factors of the MyoD family, Myf5, MyoD, MyoG and Myf6, control the differentiation of myoblast precursors, their commitment to the myogenic lineage, and the formation of myotubes. Myf5 is the first to be expressed, followed by MyoD, MyoG, and Myf6.

The most prevalent fibre type in the embryo at all ages is the fast myofiber, but there are differences between the embryonic, foetal, and adult lineages. In the embryonic muscle (embryonic day 4 to 7), both fast and slow myosin isoforms are expressed, independent of the fibre composition of the muscle they form (Crow and Stockdale, 1986). The diversification of the fibres in the embryo occurs without the involvement of the prospective nerve fibres that control the muscle later (Miller and Stockdale, 1987). Foetal muscle fibres occur between embryonic day (ED) 8 and 12 and are mainly composed of fast fibres. They form predominately in prospective muscles of fast and mixed fibre type but not of slow fibre type. Therefore, the pattern given by the first fibres prevails at large (Miller and Stockdale, 1986). M. pectoralis consists of IIB fibres (Ono et al., 1993; Verdiglione and Cassandro, 2013). Type IIB is also the most abundant in the leg muscle M. biceps femoris, but in this muscle, there are also type I and type IIA fibres that make up around one-fifth of the fibres (Papinaho et al., 1996). Other leg muscles, like M. femorotibialis medius and M. iliotibialis lateralis have a mixed composition of different fibres, involving type I, type IIA and type IIB fibres. It has been observed that the fibre ratio in these muscles is changing with age, favouring type IIA over type IIB fibres (Ono et al., 1993).

Fig. 1

4. Effect of incubation temperature on embryonic muscle development

According to the occurrence of the four myogenic cell types during specific time windows of embryogenesis, the incubation period can be divided into early, mid-term, and late. Thus, the studies were reviewed depending on the week during which the temperature manipulation was applied.

The influence of incubation temperature during early embryogenesis on the muscle development was first studied on layer chicken embryos; A temperature increase of one degree led to increased muscle fibre and myonuclei number, nuclei/fibre ratio and mass of M. gastrocnemius (Hammond et al., 2007; Al-Musawi et al., 2012). This was accompanied by increased transcripts of myogenic regulatory factors and transcription factors like Myf5, Pax7, and IGF-I (Al-Musawi et al., 2012). In broiler chicken embryos, these results could not be reproduced; The fibre number was decreased, and the expression of myogenic regulatory factors and transcription factors was delayed. This difference between broilers and layers was hypothesised to be an effect of epigenetic factors like different yolk composition between broilers and layers (Al-Musawi et al., 2012). There are few studies that examined chicken muscle development up to slaughter age after changing the incubation temperature in early embryogenesis. For instance, an increased temperature between ED 3 and 6 did not lead to differences in breast muscle fibre CSA at 33 days and 69 days post-hatch (Krischek et al., 2013). Under similar conditions, a shift in the weight ratio of M. pectoralis and M. gastrocnemius in favour of the latter was reported for 35-days-old broilers (Oksbjerg et al., 2019). A muscle ratio was not investigated in other studies, but this observation points towards differences in the susceptibility of muscles to temperature manipulation. A temperature of 44.0 °C for 1 hour on day 4 of incubation led to a decrease in the MyoD gene expression in broiler embryos, indicating a hampering effect on muscle development of such high temperatures (Gabriel et al., 2011). In summary, the results from studies investigating increased temperature in the first week of incubation are diverse, not least because of differing study design, which makes it difficult to draw conclusions. An overview of studies is given in Table 1.

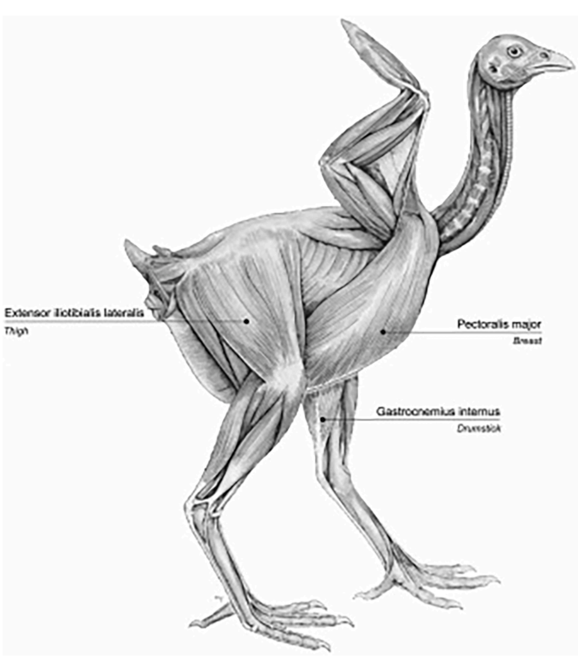

Fig. 1. Localisation of the muscles mentioned in reviewed articles (Reprinted from Baldi et al. (2021) with permission from Elsevier).

Studies on the muscle development of chickens subjected to lower temperatures during early embryogenesis are also scarce (Table 1). No change in the weights of M. pectoralis and M. gastrocnemius were found after subjecting broiler eggs to 36.5 °C from ED 4 to 7 but a tendency for a shifted ratio in favour of M. gastrocnemius could be observed (Oksbjerg et al., 2019). Reduced weight of M. pectoralis and M. semitendinosus as well as reduced fibre number and fibre size was reported in 16-day-old turkeys after subjecting eggs to 35.5 °C from ED 5 to 8 (Maltby et al., 2004). More studies are needed on the structural and genetic changes in the muscles after subjecting eggs to lower temperature in the early period of embryogenesis to get a more comprehensive picture.

The effect of increased temperature during mid-term embryogenesis on muscle development has been studied more intensively. For instance, expression of the vascular endothelial growth factor A (VEGFA) in the breast muscle, related to the vascularisation, and insulin-like growth factor-I was increased until the age of 42 days after applying 38.8 °C during ED 10 to 14 for 6 h per day (Yalcin et al., 2022a), indicating proliferation. Phenotypic changes in the fibre traits differ for this period and likely depend on the timing that was investigated. Increased fibre size in the breast muscle or thigh muscle was found when the temperature was changed for between 6 and 18 h per day of treatment (Piestun et al., 2011; Dalab and Ali, 2019; Yalcin et al., 2022a). Smaller fibres of higher number were observed in the breast muscle of female broilers compared to control females (Werner et al., 2010), when the temperature was changed continuously throughout the whole treatment period. Thus, an intermittent temperature change seems to increase fibre size in this period. Cobb broiler chickens subjected to 38.8 °C on ED 7 to 10 or ED 10 to 13 showed different muscle enzyme activities in the embryonic breast muscle. In the group that was manipulated in the earlier period, lactate dehydrogenase and cytochrome oxidase activities were increased compared to control, while in the other group, phosphofructokinase and cytochrome oxidase activity were increased (Krischek et al., 2016). This shows that a shift of the time window for temperature manipulation by a few days can alter the muscle enzyme activity. Furthermore, mitochondrial respiratory activity (MRA) increased significantly in the embryonic thigh and breast muscles for increased temperature from ED 10 to 13 and in the thigh muscles from ED 7 to 10 (Krischek et al., 2016), which may suggest a change in the composition of fibre types in these muscles towards fibres with a more oxidative metabolism. However, unlike the results on enzyme activities, the changes were not reproducible in the breast muscle and did not last up to day 35 in a following study (Krischek et al., 2018). An effect of the incubation temperature on differentially expressed genes (DEGs) in the thigh and breast muscles has been shown in the same experimental setup (Naraballobh et al., 2016a, 2016b). The changes were more profound for the time window of ED 7 to 10 than ED 10 to 13 in the breast muscle, as demonstrated by the number of DEGs. In summary, the considerable number of studies on increased incubation temperature in week 2 of incubation suggests that the temperature affects all aspects of the muscle at least temporarily, from gene expression to metabolism and phenotype, but not all effects prevail until slaughter age.

As presented in Table 1, lower temperatures have not been studied intensively during mid-term embryogenesis either. Reduced MRA in both breast and thigh muscles and lower phosphorylase and cytochrome oxidase activities compared to higher temperature were found in research subjecting broiler eggs to 36.8 °C from ED 7 to 10 or ED 10 to 13 (Krischek et al., 2016). These enzymes are involved in glycogen breakdown and cellular respiration, respectively. However, MRA was

Table 1
Overview of observations on muscle parameters in studies on temperature manipulation in broiler chickens.

Period	Temperature	Strain	Timing	Muscle	Observation				Reference
					Fibre Traits	Weight	Metabolism	MRF and TF Expression	
Early	36.5 °C	Ross	ED 4 – 7	MP, MG		–			Oksbjerg et al., 2019
	38.1 °C	Cobb	ED 0 – 5	MP		–			Piestun et al., 2013
	38.5°C	Cobb	ED 3 – 6	MP	– (FS)	–			Krischek et al., 2013
		Ross		MP, MG		–			Oksbjerg et al., 2019
		Ross, emb		MG	↓ (FN)			delayed	Al-Musawi et al., 2012
Mid-term	36.8 °C	Cobb	ED 7 – 10 or 10 – 13	MP, LM		–	+ (LM of embryo, MRA, enzymes)		Krischek et al., 2018
		Cobb, emb		BM, LM			+ (MRA, enzymes)		Krischek et al., 2016
	38.8 °C	Cobb		MP, LM		–	+ (LM of embryo, MRA, enzymes)		Krischek et al., 2018
		Cobb, emb		BM, LM			+ (MRA, enzymes)		Krischek et al., 2016
		Cobb, Ross	ED 10 – 14, 6 h/day	BM	↑ (FS) ↓ (FN)			+	Yalcin et al., 2022a
	39.5 °C	Cobb	12 h on ED 7 – 16	MP	↑ (FS)	↑			Piestun et al., 2011
			ED 7 – 16	MP		↑		+	Piestun et al., 2015
	39.5 °C	Ross	18 h, ED 7 – 11	MP, TM	↑ (FS, TM)	↑ (both)			Dalab and Ali, 2019
			18 h, ED 11 – 15	MP, TM	–	–			Dalab and Ali, 2019
Late	30 °C	Cobb	30/60 min on ED 18 and 19	MP		+			Shinder et al., 2011
	38.5 °C	Hubbard	5 h, ED 15 – 18	MP				+	Slawinska et al., 2013
		Ross	18 h, ED 12 – 18	MP		↑		↑ (ED18)	Al-Zghoul and El-Bahr, 2019
	39.0 °C	Ross	18 h, ED 12 – 18	MP		↑		↑ (ED18)	Al-Zghoul and El-Bahr, 2019
	39.5 °C	Ross	18 h, ED 12 – 18	MP		↑		↑ (ED18)	Al-Zghoul and El-Bahr, 2019
			18 h, ED 15 – 18	MP, TM	–	↓ (TM)			Dalab and Ali, 2019
		Broiler	ED 14 – 18, 12 h	MP		↓			Clark et al., 2017
		Cobb	ED 16 – 18, 3 h or 6 h	MP	↑ (FS)	↑		↑	Piestun et al., 2009
Other	40 °C	Ross	18 h, ED 12 – 18	MP		↑		↑ (ED18)	Al-Zghoul and El-Bahr, 2019
	39.5 °C	Ross	ED 7 – 18	MP, TM	↑ (FS, TM)	↓ (TM)			Dalab and Ali, 2019

ED = embryonic day; emb = embryo; MP = M. pectoralis; MG = M. gastrocnemius; TM = thigh muscle (not specified), BM = breast muscle (not specified); LM = leg muscle (not specified); FS = fibre size; FN = fibre number; MRF = myogenic regulatory factor; TF = transcription factor; MRA = mitochondrial respiratory activity; ↑ = increase; ↓ = decrease; + = effect, but cannot be specified as increase or decrease; – = investigated, but no effect.

found to be unaffected at slaughter age (Krischek et al., 2018). Furthermore, lowered temperature altered fewer genetic pathways than higher temperatures in the same experimental setup (Naraballobh et al., 2016a, 2016b). A temperature of 36.8 °C applied from ED 7 to 10 led to mostly downregulated genes related to cell signalling, gene expression, cellular function, and maintenance pathways in the breast muscle, while, applied from ED 10 to 13, it resulted in upregulated genes attributed to organ, embryonic and skeletal-muscular system development. Muscle microstructure was not investigated in studies on chickens. In turkeys, lower temperature during mid-term embryogenesis resulted in a reduced fibre number at day 16 post-hatch (Maltby et al., 2004). These results indicate that lower temperature has a less-pronounced effect than higher temperature in this period, but more studies focussing on phenotypic aspects are needed to draw a proper conclusion.

In the studies of late embryogenesis, the eggs were only subjected to temperature manipulation for part of the day, not continuously throughout the experimental period, in contrast to temperature manipulation in the first and second week of incubation (Table 1). A continuous exposition to higher temperatures during this period is considered harmful due to the embryo's own body heat production and reduced ability to lose heat (Oviedo-Rondon et al., 2008, 2009; Aviagen, 2022b). Changes in the microstructure and gene expression suggest a proliferative response of the breast muscle to an hourly increased temperature in this period; higher proliferative activity of embryonic breast muscle cells in vitro (Piestun et al., 2009), and higher expression of markers for muscle proliferation and differentiation (Slawinska et al., 2013, Al-Zghoul and El-Bahr, 2019) were found as well as increased fibre diameter of M. biceps femoris (Stojanovic et al., 2014) and M. pectoralis (Piestun et al., 2009) up to day 13 and day 42, respectively. These structural and gene expression changes were also reflected in increased breast muscle weights (Piestun et al., 2009, Al-Zghoul and El-Bahr, 2019). Thigh muscle weight, on the other hand, was lower in one study (Dalab and Ali, 2019). This suggests that leg muscles, being of different fibre composition, could be affected differently by increased temperatures in this period. In the studies mentioned, the temperature was increased for 3 to 18 h per day, while a treatment around 6 h per day seems to give the most robust results.

To the authors' knowledge, there are no comparable experiments investigating the role of lower temperatures during late embryogenesis in muscle development. A temperature of 30 °C applied for 30 to 60 min on ED 18 and 19 increased relative breast muscle weight at slaughter age (Shinder et al., 2011). This temperature is considerably lower than the temperatures used in other time windows. The apparent beneficial effect on breast muscle development could be explained by the internal heat production of the embryo in this period, which makes it susceptible to overheating (Oviedo-Rondon et al., 2009).

Studies where the temperature manipulation cannot be attributed to week 1, week 2 or week 3 are discussed in the following paragraph. Increased temperatures applied from mid-term to late embryogenesis resulted in a proliferative response of the breast muscle; Significantly higher expression of MyoG during embryogenesis and post-hatch, higher numbers of myoblasts per gram muscle during embryogenesis, at hatch and until day 6 post-hatch, and higher proliferative activity of the muscle cells in vitro were observed after subjecting eggs to 39.5 °C for 12 h and 24 h daily from ED 7 to 16 (Piestun et al., 2015). These changes seem to be transient, as a study under similar conditions found increased thigh muscle fibre size but reduced thigh muscle weight, and unaffected breast muscle weight at slaughter age (Dalab and Ali, 2019). It can also be hypothesised that longer periods of high temperatures hamper leg muscle development more than breast muscle development.

To summarise, a temperature increase of 1 to 2 °C for a short period in early, mid-term or late embryogenesis can stimulate the muscle development of both breast and leg muscles in broiler chickens. During late embryogenesis, exposure to higher temperatures should not be continuous. A difference in the susceptibility of muscle groups to temperature manipulation can be hypothesised. Leg muscles seem to be more sensitive to temperature manipulation than breast muscles. Due to the high level of genetic selection on breast muscle growth, that muscle group could be more resistant to temperature changes in embryogenesis. The underlying mechanisms are not well understood and changes in gene expression did not always result in phenotypic changes. The results suggest that all three myogenic cell lines (embryonic, foetal, adult) are susceptible to temperature manipulation. Depending on the fibre type composition, some muscles could be particularly sensitive during certain time windows, given the fact that the embryonic lineage forms slow-twitch myofibres, while the foetal lineage forms fast-twitch fibres. More studies investigating different muscles and their fibre composition in the same experimental setup are needed to test this hypothesis. The influence of lower incubation temperatures on embryonic muscle development has not been covered by as many studies to this date. Information on its effect on the phenotype, like muscle microstructure, is lacking. The available studies suggest that lower temperatures seem to have a less pronounced effect on embryonic muscle development.

5. Influence on embryonic bone development

The majority of lameness in broilers is of skeletal nature. Some tibia parameters, like width and angle, have been associated with a higher gait score (Toscano et al., 2013). The gait of modern broilers shows several differences compared to the gait of layer chickens, like an increased step width, shorter step length, and shorter periods on one foot (Paxton et al., 2013; Duggan et al., 2016). This results in a slower velocity and can be interpreted as an adaption to the shifted COM due to the massively increased breast muscle weight (Duggan et al., 2016). This suggests that the burden on the bones is different in broilers and layers. Furthermore, the rapid growth of the broilers poses a challenge for the bones. Although bone weight and length also increase rapidly, along with the thickness of cortical bone, there is a delay during the first two to three weeks after hatch, where the cortex becomes porous due to slow filling with osteoblasts (Williams et al., 2004). Another risk is that mineralisation cannot keep up with the rapid growth of the bones during the first weeks, making the bones prone to breaking and deformities at this age. Compared to laying hens, bone mineral in broilers at slaughter age is less organised, referring to the alignment of the bone minerals with the collagen fibres of the extracellular organic matrix (Sanchez-Rodriguez et al., 2019). An overview of studies investigating bone parameters organised by the incubation period during which temperature manipulation was applied can be found in Table 2.

Increased temperature applied during the first week of embryogenesis results in longer tibiae and tarsi in layer chicken embryos (Hammond et al., 2007, Al-Musawi et al., 2012). In broiler chickens, a similar effect was described by Al-Musawi et al. (2012), who used the same setup for both layers and broilers (38.5 °C from ED 4 to 7). For a temperature profile consisting of 38.9 °C from ED 4 to 7, 37.8 °C from ED 8 to 14 and 36.8 °C from ED 15 to 21, shorter femora, tibiae, and metatarsi in 18-day-old broiler embryos were reported (Sozcu et al., 2022). The effect became more pronounced at hatch, where the weight, width, and breaking strength were reduced as well. Measurements were not continued after hatch. It should be noted that the control group in this experiment was incubated at 37.8 °C from ED 1 to 14 and 36.8 °C for the remaining period. In most other experiments using temperature manipulation, the control group is subjected to 37.8 °C throughout the whole incubation period. Shim and Pesti (2011) assume that it is not the temperature itself that affects the length of the leg bones but rather the time spent in the incubator and hatcher, which, in turn, is influenced by the incubation temperature. The tibiae were observed to be longer at a

Table 2
Overview on observations on bone parameters from studies on temperature manipulation in broiler chickens.

Incubation period	Temperature	Strain	Timing	Oberservation			References
				Leg Bone Dimensions	Leg Bone Strength	Mineralisation	
Early	36.5 °C	Ross	ED 4 – 7	–	–		Oksbjerg et al., 2019
		Cobb		+		–	Shim and Pesti 2011
	38.5 °C	Cobb		+		–	Shim and Pesti 2011
		Ross		↑ (♂)	–		Oksbjerg et al., 2019
		Ross, emb		↑			Al-Musawi et al., 2012
	38.9 °C	Cobb, hatch		↓		↓	Sozcu et al., 2022
Mid-term	36.9 °C	Broiler, emb	ED 10 – 18, 6 h daily	–			Yalcin and Siegel, 2003
	39.6 °C	Broiler, emb	ED 10 – 18, 6 h daily	–			Yalcin and Siegel, 2003
	38.9 °C	Ross	ED 8 – 14	↑	↑	↑	Guz et al., 2020
Late	36 °C	Other breed, hatch	ED 17 – 21	+			Oviedo-Rondon et al., 2008
		Cobb	ED 13 – 21	–	–	–	Morita et al., 2020
	38.2 °C	Cobb, hatch	ED 15 – 21	–		↓	Sozcu et al., 2022
	39 °C	Other breed, hatch	ED 17 – 21	+			Oviedo-Rondon et al., 2008
		Cobb	ED 13 – 21	–	–	–	Morita et al., 2020
Other	36.9 °C	Ross, hatch	ED 1 – 21	–			Van der Pol et al. 2014
	38.6 °C	Ross, hatch	ED 1 – 21	–			Van der Pol et al. 2014
	38.8 °C	Ross	ED 10 – 21	↓ (until ED 15)			Oznurlu et al., 2016
	39.4 °C	Ross, hatch	ED 1 – 21	↓			Van der Pol et al. 2014

ED = embryonic day; emb = embryo; ↑ = increase; ↓ = decrease; + = effect, but cannot be specified as increase or decrease; – = investigated, but no effect.

shorter hatching time. Possibly, the biggest and most developed chickens hatch first and thus spend a shorter time in the egg. This makes the evaluation of other similar experiments difficult because they did not separate incubation time from incubation temperature. Few studies investigated the effect of early temperature manipulation on bone parameters at slaughter age. A temperature of 38.5 °C from ED 4 to 7 resulted in increased tibia diameter and a tendency for an increased breaking force in 35-day-old male broilers, but not females, and had no effect on tibia length and weight of either sex (Oksbjerg et al., 2019). No effect on feed-induced bone abnormalities (rickets) was seen (Shim and Pesti, 2011) under these conditions. Lower temperature applied in the first week of embryogenesis had no effect on bone traits in broiler embryos (Yalcin and Siegel, 2003) and 35-day-old broilers (Oksbjerg et al., 2019) and likewise did not affect feed-induced bone abnormalities (Shim and Pesti, 2011).

Research on the impact of incubation temperature in mid-term embryogenesis on bone growth is limited. The available studies suggest no effect on leg bone length in broiler chicken embryos (Yalcin and Siegel, 2003). Strength, stiffness, and mineral density of the tibia of seven-weeks-old broilers were increased after applying 38.9 °C in week 2 and 37.8 °C in week 3 of incubation compared to 37.8 °C throughout incubation (Guz et al., 2020). Regardless of temperature in week 3 of incubation, tibia weights were higher, and the lateral tibia cortex was thicker when 38.9 °C were applied in week 2. This suggests a positive effect of higher incubation temperature during mid-term embryogenesis on bone strength, which is relevant for fast-growing broiler chickens. A lower temperature from ED 10 to 18 did not affect the leg bone length and relative asymmetry of the skeleton of broiler embryos at 21 days of age (Yalcin and Siegel, 2003).

For the late incubation period, an increased tibia length has been described for broiler chicks at hatch subjected to 38 °C in the last 4 days of incubation compared to those subjected to 36 °C or 39 °C (Oviedo-Rondon et al., 2008). No difference in leg bone dimensions was reported when eggs were subjected to 38.2 °C from ED 15 to 21, compared to a control subjected to 36.8 °C (Sozcu et al., 2022). In 6-weeks-old broilers, no effect on bone dimensions or ash content for increased temperature in week 3 of incubation was found (Morita et al., 2020).

Some studies have been conducted that cannot be classified as "early", "mid-term" or "late" temperature manipulation (Table 2). Ash content, which is associated with breaking force (Sanchez-Rodriguez et al., 2019), was unaffected in newly hatched chickens after the incubation temperature had been increased or decreased throughout the whole incubation period (van der Pol et al., 2014). In layer chickens, ash content in the humeri and tibiae was increased at hatch after applying 38.5 °C from ED 7 on, but the effect did not prevail until the age of 58 weeks (Kamanli et al., 2021). Thus, it does not seem possible to achieve long-term changes in the ash content of leg bones by such temperature manipulations. Bone growth was reduced relative to the body weight after high temperatures of 38.8 °C and 39.4 °C were applied over long periods (Van der Pol et al., 2014, Oznurlu et al., 2016), indicating a disadvantageous effect of such a temperature profile.

Based on the reviewed literature, the influence of incubation temperature on bone development is diverse and likely dependant on the temperature, timing, strain, and breed. These aspects are broken down in Table 2. An increased temperature seems to have a more pronounced effect on bone parameters. Bone strength can possibly be improved by increased temperature in the first or second week of incubation.

6. Influence on walking ability and welfare

Consequences of leg weakness like experiencing pain while walking, showing reluctance to seek food and water equipment as well as performing less dust bathing behaviour (McGeown et al., 1999; Caplen et al., 2013; Sorensen et al., 2000; Riber et al., 2021) conflict with the definition of animal welfare, which Fraser (2008) defines as the ability to fulfil the needs according to the natural behaviour of the animal and the absence of pain and illness. Thus, temperature manipulation should be evaluated from a welfare perspective since it influences the development of the locomotory system. A welfare aspect was incorporated in a limited number of studies which are summarised in Table 3.

The evaluation of the gait score as a welfare parameter has so far shown diverse results. Temperature manipulation with lower or higher temperatures in early incubation did not affect gait score at 35 days of age (Oksbjerg et al., 2019). A temperature of 38.9 °C applied in week 2 of incubation resulted in lower gait score in 39-days-old broilers, likely through improved bone strength (Guz et al., 2020). Ipek and Sozcu (2016) found differences between lowering and increasing the temperature in mid-term to late incubation; while a higher proportion of high gait was found after increasing the temperature, a higher proportion of lower gait scores was observed after lowering the temperature. These studies did not always manage to elucidate the anatomical reasons for the differences in gait scores.

Dermatitis scoring is another welfare parameter that has been analysed in relation to temperature manipulation. Corresponding to their observations of gait score, Ipek and Sozcu (2016) found a higher percentage of low dermatitis scores in chickens subjected to lower temperature than higher temperature. This suggests that the chickens with lower gait scores spent less time sitting in litter and thus were less likely to develop severe dermatitis. Guz et al. (2020) on the other hand did not find a difference in hock burns despite the differences in gait score. Only males of 42 days were scored for hock burns, while both sexes at 39 days of age were involved in the gait scoring. The exact temperature profiles and information on the strains used in the mentioned studies can be found in Table 3.

In the modern commercial broiler, the leg muscles are relatively less developed in proportion to body weight compared to the adult Red junglefowl, the wild type. The Red junglefowl also has a larger cross-sectional area of the M. gastrocnemius lateralis and medialis, a postural muscle, in relation to body weight (Paxton et al., 2010). Furthermore, it exhibits a higher relative leg muscle mass than a modern commercial broiler, even though the difference is small. This could lead to the conclusion that a higher relative leg muscle mass, particularly of postural muscles like M. gastrocnemius lateralis and medialis, can improve walking ability since the Junglefowl shows no similar impairment as the broiler. Temperature manipulation could be a promising tool to achieve this, as higher temperatures have been shown to increase proliferation (Dalab and Ali, 2019), fibre size (Stojanovic et al., 2014) and mass (Janisch et al., 2015) of leg muscles. The fibre size has been associated with contractile force of the muscle in humans and rodents (Reggiani and Schiaffino, 2020), but reseach in chickens is lacking, making it difficult to relate the changes in muscle development directly to walking ability. On the other hand, larger muscles also mean a higher energy cost for maintenance and muscle activity. Thus, a balance of energy metabolism and muscle mass is needed for improved locomotion. Maintaining a standing position requires an increasing amount of energy as a boiler grows due to skeletal muscle activity to move the sternum for breathing and increased postural muscle activity to remain standing despite the high breast muscle mass and the shifted COM (Tickle et al., 2018). Therefore, a simple increase in leg muscle mass or at least mass of postural muscles is unlikely to be sufficient to increase activity and locomotion in broilers. More mass would also mean higher stress on the skeleton. A possible approach to alter muscle functionality without increasing the size and thus requiring more energy could be to modify the fibre composition of the muscle by applying the temperature manipulation in a specific time window. Slow-twitch, oxidative fibres, mainly formed by the embryonic myogenic cells in early incubation, are fitted for long-lasting contractions like maintaining a posture (Pette and Staron, 2000) and are therefore a candidate to improve broiler locomotion. Since the high growth rate and size of the breast muscle must be seen as one of the most important non-infectious factors for leg weakness, slowed growth is a possible solution. Yet only methods leading to slowed growth without severe economic deficits are promising for better

Table 3
Overview on studies on temperature manipulation in broiler chickens involving welfare assessment.

Type of Welfare Assessment	Temperature	Strain	Timing	Observation	Reference
Gait Scoring	36.5°C	Ross	ED 4 – 7	No difference in gait scores at 35 days of age	Oksbjerg et al., 2019
	38.9 °C	Ross	ED 8 – 14	Lower gait score at 39 days of age	Guz et al., 2020
	33.3 – 36.7 °C	Cobb	ED 10 – 18	Higher percentage of lower gait scores (1 and 2) than control and higher temperature at 42 days of age	Ipek and Sozcu, 2016
	38.9 – 40.0 °C			Higher percentage of higher gait scores (3 and 4) than control and lower temperature at 42 days of age	Ipek and Sozcu, 2016
Dermatitis Scoring	38.9 °C	Ross	ED 8 – 14	No difference in hock burns and foot pad dermatitis in males aged 42 days	Guz et al., 2020
	33.3 – 36.7 °C	Cobb	ED 10 – 18	Higher percentage of low HD scores at 42 days of age	Ipek and Sozcu, 2016
	38.9 – 40.0 °C			Lower percentage of low HD scores at 42 days of age	Ipek and Sozcu, 2016
	15 °C	Ross	30 min, ED 18 – 19	Higher occurrence of hock burns and food pad dermatitis, when low incubation temperature was combined with lower rearing temperature	Nyuiadzi et al., 2020
Bone Abnormalities	36.5 °C, 38.5 °C	Cobb	ED 4 – 7	No difference in feed-induced rickets compared to control	Shim and Pesti, 2011

ED – embryonic day.

leg health and walking ability. Temperature manipulation could be a tool to achieve this by slowing the development and growth after the application of a lower incubation temperature.

As another factor, longer legs are said to create more instability while walking due to the elevated COM (Duggan et al., 2016). Therefore, shorter leg bones could be deemed favourable for improved walking ability of broilers. Increased incubation temperature has been shown to increase leg bone length in several cases (Hammond et al., 2007; Al-Musawi et al., 2012; Morita et al., 2020), as described earlier in this paper and could thus be presumed unfavourable. A shortening of the leg bones was described for very high temperatures for longer periods (Van der Pol et al. 2014, Oznurlu et al., 2016), which are not suitable in a production setting due to expected deficits in chick quality (Tainika, 2022).

In summary, temperature manipulation influences the locomotory system in several ways, making it a possible tool to improve walking ability and thereby welfare, but the results are diverse, and few studies address this aspect. Future studies should incorporate an assessment of the walking ability to deepen the understanding of the relationship between muscle, bone parameters, and walking ability and the role of the incubation temperature in this relationship.

Summary

Changes in the incubation temperature of chicken eggs result in lasting effects on locomotory system development and have potential consequences for the chickens' welfare. However, no temperature manipulation protocol has so far been established to show a clear positive or negative impact on broiler locomotory ability and subsequent welfare.

The underlying mechanisms for the changes in muscle development, like increased fibre cross-sectional area and number, and the subsequent effects on muscle functionality, walking ability and welfare remain to be elucidated. Lasting effects on bone growth and -strength could rarely be demonstrated. Future experiments should combine metabolic, anatomical, and functional analysis of the locomotory system. By closing these gaps in our knowledge and fine-tuning of temperature protocols, temperature manipulation could be developed into a tool to improve locomotion and thereby welfare of chickens in commercial production systems. As there is no additional cost for hatcheries and farms in adjusting the incubation temperatures, economic benefits can be expected immediately.

Declaration of Competing Interest

None

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Supplementary materials

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