

# Analysis of the Impact of Heat Stress on Embryo Development of Broiler: A Literature Review

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## ABSTRACT

Temperature is an important factor in embryonic development because temperature plays a role in determining the overall development of the embryo. The effect of high temperature on the embryo will cause the embryo to make a defense by diverting some of its energy to form thermal homeostasis, which is a physiological response. The purpose of this study was to analyze heat stress on embryonic development. This study used a systematic literature review (SLR) following PRISMA guidelines and gathered the sample of papers through the PubMed databases using keywords categorized as *heat*, *stress*, and *embryo*. There were 31 papers used as samples. The result showed that hyperthermia in chickens caused an increase in heart rate per minute as a result of temperature pressure. It affects the hatching percentage, hatching time, weight, and mortality rate in chicken embryos. In summary, heat stress has an impact on embryonic development heat stress gives an impact on the development of embryos.

**Key words:** Chicken, Embryo Development, Food Security, Health risk, Heat Stress.

## INTRODUCTION

Incubation temperature is an important factor in the development of chicken embryos. This is because temperature plays a role in determining the overall development of the embryo. In addition, temperature also affects the ability of chicken embryos to hatch from eggs (hatchability) and embryo survival after hatching.<sup>1</sup> Incubation temperature that is very high and far exceeds the normal incubation temperature is called hyperthermia.<sup>2</sup> Since the development of chicken embryos is highly dependent on temperature, hyperthermia can harm the embryo.<sup>3</sup>

The optimum temperature for the development of chicken embryos is around 37-38°C and temperatures that exceed this optimum temperature can affect the overall development of chickens.<sup>3</sup> The effect of hyperthermia could be tested on chicken embryos easily because the incubation temperature of chicken eggs can be adjusted by changing the temperature of the incubator. The organ that can be affected by incubation temperatures that are too high is the heart.<sup>4</sup> The heart is a very vital organ for an organism so failure in its development is something fatal. This can determine the survival of the embryo. The effect of hyperthermia that can be observed on the heart is the heart rate of chicken embryos.<sup>5</sup> Hyperthermia can accelerate the heart rate of chicken embryos per minute.<sup>4</sup>

High temperatures can also occur in the aves group, aves will respond physiologically by changing some of the energy in the body and forming a homeostatic state of temperature or balance.<sup>6</sup> High temperatures trigger the emergence of heat shock proteins<sup>7</sup> heat shock proteins or Hsp may be associated with tolerance to high-temperature stresses, generally heat shock proteins or Hsp that appear are proteins with a molecular weight

of 70 kDa,<sup>7</sup> research shows that *in vitro* anti-Hsp70 antibodies cause death in fibroblast cells.

In the Aves group, heat shock protein has a BM between 22 kDa to 108 kDa, heat shock protein will be expressed in different cells and tissues when there is pressure in the form of heat or high temperature both *in vivo* and *in vitro*. Expression of heat shock protein or Hsp, especially with a molecular weight of 70 kDa, often appears in broilers and other aves groups, Hsp70 expression occurs more often during stress with high temperatures and heat compared to groups that do not experience high-temperature pressure or heat. Hsp is also expressed in embryos but the mechanism of Hsp expression is not known when pressure occurs with high temperature or heat.<sup>8</sup>

There were no previous studies that observed the impact of heat stress on the embryo of chicken using a systematic literature review. Moreover, the urgency of this study was to ensure that the appropriate heat could nurture the growth of the embryo of the chicken. The purpose of this study was to analyze heat stress on embryonic development.

## MATERIALS AND METHODS

This study used systematic literature review (SLR) and gathered the sample of papers through the PubMed databases using keywords, namely "heat, stress, embryo". Based on these keywords, the articles obtained were selected first by setting several inclusion criteria including journals that are not paid / free articles, and papers that focus on "heat, stress, and embryo" as the topics. Articles that do not meet the inclusion criteria are eliminated and articles that meet the criteria will be analyzed to obtain data.<sup>9</sup>

This study used PubMed because PubMed gives free meta-data of papers to the users. Unlike Scopus or Web of Science which could not give free meta-data

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and have to pay subscription fees to the academicians,<sup>10</sup> PubMed offers the form of meta-data to be downloaded in the form of the abstract itself or the details of each selected paper.<sup>11</sup> PubMed also could reveal the tools for identifying the metric of the indexed papers itself.<sup>12</sup>

Table 1 shows the keywords that were divided based on the category. To sum up the keywords of those three categories, between the last keywords of *heat* categories to the beginning part of keywords of *stress* categories, continued to the last keywords of *stress* categories to the beginning part of keywords of *embryo* categories was added a keyword *AND*. It was to do so that the population data gathered was not too many.<sup>14</sup>

There was a total of 624.129 papers found in PubMed and they were saved into two different forms of files, namely a txt file for the abstract of each paper, and a CSV form of file for the detail of each paper. The articles then were re-selected based on inclusion criteria and exclusion.<sup>15</sup> According to the inclusion criteria, this study found there were 28.181 papers to be added and downloaded into TXT and CSV form of file.<sup>16</sup> The inclusion criteria for the samples of this study are as follows:

- The papers must be in the English Language,
- Had full abstract
- Discussed about embryo of chicken and heat
- Inside the abstract, two keywords, *heat* and *embryo* must be added in the title of the papers,
- Papers must be used in clinical trials or controlled group studies to be used for analysis
- Samples must be developed embryos of chicken inside the egg
- Papers had no conflict of interest and followed ethical principles in conducting the research process.

## RESULTS AND DISCUSSION

Before processing the data using SLR, the authors screened again the papers according to the scope of the discussion following the PRISMA guidelines.<sup>18</sup> This guideline should be followed to reduce the bias in selecting the appropriate studies for SLR as well as in proposing the result according to the selected papers as the basis of analysis.<sup>19</sup> Because the papers were too many to be included as final samples of SLR, which were more than 28 thousand; the authors helped the reviewers, who were experts in the field of veterinary, review the papers one by one by looking at the abstracts first.<sup>15,20</sup> If the abstract failed to give the expectation of the result of the study, the reviewers and authors looked up to the conclusion of the papers. It was to do so to save time for screening the papers.<sup>21</sup> There were 31 articles included as the final samples for this study.

Figure 2 can be displayed after the metadata of the downloaded documents has been analyzed using Vosviewer software. In Figure 2, it is shown that each word has a different color, depending on the novelty of the words used. The newest words in 2020 are oxidative stress, antioxidant, I-leucine, *in ovo*, and liver. These words were often used in previous research starting in 202 and after. I-leucine is an essential amino acid that is important for embryo growth and development. Heat stress can cause a decrease in I-leucine levels in embryos, which can exacerbate the negative impact of heat stress on embryo growth and development. This decrease in I-leucine levels was associated with increased embryonic lethality and reduced growth.<sup>24</sup>

## DISCUSSION

### Stress temperature on embryonic development

In general, the effects of hyperthermia vary depending on the species of animal experiencing it.<sup>21</sup> Hyperthermia in experimental animals

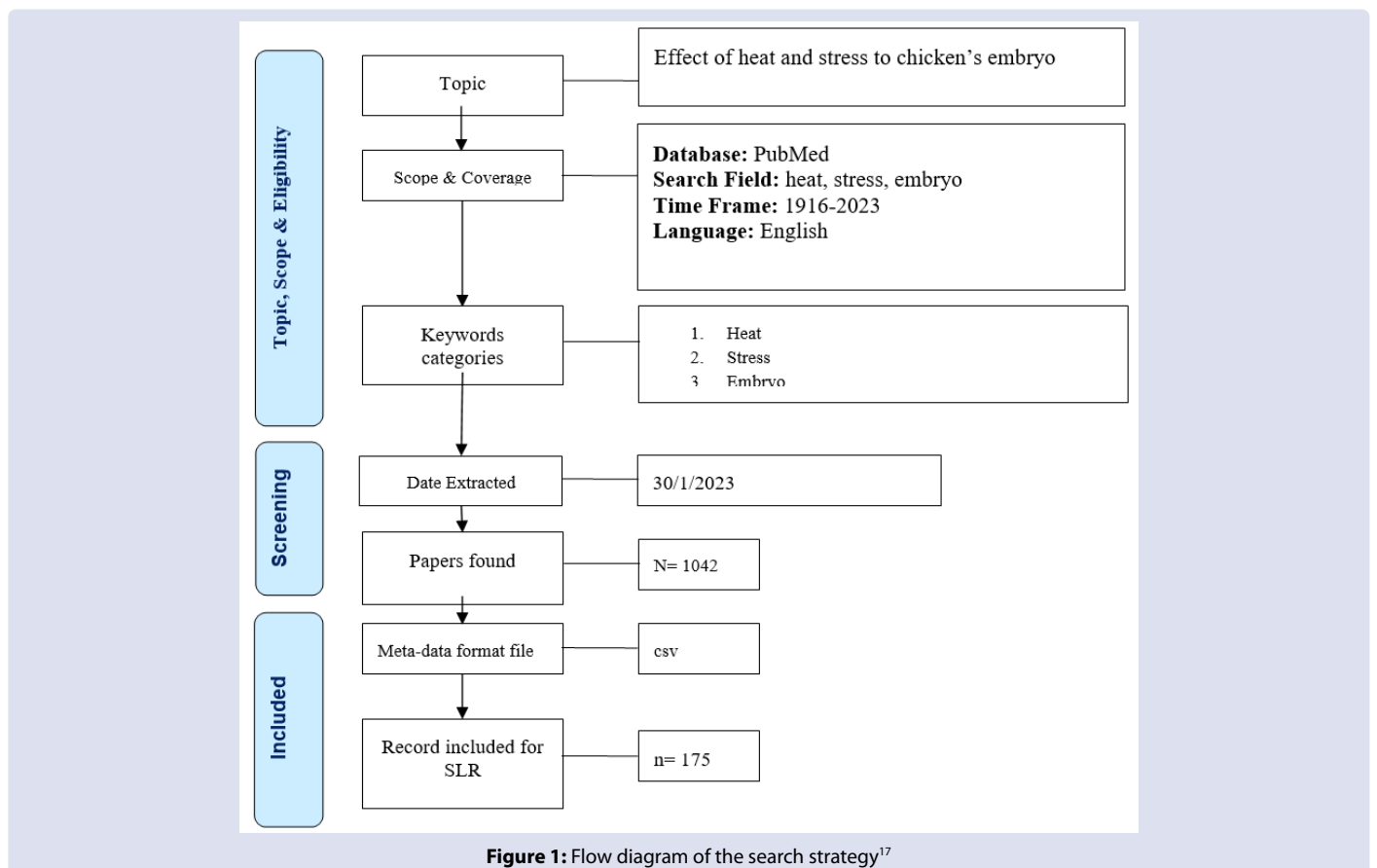
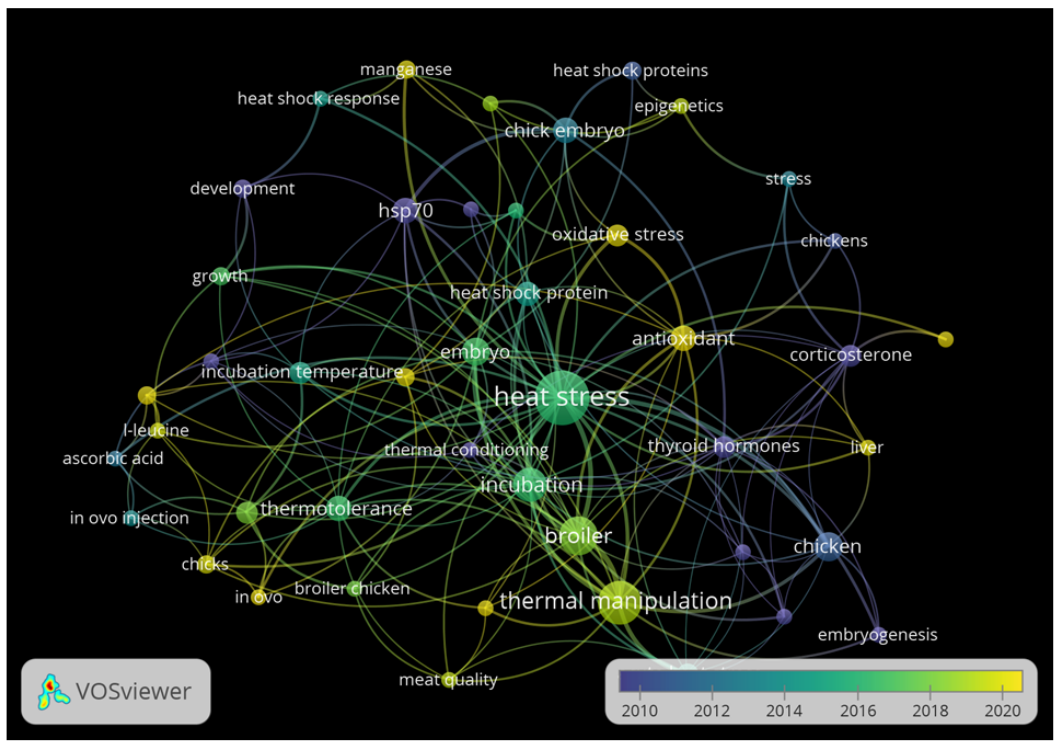


Figure 1: Flow diagram of the search strategy<sup>17</sup>



**Figure 2:** Most used words in author's keyword of previous studies related to heat stress on embryo of chicken  
**Source:** Data Processed by Vosviewer

**Table 1: The search strategy of papers using keywords.**

Heat	"warm(up)"OR"cook"OR" fry"OR" boil"OR "roast" OR"reheat"OR "make hot"
Stress	"pressure"OR"strain" OR"tension"OR"accent"OR"accentuation"OR" emphasis"OR"underscoring"OR"weight"
Embryo	"budding"OR"germinal"OR"infant"OR"ancient"OR"early"OR"pri ma"OR"primeval"

**Source:** According to the study by<sup>13</sup>

**Table 2: Development time of broiler chicken embryos after being shocked at 360°C for 10 minutes.<sup>22</sup>**

Stages	Development time (hours)				
	A	B	C	D	E
2 cells	1,30	1,10	1,18	1,00	1,27
4 cells	2,00	1,15	1,20	1,15	1,24
Morula	3,15	2,15	2,30	2,00	2,40
Blastula	8,00	6,15	6,35	6,00	7,05
Gastrula	11,00	9,17	9,45	9,00	10,00
Initial neural	14,00	10,45	11,00	10,30	11,26
Final neuron	16,50	12,20	13,00	12,00	13,25
tail bud	24,00	20,00	20,40	19,00	21,33
Hacking	81,00	73,00	75,20	67,30	78,30

**Table 3: Results of measuring the heart rate of chicken embryos.<sup>23</sup>**

Treatment	Heart rate (bpm)
Control	63.97
Treatment I	99
Treatment II	45.17

can cause anencephaly, encephalocele, micrencephaly, and others. These defects were observed in the embryos of tested mammals such as hamsters, mice, rats, monkeys, and humans.<sup>25</sup> Hyperthermia in chickens, which belong to the Aves class, has a different effect. One of them is an increase in heart rate per minute.<sup>26</sup>

The use of chicken embryos as experimental animals has the advantage that the egg incubation temperature greatly affects the temperature of the embryo so deviations from the incubation temperature from the optimum temperature can interfere with embryo development.<sup>5</sup> Hyperthermia given for a short period can cause a short-term stress response, one of which is an increase in heart rate. Mechanisms that occur in the short-term stress response include stimuli such as hyperthermia causing nerve cell activation in the hypothalamus.<sup>3</sup> These cells will send signals to the adrenal medulla through the spinal cord. This will stimulate the release of the hormone epinephrine which causes a stress response, namely an increase in heart rate.<sup>27</sup> The effects of long-term stress response include the inhibition of the immune system response which can then cause the death of the embryo so that the embryo's heart rate cannot be observed.<sup>28</sup>

According to a study conducted by,<sup>29</sup> hyperthermia can accelerate the embryo's heart rate per minute. In general, hyperthermia can increase the rate of metabolism in the body. Because the metabolic process increases, the heart activity will increase due to the increased need for oxygen and nutrient transportation so the heart rate will increase.<sup>28</sup> In addition, an excessive increase in metabolism can cause stress.<sup>30</sup>

The incubation temperature of 42°C to 44°C is a lethal or embryotoxic temperature for the development of chicken embryos.<sup>31</sup> Incubation of chickens at these temperatures can cause malformations, even death of the embryo. Therefore, it can be assumed that because the incubation temperature of 42°C and above can cause death, the heart rate observed is the heart rate of chicken embryos that have started to die. This causes the embryo's heart rate to be lower than the normal embryo's heart rate.<sup>32</sup>

The length of incubation of frog eggs depends on the species and several external factors. When the temperature is low, the chorion enzyme does not work and makes the old embryo dissolve the eggshell so that the old hatching process occurs.<sup>33</sup> The external factor that affects egg incubation is water temperature.<sup>18</sup> Temperature is an important factor in influencing the process of embryo development and egg hatchability.<sup>34</sup> It should be noted that at too low a temperature, the embryo will grow

and develop slowly. This can cause the embryo to be hatched smaller than a normal embryo and have a weak immune system. At too high a temperature, the embryo will die due to dehydration or overheating.<sup>35</sup>

### HSP70 expression in embryo development

Based on research conducted by Romanoff and Yahav *et al*, stress temperature affects embryo development including embryo weight, especially in broilers. The results of the research conducted by Morgan and Tucker<sup>36</sup> and Suarez<sup>37</sup> that temperature pressure affects hatching percentage, hatching time, weight, and mortality rate in chicken embryos. In addition, research conducted by<sup>38</sup> showed an interaction between the stage of embryo development and incubation temperature, a low temperature will prolong the incubation period in chicken embryos.<sup>39</sup> Decreased embryo growth with lower incubation temperatures appears to be related to thyroid status because lower temperatures result in reduced plasma triiodothyronine levels.<sup>40</sup>

The pressure of hot temperature will cause the expression of heat shock protein or Hsp with a molecular weight of 70 kDa known as Hsp70. Related research on chicken embryos has been carried out by,<sup>41</sup> mice there were differences in the expression of Hsp70 in embryos incubated on day 19 and day 20 incubation. Based on research conducted by Morales *et al* there was a decrease in the amount of expression on days 4 to 12 in chicken embryos (Mirke) in mouse embryos and a decrease in the number of Hsp70 on day 9 to day 12.

Based on research conducted by Manzerra *et al* in rabbits, Hsp70 was more commonly found in neural tissue than in non-neural tissue, but there was no difference in Hsp70 expression in embryos when subjected to temperature stress treatment.<sup>43</sup> Based on research conducted by<sup>44</sup> pig embryos that did not express Hsp70 when treated with high temperatures, the same results were also obtained by<sup>45</sup> that Hsp70 expression was not found in pre-implantation bovine embryos that were treated with high temperatures.

### CONCLUSION

Based on the results above, it can be concluded that the effects of hyperthermia vary depending on the species of animal experiencing it. Hyperthermia in chickens caused an increase in heart rate per minute as a result of temperature pressure. It affects the hatching percentage, hatching time, weight, and mortality rate in chicken embryos. In summary, heat stress has an impact on embryonic development. The implication of this study is to be hoped as a reference for further study that heat pressure could affect the embryo development of poultry type of animals. Further studies are needed, especially those related to other types of stress that can affect embryonic development in various poultries, such as various types of chicken, quail, and ducks.

### AUTHORS' CONTRIBUTION

Maslichah contributed to the project funding, project analysis, formal analysis, writing and editing paper, along data collection. Nor Hayati Othman contributed to the proofreading and reviewing of the draft of the paper. Akhmad Kusuma contributed to the data analysis and methodology.

### CONFLICT OF INTERESTS

This study has no conflicts of interest. Any process of this study has not involved with third party. This paper has never been published in any journal before.

### STATEMENT OF FUNDING

The funding was in the form of reimbursement after this paper was published in a Scopus-indexed journal. The Article processing charge would be reimbursed by Airlangga University upon publication.

### STATEMENT OF DATA AVAILABILITY

This study has uploaded the raw data used for this sample of the study in Zenodo. Zenodo is a platform for disseminating the data used for academic purposes. Here is the link:

[https://zenodo.org/record/7683448#.Y\\_12O3ZBwdU](https://zenodo.org/record/7683448#.Y_12O3ZBwdU)

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