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Rectal temperature and respiration rate as indicators of heat stress in broiler chickens subjected to early-age thermal conditioning and vitamin C supplementation

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Abstract: One of the major concerns in poultry production presents heat stress (HS) and it is followed by significant economic loss manifested by reduced growth, decreased immune response, increased mortality, etc. The objective of this study was to investigate whether the early-age thermal conditioning (ETC) and vitamin C (Vit.C) treatments, individually and in combination, produce their beneficial effects through increased heat release manifested as a change in respiration rate consequentially leading to a decrease in the chicken's body temperature and thus reduce the harmful effects of HS. A 400 broiler chicks mixed sex male and female were split into four groups. From day 22 through the end of breeding, Group C received Vit.C (2 g/L) diluted in water. On the fifth day of production, group T was exposed to ETC for 24 h at 38 ± 1 °C. Group TC was formed by combining treatments of groups T and C, and group K served as the control. All chicks were exposed to chronic HS in the last two weeks breeding period. On 11 out of 14 days, the highest respiration rate was observed in group TC with a statistically significant difference in comparison with the group in which the lowest values were measured. The lowest rectal temperature in the morning was measured in group TC on all 14 days. During the evening measurements, the average rectal temperature was the lowest in group TC on 13 out of 14 testing days. The ETC and Vit.C reflected to increase in respiration rate resulting in more heat being released to the environment, achieving lower body temperature in broiler chickens and the best results are seen when these methods are combined.

Key words: Vitamin C, early-age thermal conditioning, heat stress, broiler chickens, rectal temperature, respiration

1. Introduction

Heat stress (HS) is one of the greatest problems in modern breeding of broiler chickens [1], and it is followed by significant economic loss manifested by decreased immune response, altered gut flora, increased mortality, decreased growth, etc. [2]. In our previous study, we investigated the influences of the application of vitamin C (Vit.C) and early-age thermal conditioning (ETC) as methods for mitigating the effects of chronic HS and reached a conclusion that a combined application of these two methods results in a synergistic effect and improves the production results, leads to an increase in the volume of certain parts of anatomy relevant in terms of production, as well as to the improvement of meat quality with regards to the color and pH [3].

The mechanisms which help to achieve this beneficial effect have not been definitely confirmed, especially considering the synergistic effect of individual treatments. The studies published so far usually indicate that these treatments, when applied individually, achieve their effects with the help of the hormones of the adrenal glands—corticosterones [4] and thyroid hormones [5].

It has been shown that Vit.C is necessary for broiler chickens to achieve the appropriate metabolism of amino acids and minerals, as well as to synthesize hormones involved in stress resistance [6]. It has also been observed that ETC can influence the thermoregulation system, i.e. thermoregulation center in animals. The preoptic area of the hypothalamus contains thermosensitive neurons which receive temperature signals from the entire body and integrate them to produce an adequate thermoregulatory response [7]. This response includes physiological, endocrinological, and behavioral changes aimed at maintaining a relatively constant body temperature.

In the conditions of HS, birds experience rapidly and shallow breathing – panting, aimed at releasing larger

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amounts of heat through evaporation. Panting is seen as the most important heat loss mechanism in the conditions of HS [8] which is why the number of respirations can be a valid indicator of the animal's condition during HS.

In addition, other physiological parameters can be considered indicators of the animal's thermal condition, i.e. indicators of whether the animal is exposed to HS and of its intensity. One of the most commonly used parameters, as well as one of the simplest ones to determine, is body temperature. Body temperature in chickens is usually measured rectally and this method has been considered the standard one [9].

The objective of this research is to investigate whether the ETC and Vit.C treatments, individually and in combination, produce their beneficial effects through increased heat release manifested as a change in the number of respirations consequentially leading to a decrease in the chicken's body temperature.

2. Materials and methods

2.1. Animals and experimental design

The experiment involved 400 day-old Cobb 500 heavy hybrid chicks. The broiler chicks were divided into 4 groups in 4 repetitions, each group including an equal number of male and female chicks. Throughout the process of broiler production, the chickens were kept in controlled ambient conditions.

Group C received vitamin C (Veterinary Institute Subotica, Serbia) through water from day 22 until the end of the production period (day 42) in the amount of 2 g/L (1 g of vitamin C contained 100 mg of active substance). Group T was exposed to ETC on the 5th day of production over 24 h at a temperature of 38 ± 1 °C and relative humidity of 40%–60%. Group TC was exposed to ETC in the same manner as group T, but this group also received Vit.C through water in the same manner as group C, starting from the 22nd day of fattening. Group K was the control group, and the broiler chickens in this group were neither exposed to ETC nor did they receive Vit.C supplementation.

Since this research was conducted in the summer of 2018, high temperatures were expected, and they did occur on the 29th day of production, continuing until the end of the production cycle (day 42). The summer of 2018 was determined to have been the warmest summer according to the lowest air temperature since temperature measurement started in this area. On as many as 74 days the subjective temperature experience was over 30 °C.[1] Ambient temperature in the facility was measured from 08:00 h to 20:00 h in 2-h intervals. Figure shows the average temperature in the facility for the period between days 29 and 42 of breeding. These temperatures correspond to chronic HS. RH remained between 40% and 70%. On the remaining days (except for day 5 for groups T and TC) the ambient conditions were within the recommended values for this chicken hybrid and equal for all test groups. During the entire production period, the chickens received commercial broiler feed and water ad libitum.

2.2. Measuring rectal temperature

Body temperature was measured on the days when HS occurred (between days 29 and 42 of breeding) in the morning (10 h) and in the evening (18 h), i.e. before the rise of the temperature in the facility and in the evening, after the temperature had started to fall. Body temperature was measured in 20 broiler chickens in each group (5 chickens in each repetition, ensuring an equal number of male and female chickens in each group). Body temperature was

[1] Annual Bulletin for Serbia the Year of 2018. Republic Hydrometeorological Service of Serbia [online]. Website: https://www.hidmet.gov.rs/data/klimatologija/eng/2018.pdf

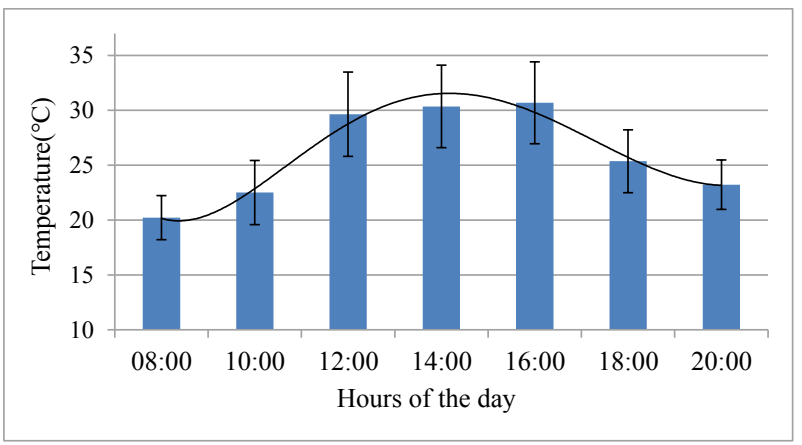

Figure. The facility's air temperature in the last two weeks of production.

measured rectally and these values were taken as indicators of thermotolerance. The instrument used for measuring was the dual-channel digital thermometer GM1312 (Benetech, China) with a K type probe, model SD0218-2 (Ootdty, China). Each rectal temperature measurement was followed by a control measurement on the second channel using a second probe to prevent potential errors in measurement. The tips of the metal probes were marked at 3 cm, to introduce the probe equally to all chickens, because different temperatures may be measured at different depths of the cloaca. The broiler chickens were captured and settled carefully to avoid exposing them to additional stress during the procedure.

2.3. Measuring respiration rate

The respiration rate was measured on the same days as body temperature (days 29–42), as well as at the same time (10 h and 18 h). Counting was performed on 16 broiler chickens in each group (4 chickens in each repetition, ensuring an equal number of male and female chickens). The counting process involved making a video at the specified time using the Canon EOS M50 4K camera (Canon, Japan) with the Canon EF-M 15-45mm f/3.5-6.3 IS STM detachable lens (Canon, Japan). The videos were subsequently visually analyzed, and the respirations were counted over the course of one minute for each observed chicken. The respirations were counted by following the respiratory movements best observed in the abdominal region, as the birds have an abdominal type of breathing due to the absence of a diaphragm.

2.4. Statistical analysis

The results are presented in the form of arithmetic mean with standard deviation. The statistic processing of data was performed using the "R" software using models of analysis of variance (ANOVA) and Duncan's posthoc test where the statistical significance was presented at the $p < 0.05$ level.

This paper is an integral part of the research for a doctoral dissertation and includes the opinion of the Ethics Committee for the Welfare of Animals Used in Animal Testing of the University of Novi Sad (EK: II-2018-02).

3. Results and discussion

The genetic advancement in the increase of production potential in broilers resulted in a significantly higher weight gain in a short time period. However, this growth was not entirely followed by the development of the cardiopulmonary system [10]. This is a result of an increase in metabolic heat production and body temperature due to larger body mass and higher metabolic processes [11], usually during the growth and final phases, when the process of heat loss is in decline [12,13].

The results regarding respiration rate are shown in Table 1 for each day when they were measured (days 29–42) for

Table 1. Respiration rate for the examined days in the morning hours (10:00 h).

Days	K	C	T	TC
29.	57.19 ± 10.48	53.63 ± 9.73	53.44 ± 11.18	53.06 ± 11.83
30.	51.38 ± 9.42	46.19 ± 7.52	50.06 ± 6.55	49.13 ± 6.65
31.	48.94 ± 7.00	48 ± 6.20	48.56 ± 10.23	48.38 ± 5.68
32.	43.13 ± 7.17	46.5 ± 3.79	44.06 ± 7.57	43.06 ± 5.84
33.	44.25 ± 7.94	45.38 ± 6.65	43.88 ± 9.79	47.06 ± 8.88
34.	41.81 ± 4.83	41.81 ± 4.71	40.12 ± 5.46	42.56 ± 8.43
35.	40.44 ± 5.78	43.69 ± 9.55	42 ± 6.75	45.94 ± 7.95
36.	41.06 ± 6.55	44.94 ± 7.22	38.81 ± 10.81	41.25 ± 8.66
37.	43.69 ± 8.90	46.88 ± 6.38	43.69 ± 9.29	47.06 ± 11.73
38.	52.88 ± 7.58	56.63 ± 15.87	50.63 ± 6.83	53.25 ± 15.08
39.	46.13 ± 6.09	48.19 ± 6.79	50.25 ± 6.62	52.5 ± 12.63
40.	50.44 ± 7.99	56.63 ± 13.00	49.31 ± 13.05	53.44 ± 10.58
41.	49 ± 9.18	48 ± 11.17	55.88 ± 18.31	49.5 ± 9.22
42.	65.13 ± 21.65	65.13 ± 26.04	63.38 ± 25.78	63.63 ± 16.99

* K: control group, without any treatment. C: group received Vit.C (2 g/L) diluted in water from 22nd to 42nd days of age. T: group exposed to ETC for 24 h on the fifth day of age. TC: group exposed to ETC for 24 h on the fifth day of age and received Vit.C (2 g/L) diluted in water from 22nd to 42nd days of age.

a,b (if present): Mean values within a column with different lower-case symbol differ significantly ($p < 0.05$) in respiration rate.

values measured in the morning (10 h) and in Table 2 for the evening (18 h) measured. If we consider the results of the morning measurements (10 h), there is no statistically significant difference between the test groups on the days of testing. The results indicate that in the evening (18 h) there was a statistically significant difference between the test groups on 13 of 14 days of tracking these data. On 11 out of 14 days, the highest values were observed in group TC with a statistically significant difference in comparison with the group in which the lowest values were measured, which was predominantly group K. On the remaining 3 days on which group TC did not have the best results in the evening, it was observed that there was no statistically significant difference between group TC and the group which had the best results that day.

Since broiler chickens do not have sweat glands to release heat through perspiration and evaporation and they do not have enough body areas which are not covered in feathers to efficiently release heat by means of conduction, radiation and convection [14], the most efficient heat loss mechanism is the increase in respiratory rate (tachypnea) and shallow breathing (polypnea) leading to panting which results in efficient heat release through the evaporation of water from the respiratory tract [15]. Respiration rate, as a physiological parameter, changes throughout life, and most authors define a range from 20 respirations per minute [16] to 40 respirations per minute [9]. Marchini et al. [17] define the values for the number of respirations of broiler chickens according to the number of weeks of breeding, and this number varies from 48 respirations per minute in the first week of breeding to 42 respirations in the sixth week of breeding. The values in this study were significantly higher than the aforementioned, especially in the evening, due to the presence of HS during the process of breeding at the time when this parameter was determined. With regards to the number of respirations in the morning, the values were within physiological ranges or slightly higher, which can be attributed to the effects of chronic HS as well.

The number of respirations changes significantly depending on the ambient temperature, so the authors Silva et al. [18] observed values of up to 165 respirations per minute in the final phases of production when the HS was present in the process of breeding. In our study, the highest observed single value of the number of respirations was 210 respirations per minute and this value was observed in a chicken in group C, in the evening of the 38th day of breeding. Apart from heat release, such high values of the number of respirations may lead to dehydration, as well as to a change in acid-base balance, since increased CO_2, exhalation can result in hypocapnia and potential respiratory alkalosis [15].

Rectal temperature values are shown in a similar way as the number of respirations, in Table 3 for morning measured and in Table 4 for evening measured. A statistically significant difference was observed between

Table 2. Respiration rate for the examined days in the evening hours (18:00 h).

Days	K	C	T	TC
29.	130.5 ± 25.29^{b}	131.81 ± 22.91^{b}	129 ± 24.32^{b}	150.56 ± 13.38^{a}
30.	123.56 ± 16.07^{b}	133.69 ± 23.62^{ab}	134.44 ± 24.08^{ab}	148.88 ± 27.67^{a}
31.	102.75 ± 28.81^{b}	128.44 ± 26.93^{ab}	133.88 ± 24.27^{a}	138.19 ± 29.42^{a}
32.	117.38 ± 26.90^{b}	138.19 ± 30.60^{ab}	138.38 ± 25.36^{ab}	154.5 ± 31.86^{a}
33.	112.13 ± 22.66^{b}	135.19 ± 28.41^{a}	134.06 ± 20.92^{a}	150.19 ± 26.39^{a}
34.	113.25 ± 16.60^{b}	141.38 ± 19.16^{a}	139.5 ± 20.76^{a}	151.88 ± 23.69^{a}
35.	114.56 ± 22.43^{b}	145.69 ± 19.41^{a}	135.38 ± 23.82^{a}	146.63 ± 30.87^{a}
36.	95.44 ± 26.39^{b}	147.19 ± 23.07^{a}	139.5 ± 24.35^{a}	150.19 ± 27.84^{a}
37.	95.06 ± 42.62^{b}	142.13 ± 22.37^{a}	131.15 ± 41.10^{a}	139.5 ± 38.34^{a}
38.	121.31 ± 25.92^{b}	163.5 ± 21.18^{a}	145.69 ± 22.40^{a}	154.5 ± 26.61^{a}
39.	140.44 ± 24.15^{b}	160.5 ± 29.41^{a}	152.06 ± 22.09^{ab}	161.63 ± 20.92^{a}
40.	142.88 ± 21.29^{ab}	151.13 ± 27.10^{a}	130.88 ± 18.87^{b}	151.5 ± 22.69^{a}
41.	125.44 ± 21.33	131.63 ± 18.42	134.5 ± 11.73	122.38 ± 16.04
42.	110.13 ± 14.41^{b}	118.75 ± 11.73^{b}	119.38 ± 31.56^{b}	134.13 ± 9.95^{a}

* K: control group, without any treatment. C: group received Vit.C (2 g/L) diluted in water from 22nd to 42nd days of age. T: group exposed to ETC for 24 h on the fifth day of age. TC: group exposed to ETC for 24 h on the fifth day of age and received Vit.C (2 g/L) diluted in water from 22nd to 42nd days of age.
a,b,c (if present): Mean values within a column with different lower-case symbol differ significantly ($p < 0.05$) in respiration rate.

Table 3. Rectal temperature for the examined days in the morning hours (10:00 h).

Days	K	C	T	TC
29.	41.23 ± 0.69^{b}	41.82 ± 0.48^{a}	41.34 ± 0.51^{b}	40.98 ± 0.71^{b}
30.	42.07 ± 0.29^{a}	42.2 ± 0.52^{a}	40.88 ± 0.26^{b}	40.17 ± 0.44^{c}
31.	42.22 ± 0.36^{a}	42.45 ± 0.46^{a}	41.55 ± 0.52^{b}	41.06 ± 0.35^{c}
32.	42.73 ± 0.57^{b}	43.68 ± 0.42^{a}	42.63 ± 0.89^{b}	42.11 ± 0.72^{c}
33.	42.11 ± 0.56^{a}	42.14 ± 0.49^{a}	41.23 ± 0.30^{b}	41.12 ± 0.47^{b}
34.	41.27 ± 0.54^{a}	40.82 ± 0.38^{b}	40.59 ± 0.49^{b}	40.56 ± 0.33^{b}
35.	42.43 ± 0.49^{a}	41.97 ± 0.27^{b}	41.97 ± 0.30^{b}	41.2 ± 0.27^{c}
36.	41.73 ± 0.64^{a}	41.08 ± 0.52^{bc}	41.21 ± 0.56^{b}	40.75 ± 0.34^{c}
37.	42.08 ± 0.24^{a}	41.71 ± 0.38^{b}	41.41 ± 0.38^{c}	41.11 ± 0.29^{d}
38.	41.97 ± 0.48^{a}	41.97 ± 047^{a}	41.36 ± 0.33^{b}	41.04 ± 0.38^{c}
39.	41.55 ± 0.53^{a}	41.55 ± 0.34^{a}	41.2 ± 0.49^{b}	40.92 ± 0.35^{c}
40.	41.07 ± 0.29^{bc}	41.35 ± 0.69^{ab}	41.44 ± 0.37^{a}	40.99 ± 0.36^{c}
41.	41.7 ± 0.47^{a}	41.5 ± 0.45^{a}	41.61 ± 0.62^{a}	40.66 ± 0.33^{b}
42.	42.12 ± 0.45^{a}	42.09 ± 0.45^{a}	41.89 ± 0.46^{a}	41.08 ± 0.29^{b}

* K: control group, without any treatment. C: group received Vit.C (2 g/L) diluted in water from 22nd to 42nd days of age. T: group exposed to ETC for 24 h on the fifth day of age. TC: group exposed to ETC for 24 h on the fifth day of age and received Vit.C (2 g/L) diluted in water from 22nd to 42nd days of age.
a,b,c (if present): Mean values within a column with different lower-case symbol differ significantly ($p < 0.05$) in rectal temperature.

Table 4. Rectal temperature for the examined days in the evening hours (18:00 h).

Days	K	C	T	TC
29.	42.71 ± 0.42^{a}	42.44 ± 0.42^{a}	42.01 ± 0.40^{b}	41.580.45^{c}
30.	43.25 ± 0.45^{a}	42.94 ± 0.74^{ab}	43.02 ± 0.28^{ab}	42.84 ± 0.49^{b}
31.	43.29 ± 0.36^{a}	43.06 ± 0.57^{ab}	42.16 ± 0.57^{c}	42.96 ± 0.46^{b}
32.	43.19 ± 0.47^{a}	43.04 ± 0.43^{a}	43.13 ± 0.47^{a}	42.67 ± 0.41^{b}
33.	43.31 ± 0.61^{a}	43.09 ± 0.70^{a}	42.62 ± 0.58^{b}	42.5 ± 0.56^{b}
34.	43.02 ± 0.60^{a}	42.76 ± 0.45^{ab}	42.66 ± 0.44^{b}	41.94 ± 0.44^{c}
35.	43.18 ± 0.98^{a}	43.23 ± 0.62^{a}	43.04 ± 0.29^{a}	42.4 ± 0.49^{b}
36.	43.4 ± 0.68^{a}	42.97 ± 0.50^{b}	43.1 ± 0.59^{ab}	42.52 ± 0.65^{c}
37.	42.87 ± 0.40^{a}	42.63 ± 0.48^{a}	42.8 ± 0.30^{a}	42.08 ± 0.64^{b}
38.	42.97 ± 0.33^{a}	42.97 ± 0.48^{a}	42.69 ± 0.47^{a}	42.22 ± 0.43^{b}
39.	43.14 ± 0.50^{a}	43.28 ± 0.58^{a}	43.07 ± 0.51^{a}	42.51 ± 0.61^{b}
40.	43.24 ± 0.43^{a}	42.77 ± 0.50^{b}	42.81 ± 0.78^{b}	42.51 ± 0.44^{b}
41.	43.43 ± 0.30^{a}	43.19 ± 0.27^{a}	43.34 ± 0.48^{a}	42.48 ± 0.37^{b}
42.	43.96 ± 0.47^{a}	43.88 ± 0.77^{a}	43.11 ± 0.53^{b}	42.44 ± 0.61^{c}

* K: control group, without any treatment. C: group received Vit.C (2 g/L) diluted in water from 22nd to 42nd days of age. T: group exposed to ETC for 24 h on the fifth day of age. TC: group exposed to ETC for 24 h on the fifth day of age and received Vit.C (2 g/L) diluted in water from 22nd to 42nd days of age.
a,b,c (if present): Mean values within a column with different lower-case symbol differ significantly ($p < 0.05$) in rectal temperature.

test groups on all testing days, both in the morning and in the evening. With regards to morning measurements (10 h), the lowest rectal temperature was measured in group TC on all 14 days. During the evening measurements (18 h), the average rectal temperature was the lowest in group TC on 13 out of 14 testing days. The only exception was the 31st day of breeding when group T achieved the lowest average rectal temperature.

Published research suggests several ideal body temperature values and they are usually in the range between 41 ˚C and 42 ˚C for broiler chickens within the thermoneutral zone [9,19]. In the conditions of HS, these values can reach a temperature of up to 46 °C in the final days of breeding [18], resulting in the death of broiler chickens. In our study, it can be observed that the average morning temperature values measured rectally were mostly within the range considered physiological or they differed slightly from the optimal values, which was particularly the case in group K. With regards to evening temperatures, it can be observed that they were higher than the morning ones which can clearly be attributed to HS. On most days when this parameter was tracked, these values were above physiological, i.e. above 42 °C. These values reached their highest average on the last day of breeding in group K (43.96 ± 0.47), while the lowest values were observed on the first day of HS in group TC (40.98 ± 0.71). These results suggest that chronic HS gradually leads to the fatigue of thermoregulatory mechanisms and that it eventually becomes more difficult for the broiler chickens to keep their body temperature within physiological limits for longer periods of the duration of these ambient conditions. This is particularly prominent in group K which had the least favorable results in terms of body temperature measured both in the morning and in the evening during the period of HS. It is also evident that the best body temperature results were achieved by group TC with a statistically significant difference on most days during the period of HS. These results of the measurement of body temperature are in accordance with the number of respirations, suggesting that this is the mechanism which birds use to reduce HS and maintain their body temperature. Groups T and C had results in the range between K and TC, suggesting that the combination of individual treatments achieves a synergistic effect and leads to the best results. This can also explain the results observed in our previous study [3], where the best results with regards to production results, volume of certain parts of anatomy and the quality of chicken breast meat were observed precisely in group TC, which is in accordance with the results for the measured body temperature and the number of respirations.

It has been shown that the application of ETC at a particular age influences the thermoregulatory center in the front part of the hypothalamus where certain genes, such as *R-Ras3*, *BDNF* and 14-3-3Ɛ, are increasingly expressed, suggesting that they could play a role in this epigenetic regulation [20,21]. It is worth mentioning that the neurotrophic factor *BDNF* is specifically bound to the *TrK-B* receptor and it initiates the phosphorylation of tyrosine which activates the phosphotyrosine-binding domain. This initiates the *RAS* intracellular pathway and leads to the transduction of genes involved in the growth and maintenance of nerve cells, which could be significant from the aspect of the preservation of the function of the nerve cells of the thermoregulatory center. It has also been shown that Vit.C plays an important role in the synthesis of the hormones involved in stress resistance [6]. Therefore, the physiological mechanisms of the effects of these two methods differ significantly, which accounts for the best results in group TC which received both treatments.

4. Conclusion

Based on the observed results, we can conclude that with regards to the application of Vit.C and ETC in the breeding of broiler chickens when HS is present in the process of breeding, these two methods achieve the best results when combined. The mechanism of their effect is reflected in the increase in the number of respirations resulting in more heat being released to the environment, achieving lower body temperature in broiler chickens subjected to both ETC and Vit.C supplementation. We can thus claim that those chickens had the best response to HS and were therefore able to achieve the best production results, as well as an increase in the volume of certain parts of anatomy and better meat quality observed in our previous study [3].

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