# Research Note: Effects of the in ovo injection of organic zinc, manganese, and copper and posthatch holding time before placement on broiler body temperature during grow out[1,2,3]

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**ABSTRACT** Effects of the in ovo injection of organic microminerals (**OM**) (zinc, manganese, and copper) and posthatch holding time (**HT**) on the daily body temperature (**bt**) of broilers during grow out were determined. The hatching eggs from a Ross 708 breeder flock at 32 wk of age were incubated under standard commercial conditions. At 17 d of incubation, eggs were randomly allocated to 3 in ovo OM injection treatment (**TRT**) groups, and at 21 d of incubation, male hatchlings were randomly allocated to 2 posthatch HT treatment groups. Eggs were either not injected or were in ovo injected with diluent only or diluent containing the OM mixture. A 0-hour HT group had immediate access to water and feed, and a 24-hour HT (**24HT**) group contained birds that were kept in transport baskets in their pens without access to feed and water for 24 h before being released. Fifteen male birds were placed in each of 36 litter floor pens in a temperature-controlled facility. Approximately 2 birds in each of 6 replicate pens belonging to each TRT-HT combination had temperature transponders inserted subcutaneous in the mid-dorsal region of the neck. All birds were brooded under standard commercial conditions and had ad libitum access to feed and water after their respective HT. The bt of the same birds were determined daily at the same time each day beginning at hatch and ending on 39 d of posthatch age (**AGE**). There were no significant main or interactive effects involving TRT or HT for bt. However, there was a significant ($P \leq 0.0001$) main effect because of AGE. A general increase in bt occurred during the 39 d grow out period. At hatch, bt was 40.54 ± 0.056°C and at AGE 39 was 41.46 ± 0.055°C. Under standard brooding conditions, a general increase in bt occurred in the Ross 708 broilers. However, these birds did not exhibit a significant bt response to TRT or a 24HT before placement.

**Key words:** body temperature, broiler, feed and water restriction, holding time, posthatch

2021 Poultry Science 100:755–759
https://doi.org/10.1016/j.psj.2020.10.048

## INTRODUCTION

Yair and Uni (2011) reported that the yolk concentrations of zinc (**Zn**), copper (**Cu**), and manganese (**Mn**) are very low at hatch. Sirri et al. (2016) noted that male Ross 308 broiler chicks provided organic rather than inorganic sources of Zn, Cu, and Mn in their diets were heavier, had a higher daily BW gain, and a better feed conversion ratio at 31 and 51 d of age. In ovo feeding of the chicken embryo has also been shown to improve their subsequent BW through 35 d after hatch (Uni and Ferket, 2004). In a companion study by Oliveira et al. (2015a), it was observed that a posthatch holding time (**HT**), involving a 24-hour delay in access to feed and water (**24HT**) before chick placement negatively affected performance through 2-week posthatch in comparison with hatchlings that were provided immediate access to feed and water (**0HT**).

Nascimento et al. (2017) reported that the metabolic heat production of broiler chickens increased linearly with increasing BW. It is well documented in the



Received July 7, 2020.
Accepted October 20, 2020.
[1]This publication is a contribution of the Mississippi Agricultural and Forestry Experiment Station.
[2]This material is based on work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch project under accession number 329260.
[3]Use of trade names in this publication does not imply endorsement by Mississippi Agricultural and Forestry Experiment Station of these products, nor similar ones not mentioned.
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literature that a delay in feed access after hatch is detrimental to posthatch chick performance, which includes growth, organ development, and immune and digestive functionality (Willemsen et al., 2010). Therefore, the objective of this study was to investigate the effect of the in ovo injection of an organic mineral (**OM**) solution containing Zn, Cu, and Mn, in association with posthatch feed and water restriction, on the body temperature (**bt**) of a modern commercial strain of broilers throughout a posthatch grow out period.

## MATERIALS AND METHODS

### Eggs and Incubation

The protocols for the present study were approved by the Institutional Animal Care and Use Committee of Mississippi State University (approval number was #13-016). Hatching eggs were obtained from a breeder flock (Ross 708) at 32 wk of age and then stored under commercial conditions (19°C and a 75% RH) for a maximum of 2 d. The mean weight of all the eggs before being set was 64.6 ± 0.15 g. The individual weights of all the eggs were subsequently obtained, and only those that weighed within 10% of the mean weight of all the eggs obtained were set for incubation. The materials and methods of egg handling and incubation were as described in detail by Oliveira et al. (2015b). Briefly, 26 eggs were randomly assigned to each prespecified treatment group on each of 6 replicate tray levels in 3 NatureForm incubators (Model 2340; NatureForm, Jacksonville, FL). Eggs were incubated under standard commercial conditions. On 12 d of incubation, all eggs were candled, and those eggs with shells that were cracked or that were unfertilized or contained dead embryos were discarded.

### Injection Solutions

Three in ovo injection treatment (**TRT**) groups were designated at 17 d of incubation. The first was a noninjected control group containing eggs that were not injected but were subjected to the same handling procedures, including all environment and temperature changes, as the following TRT groups. The second group contained fertile eggs injected with 150 μL of commercial diluent (Poulvac Sterile Diluent; Pfizer, Exton, PA) and were designated as diluent-injected controls. The third group comprised fertile eggs injected with 150 μL of diluent enriched with the added OM containing organic Zn, Cu, and Mn (Mintrex Zn, Cu, and Mn; Novus, Saint Louis, MO). The injected solution volume (150 μL) was the same as that used by Oliveira et al. (2015a,b). The concentrations of the organic Zn, Cu, and Mn in the enriched solution containing diluent were 0.544, 0.030, and 0.260 mg/mL, respectively, and the total amounts of organic Zn, Cu, and Mn injected into each egg were 0.0816, 0.0045, and 0.0390 mg, respectively. The respective osmolality and pH of the solution used in the diluent-injected control TRT were 293 mOsm and 6.89, and the respective osmolality and pH of the solution used in the TRT that received diluent enriched with the OM were 311 mOsm and 6.85. The approximate average weight of the yolks in the hatching eggs of this study were 20 g (0.31 [30%] × 64.6 g). Subsequently, the respective concentrations of Zn, Cu, and Mn that occur naturally in yolk are 0.038, 0.0013, and 0.0011 mg/g, and those that were supplemented by in ovo injection on a similar yolk basis were 0.0041, 0.00023, and 0.0020 mg/g, respectively. Therefore, the supplemental levels of Zn and Cu were approximately 10-fold lower, whereas that for Mn was approximately 2-fold higher than the preexisting levels in the yolk.

The chelated trace minerals combine HMTBa (hydroxy analog of methionine) with an essential trace mineral in a two-to-one chelated molecule. The advantage of organic compared with inorganic trace minerals is that the binding of the mineral to the organic ligand provides stability of the complex in the upper gastrointestinal system. The injection procedure was as previously described in detail by Oliveira et al. (2015b). In brief, using an Embrex Inovoject M (Zoetis; Florham Park, NJ) multiegg injector, embryonated eggs were injected at an approximate 2.54 cm depth from the top of the large end of the egg to target the amnion. Using a water-soluble dye to validate the site of injection in 112 test eggs, 88.4% were injected in the amnion and 8.0% were injected intramuscularly. After injection, the eggs were transferred to a Jamesway model PS 500 hatcher unit (Jamesway Incubator Company Inc. Cambridge, Ontario, Canada) and were incubated under standard commercial conditions. Egg injection and handling before transfer required a maximum of 5 min at a room temperature of 23.9°C, and the incubator doors were kept closed during egg injection and handling in the room. The positions of the TRT replicate groups in the hatcher corresponded to their positions in the setter.

### Grow Out Phase

At hatch, chicks belonging to a common TRT replicate group from each incubator were pooled together and were subsequently sexed. Each of the 3 TRT groups from the incubation phase were then subdivided into another 2 posthatch HT groups, which resulted in a total of 6 treatments (3 TRT × 2 HT). Fifteen male birds were randomly allocated to each of 6 mini pens (0.914 m × 1.219 m) representing an individual TRT-HT treatment combination. The mini pens were floor pens containing pine shavings litter. Initial male bird density in each of the 36 mini pens was approximately 0.074 $m^2$ per bird. The first HT group, designated as having a 0HT, had immediate access to water and feed, and the second HT group, designated as having a 24HT, contained birds that were kept in transport baskets for 24 h before being placed inside their respective treatment-replicate pen. For birds in the 0HT treatment group, standard brooding conditions and ad libitum feed and water were provided from 0 to 39 d after hatch. Birds in the 24HT treatment group were likewise provided

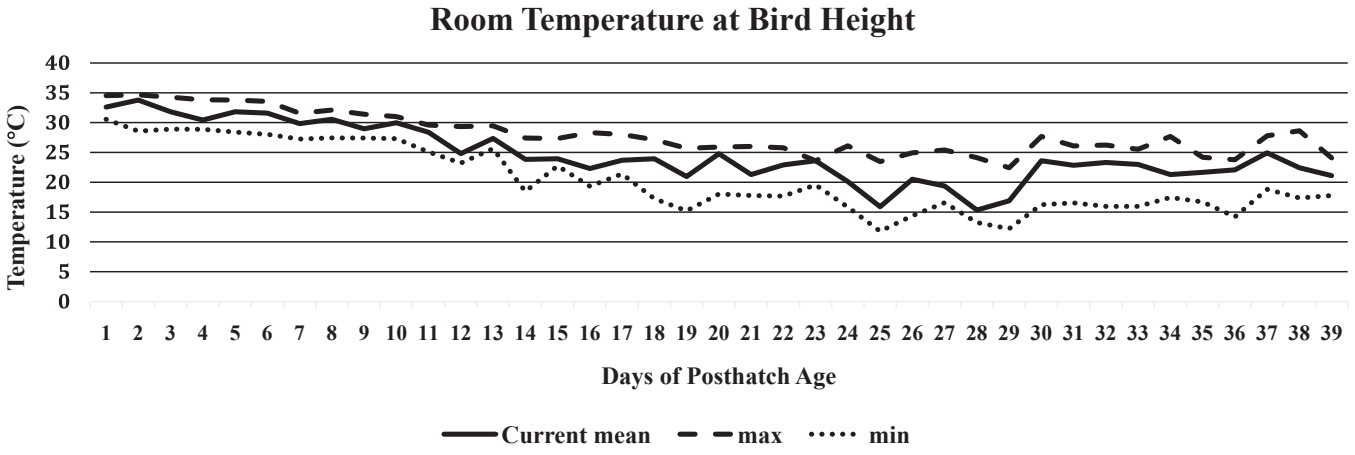


**Figure 1.** Room temperature at bird height.

the same conditions and were later provided ad libitum access to feed and water after the HT period. There were 6 replicate groups of pens (blocks) in which all 6 TRT-HT treatment combinations were randomly represented. The replicate groups of pens were arranged throughout the grow out facility in a manner that prevented potential environmental positional effects.

In each replicate pen belonging to each TRT-HT combination, the bt of 2 birds that had transponders inserted subcutaneous in the mid-dorsal region of the neck were recorded at the same time (2:00 PM) each day beginning immediately at hatch and ending at age (**AGE**) 39. Transponder data were read by a probe reader-programmer. The IPTT-300 implantable programmable temperature transponders and DAS-6006/7 Smart probe were manufactured by Bio Medic Data Systems Inc. (Seaford, DE). A single bt reading was taken for each bird each day to minimize handling stress in the birds, and readings were recorded at the same time each day to avoid the potential effects of circadian bt fluctuations. In addition, temperature sensors (HOBO ZW Series wireless data loggers [Onset Computer Corporation, Bourne, MA]), set at chick height and that had their positions adjusted weekly for subsequent increases in chick height, were placed on opposite ends of the facility to record current, minimum, and maximum temperatures beginning at 1 d and ending at 39 d after hatch. Current sensor temperature readings were taken at the same time (10:00 AM and 2:00 PM) each day. The daily means of these morning and afternoon readings are provided in a graphical form in Figure 1. In each pen, mortality was recorded daily, and selected birds were necropsied to confirm their sex.

## Statistical Description

The trial included 6 experimental treatments that were arranged in a 3 × 2 factorial design (3 TRT groups and 2 posthatch HT), with each experimental treatment replicated 6 times. A randomized complete block design

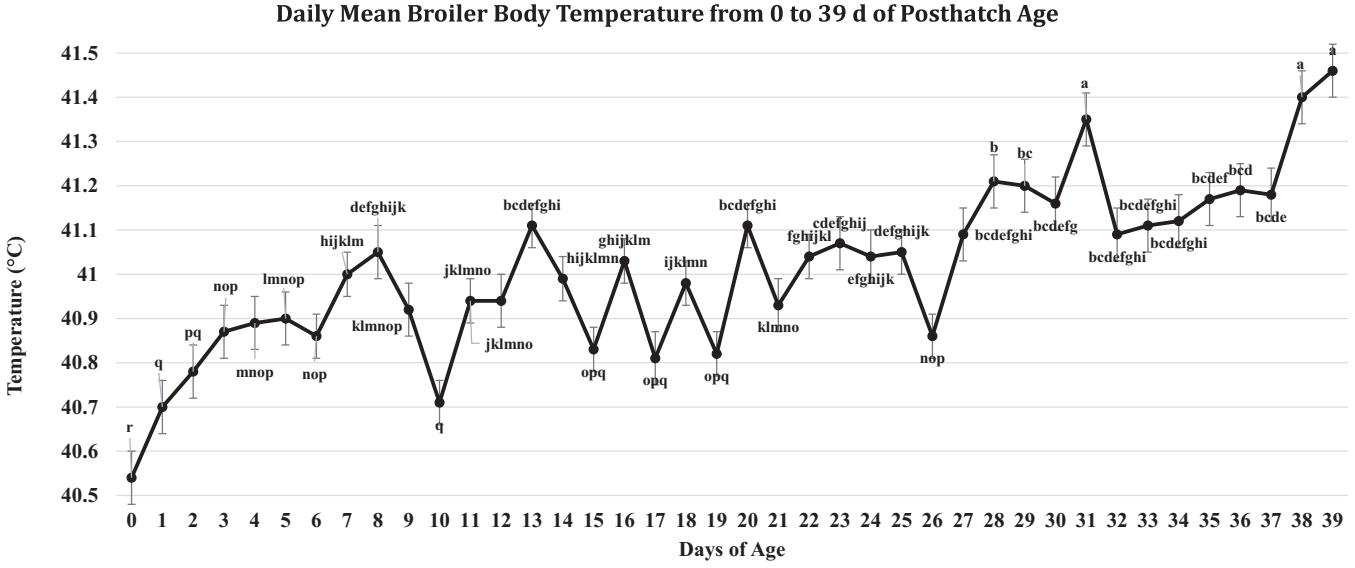


**Figure 2.** Daily mean broiler body temperature from 0 to 39 d of posthatch age. [a–r]Means with no common superscript differ ($P \leq 0.05$). N = 72 (2 birds × 6 replicate pens × 6 treatment combinations) at each day of age.

was used in the arrangement of the placement of chicks in the floor pens. Each of the 3 TRT groups were allocated to each of 2 HT groups at hatch. Each of the 6 groups of grow out floor pens (blocks) represented a replicate unit for each of the 6 TRT-HT treatment combinations. The TRT and HT treatments were designated as fixed effects and block as a random effect, with the main and interactive effects of TRT, HT, and AGE being tested. The daily bt of each of the individual experimental birds were subjected to repeated measures analysis between posthatch AGE 0 (hatch) and 39. Least squares means were compared in the event of significant global effects. The MIXED procedure of SAS software 9.2 (SAS Institute, 2010) was used for all data analyses. Global effects and least squares means differences were considered significant at $P \leq 0.05$.

## RESULTS AND DISCUSSION

Oliveira et al. (2015b) previously reported that in comparison with diluent-injected controls, fertile egg hatchability at 20.5 and 21.5 d of incubation was not significantly affected, but in comparison with noninjected controls, the OM treatment significantly ($P = 0.04$) reduced fertile egg hatchability at 21.5 d of incubation but not at 20.5 d of incubation. Nevertheless, there were no significant TRT × HT ($P = 0.3667$), TRT × AGE ($P = 0.9309$), HT × AGE ($P = 0.0932$), or TRT × HT × AGE ($P = 0.7796$) interactions on bird bt. Furthermore, there were no significant main effects because of TRT ($P = 0.8114$) or HT ($P = 0.7106$) on bt. Mean bt in the noninjected and diluent-injected controls and OM-injected TRT groups were 41.01 ± 0.04, 41.03 ± 0.04, and 41.00 ± 0.04°C, respectively. Mean bt in the 0HT and 24HT groups were 41.00 ± 0.03 and 41.02 ± 0.03°C, respectively. However, there was a significant ($P < 0.0001$) main effect because of AGE. Daily mean broiler bt from AGE 0 to 39 are provided in Figure 2. An overall increase in bt occurred during the 39 d grow out period, with a bt of 40.54 ± 0.06°C observed at hatch and a bt of 41.46 ± 0.06°C observed at AGE 39.

Across HT and TRT, it was observed in the present study that chick bt exhibited an overall increase between 0 and 39 d after hatch. Metabolic heat production is influenced by live broiler chick BW and has been reported by Nascimento et al. (2017) to increase from 56.62 ± 1.64 W/m$^2$ at hatch to 67.89 ± 1.90 W/m$^2$ on the sixth day after hatch. A higher level of heat production was also observed at 42 d of age in that report and was noted to be associated with an increase in live BW. The overall increase in bt between 0 and 39 d after hatch in the present study was likewise related to an increase in BW between 0 and 42 d of age, as reported in the companion article by Oliveira et al. (2015a).

Although the heat production of chicks increases with their posthatch age, this increase has been observed to be lower for those that hatch early (Willemsen et al., 2010). Time of hatch is related to the duration of the delay between time of hatch and the bird's access to feed. Nevertheless, subjection of the hatchlings in the present study to a 24HT, where access to feed and water were denied but where standard brooding conditions were provided, did not significantly affect their bt throughout the 39 d period in which bt was recorded. Body temperature is influenced by environmental temperature, feed intake, and composition of the diet. Conversely, these results suggest that the birds in the 24HT treatment were able to maintain a bt that was similar to that of those in the 0HT treatment when standard brooding conditions were provided and despite a 24-hour delay in feed and water access.

Delays in access to feed for 24 and 48 h has depressed the growth of broilers through 7 d after thatch, with the extent of effect being influenced by the time of hatch within the hatching window (Careghi et al., 2005). In the companion article by Oliveira et al. (2015a) in which the effects of HT and TRT on broiler performance were evaluated, it was observed that there were no significant TRT × HT interactions or main effects because of TRT on posthatch bird mortality, BW, BW gain, fed intake, or feed conversion. However, in comparison with birds in the 0HT treatment, those in the 24HT treatment had a significantly lower feed intake and gain in BW between 0 and 7 and between 0 and 14 d of posthatch age, whereas BW gain did not differ significantly because of HT between 0 and 21, 0 and 35, and 0 and 42 d after hatch. Therefore, the birds in the 24HT treatment were able to compensate by 21 d after hatch for the reduction in BW at 14 d after hatch.

Maman et al. (2019) observed that chicks with an elevated bt (42.6°C) at 1 d of age exhibited a lower BW and a poorer level of posthatch performance. Nevertheless, in this present study, it appears that during the first 2 wk after hatch, when growth was adversely affected by a 24 h delay in the chick's access to feed and water, the bt of the birds was not an appreciable factor in that effect. The birds were also apparently able to maintain homeothermy, despite a decrease in room temperature during this time, as depicted in Figure 2. The lack of an OM TRT effect on bt in the present study, therefore, coincides with the lack of an associated effect on overall performance, including BW gain, that was reported for these birds by Oliveira et al. (2015a). It was expected that by increasing mineral availability to the embryo through in ovo injection, that it would subsequently enhance overall growth, particularly when mineral injection was used in conjunction with an imposed negative influence on growth rate owing to the 24HT TRT. However, Oliveira et al. (2015a) showed that there were no TRT × HT interactions for BW gain. This would indicate that the subjection of the birds to the 24 h HT did not influence the effects of the in ovo OM TRT on broiler growth or bt.

## ACKNOWLEDGMENTS

We express appreciation for the financial support of the United States Department of Agriculture (USDA;

Grant No. 58-6406-4-016), and of the Brazilian Council for Scientific and Technological Development.

## DISCLOSURES

The authors declare that there is no conflict of interest.

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