"Temperature training" during the last days of incubation: a new method to improve poultry performance

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Introduction

Incubation climate can significantly influence hatchability, chick quality, and later performance in poultry. One of the most important factors is the incubation temperature (Decuypere and Michels, 1992). Changes of only 1°C from the optimum have a major impact, for instance, on hatching results in turkeys (French, 1994), but the strength of this influence depends on the time frame used and the duration of changes in incubation temperature during embryogenesis (French, 2000). Further, during 'critical periods' of early development incubation climate may cause life-long modifications in body functions (Tzschentke and Plagemann, 2006).

Questions of interest are, whether the development of body functions can be improved by short-term prenatal temperature stimulation and which time window of the incubation period is optimal for the application of such stimulations. In poultry, the final incubation period is characterized by well-developed body functions (e.g. temperature regulation, Tzschentke 2007) as well as dramatic changes in the quality of regulatory processes (Tzschentke and Plagemann, 2006). On the one hand, environmental stimulation could improve the maturation and adaptability of body functions ('training effect', Nichelmann and Tzschentke, 2002). The end of the incubation period seems to be a 'critical developmental period' so that long-lasting effects of temperature stimulation on body functions and, finally, on the post-hatching performance can be expected. Results from a previous study with a small sample of broiler chicks indicate that short-term variation in incubation temperature during the last 4 days of incubation may have a long-lasting stimulating influence on broiler performance at age of slaughter (Janke *et al.*, 2006).

So far, different incubation programs for broiler chicks and other poultry species have not included modification of daily incubation temperature as a possibility to stimulate the development of body functions and/or to increase post-hatching adaptability to different environmental conditions and thereby to improve performance. To test our hypothesis that short-term variation in incubation temperature during the last days of incubation can improve hatching results and have long-lasting effects on the performance in poultry, studies were carried out in different modern poultry breeds under various experimental conditions and are still in progress. The questions of interest are: (1) which pattern of temperature stimulation is optimum, (2) are differences between strains or sexes in their response to 'temperature training' significant and (3) to what extent are breeder age and egg storage time affecting the outcome of prenatal 'temperature training'?

In this paper we report how commercial male and female broiler chicks (ROSS 308) responded to (1) a mild short-term and (2) chronic increase in incubation temperature at the end of incubation (day 18 until hatching). The traits studied under experimental conditions were hatchability, sex ratio at hatch (secondary sex ratio), chick quality and growth performance to age at slaughter.

Materials and methods

Incubation

In 6 trials a total of 9,883 hatching eggs of ROSS 308 broilers from breeders aged between 30 to 50 weeks were incubated from days 1 to 17 under normal incubation temperature (37.2 – 37.4 °C). On day 18 the eggs were transferred to hatchers with three different temperature programs:

- 37.2 – 37.4 °C (control),
- 38.2 – 38.4 °C (chronic warm incubation),
- 38.2 – 38.4 °C for 2 hours daily (short-term stimulation, 'temperature training').

After analysis of the results from the first two trials with three incubation treatments, we used only two different incubation treatments (short-term warm stimulation vs. control) in trials 3 to 6, because this method seemed to be the best to improve hatchability and later performance.
In all trials, the one-day-old chicks were sexed. Random sampling of chicks (30 males and 30 females from all incubators) were analysed by the Pasgar©score (vitality, navel, legs, beak, and belly). The highest chick quality has a score of 10 and one point is subtracted for each abnormality recorded in one of the above-mentioned five criteria (www.pasreform.com).

Growing period until slaughter age

Day-old chicks from incubation trial 2 were used for a follow-up growth trial. A total of 120 male and 120 female chicks from every incubator were randomly assigned to 20 pens with 12 chicks each (10 pens per sex) and grown to 35 days of age. The chicks were kept at temperatures recommended by the breeding company: 35–34 °C on days 1-2, 33-32 °C on days 3 and 4, 30 °C on days 5 to 7, 29 °C during week 2, 26 °C during week 3, 22 °C during week 4, 20 °C during week 5.

Feed (composition as shown in table 1) and water were provided *ad libitum*. Body weight and feed intake were recorded on days 1, 14, 21 and 35. Body weight was recorded individually and analysed on per pen. Feed was weighed back weekly per pen. One bird per pen with close to average body weight of this pen was slaughtered at the end of the trial (10 males and 10 females per treatment group) to determine carcass composition. Weights of total breast meat (without skin), complete right leg, liver, heart, gizzard, spleen and sum of abdominal and viscera fat were individually recorded. All parts were expressed as percentage of body weight.

Table 1: Feed formulation in the growth trial with nutrient content (g/kg)

Ingredient	Trial 1
Wheat	200.0
Corn	336.1
Soya bean meal	376.7
Soya oil	42.9
Di-calcium-phosphate	22.0
Calcium carbonate	4.6
Sodium chloride	3.2
DL-methionine	2.8
L-lysine-HCl	1.7
Premix [1)]	10.0
Dry matter [2)]	903
Crude protein [2)]	208
ME, MJ/kg [3)]	12.8
Lysine [4)]	12.5
Methionine+Cystine [4)]	9.6

1) Vitamin- mineral premix provided per kg of diet: Fe, 32 mg; Cu, 12 mg; Zn, 80 mg; Mn, 100 mg; Se, 0.4 mg; I, 1.6 mg; Co, 0.64 mg; retinol, 3.6 mg; cholecalciferol, 0.088 mg; tocopherol, 40 mg; menadion, 4.5 mg; thiamine, 2.5 mg; riboflavin, 8 mg; pyridoxine, 6 mg; cobalamin, 32 µg; nicotinic acid, 45 mg; pantothenic acid, 15 mg; folic acid, 1.2 mg; biotin, 50 µg; choline chloride, 550 mg

2) Analysed values;

3) Calculated values (WPSA; 1985);

4) Calculated values

The results from six hatching trials and one growth trial were analyzed statistically by one-way analysis of variance, using the following model for all traits:

$$y_{ij} = \mu + a_i + e_{ij}$$

where
μ = overall mean
a_i = treatment group (hatcher, temperature regime)
e_{ij} = error term.

The significance of differences was evaluated by the Student-Newman-Keuls Test ($P \leq 0.05$), using SAS operating system (Version 9.1, 2002/03).

Results and Discussion

Influence of "temperature training" on hatchability and chick quality

The results from incubation trials 1 and 2 showed that chronic warm incubation (+ 1 °C during the last 4 days of incubation) had no negative effect on hatchability (96.4% and 94.1% in the experimental group vs. 96.2% and 94.6% in the control) as well as chick quality. But only short-term warm stimulation (+ 1°C for 2 h per day during the last 4 days) improved hatchability by more than 1.5%. The results of trials 1 and 2 were confirmed in trials 3-6: short-term warm stimulation during the last 4 days of incubation improved hatchability of broiler chicks significantly (Tab. 2). Further, in all of the 6 incubation trials short-term warm stimulation consistently changed the sex ratio in the direction of more males: the ratio of 52.4% males to 47.6% females was significantly different from the control group (50.2% males to 49.8% females).

Other authors who studied the influence of short-term warm loads during incubation on hatching results used different warm-loads (39-39.5 °C) for different lengths of time (3-24 h per day during incubation days 16-18). The results were contradictory (e.g. Moraes *et al.*, 2004; Yahav *et al.*, 2004; Collin *et al.*, 2005). In our study we used a milder and shorter warm load during a later period (day 18 to hatch) of embryonic development for the application of 'temperature training'.

In poultry embryos, peripheral and central nervous thermoregulatory mechanisms as well as other body functions are well developed at the end of incubation (Tzschentke, 2007). Therefore no negative side effects would be expected after mild temperature variations during this stage. During the final stages of embryonic development, body functions develop feedback mechanisms, which change the quality of regulatory processes dramatically. This seems to be a 'critical period' in the development of body functions (Tzschentke and Plagemann, 2006).

Our hypothesis is that environmental 'training' of bodily functions during the last days of incubation can be a tool to improve their maturation and reactivity to environmental variations life-long and thus improve adaptability, vitality, health and productivity of the chickens throughout their life cycle.

Chick quality sensitively reflects incubation problems and is recommended as an indicator in the evaluation of incubation conditions (Boerjan, 2004). For evaluating the chick quality of day-old chicks (www.pasreform.com) the Pasgar©score was used. With this score the vitality of individual chicks can be evaluated using morphological parameters as well as the chicks' alertness (reflexes). In all trials, the Pagar©score was higher than 9 (of maximum 10) for male and female chicks, in some trials suggesting a beneficial effect of short-term stimulation with increased temperature. However, the mean value of the Pasgar©score was 9.6 for all groups, with no difference between sexes or temperature treatment (Tab. 2).

Table 2: Summary of results from 6 incubation trials: means and standard deviations (9883 hatching eggs set; 5.7 % infertile; egg weight 62.2 ±.2.4 g)

Parameter	Control	4 days, 2 h, +1°C
Eggs per hatcher	4323	3710
Hatched live chicks, %	94.1 ±1.7	95.4 ±2.6
Female chicks, %	49.8 ab ±2.0	47.6 b ±1.4
Male chicks, %	50.2 ab ±2.0	52.4 a ±1.4
Pasgar©score		
Female chicks	9.6 ±0.3	9.6 ±0.3
Male chicks	9.6 ±0.2	9.6 ±0.3

a; b; c – Means with different letters differ significantly

We interpret the consistently increased male:female ratio after short-term stimulation as a clear sign for improved chick vitality. It is known that male embryos are more sensitive to environmental factors than female embryos (Bogdanova and Nager, 2008, Catalano *et al.*, 2008). Related to this fact, our hypothesis is that prenatal 'temperature training' with short-term warm loads improves chick vitality, especially in the late-term male embryos. For the male chicks of ROSS 308 broilers used in our study the short-term warming resulted in a significant increase in hatchability due to the fact that relatively more male chicks hatched than in the control group.

Influence of 'temperature training' on performance until slaughter age

In the Growth Trial, the feed intake of male broilers from all three incubation groups was significantly higher during the entire fattening period than for the females. The 24 h chronic warm incubation during the last 4 days of incubation had no negative effect on the later performance of male or female broilers. But only prenatal short-term warm stimulation improved the performance until slaughter age, especially in the male broilers (Tab. 3). Daily feed intake of male broilers in the short-term warm stimulated group was higher from the first day in comparison to the control and the chronic warm incubated group. The male broilers from the short-term warm stimulated group gained 64.8 g per day to 35 days of age and reached a final weight of 2336 g, which was a significant increase by 2.9% over the control group. Feed conversion of the male but also female broilers from short-term prenatal warm stimulation was improved in comparison with the females of the control and chronic warm incubated groups.

Table 3: Performance of male broilers - Incubation trial 2 (Mean, SD)

Treatment Group	1 - control		2 - chronic warm		3 - 2h warm per day	
Age, days	Mean	SD	Mean	SD	Mean	SD
Feed intake, g/d						
1 - 14	35.0	0.9	35.4	0.6	35.9	0.7
15 - 21	89.9	3.4	93.0	2.4	92.5	3.6
22 - 35	155.3	4.0	155.3	4.8	156.3	4.5
1 - 35	93.3	3.2	94.3	2.4	95.2	3.0
Body weight gain, g/d						
1 - 14	28.7	0.6	29.2	0.9	29.6	0.7
15 - 21	66.8	3.8	68.3	2.3	69.6	3.4
22 - 35	94.4 b	4.0	95.0 b	3.1	97.9 a	2.6
1 - 35	62.2 b	2.9	63.0 ab	2.0	64.8 a	2.0
Feed conversion, kg feed/kg gain						
1 - 14	1.22	0.02	1.22	0.02	1.22	0.01
15 - 21	1.35	0.04	1.36	0.03	1.33	0.04
22 - 35	1.65	0.08	1.64	0.05	1.60	0.03
1 - 35	1.50 a	0.04	1.50 a	0.02	1.47 b	0.02
Body weight in g						
14	445	45	453	50	455	44
21	919	100	937	118	942	118
35	2270 b	203	2292 ab	243	2336 a	191

a; b - Means with different letters differ significantly

The long-lasting stimulating effect of the prenatal 'temperature training' with short-term warm load on the performance at the end of the fattening period can be explained similarly to that already described for the hatching results. Besides 'training effects', which appears to improve the vitality and health especially in male broiler chicks, a stimulating effect on muscle development can be assumed. In embryos as well as chicks of meat-type poultry mild heat exposure stimulates skeletal muscle growth due to an immediate increase in satellite cell proliferation and accelerated differentiation (Halevy *et al.*, 2001, 2006a,b; Maltby *et al.*, 2004). Further, if applied during developmental 'critical periods' environmental influences can change the programming of respective body functions (Tzschentke and Plagemann, 2006), so that 'temperature training' can also induce life-long alterations in the regulation of food intake and body weight.

The carcass analyses based on the small sample of ten male and ten female broilers from each group did not reveal any difference due to hatching egg treatment. No differences could be found in the carcass composition (meat, organs, fat) of both sexes between the groups with different incubation temperature. For marketing it is especially important that body fat content is not increased by changes in the incubation environment.

The complete results from the male and female chickens of this study were published recently (Tzschentke and Halle, 2009).

Conclusions

An incubation temperature profile including short-term temperature variation can improve broiler performance. The final incubation period seems to be a very sensitive and relatively safe period for 'temperature training'; this also holds for chronic elevated temperature. However, different goals (e.g. improvement of body weight or improvement of temperature adaptation) need different temperature manipulation. Short-term 'temperature training' of the embryos improved post-hatch performance under our conditions, whereas chronic temperature changes during the last days of incubation may improve adaptation to high temperature during the fattening period. The effect of temperature manipulations at the end of incubation is sex-specific and may also depend on the strain of chickens used, breeder age (Yalcin *et al.*, 2005) and egg storage conditions.

Summary

Incubation climate can significantly influence hatchability, chick quality, and later performance in poultry. One of the most important factors is the incubation temperature. For practical application of prenatal 'temperature training' the questions of interest are: (1) which pattern of temperature variation is the optimum one, (2) are there differences in the effect of prenatal 'temperature training' between poultry breeds and lines as well as between male and female animals, and (3) what is the influence of factors like breeder age and egg storage time on the outcome of prenatal 'temperature training'? In an exemplary study carried out in male and female broiler chicks (ROSS 308) the influence of a mild (1°C over standard) short-term (2 h daily), as well as chronic (over 4 days) increases in incubation temperature at the end of incubation (day 18 until hatch) on hatchability, sex ratio of the hatched chicks (secondary sex ratio), chick quality and performance until age of slaughter was investigated. Chronic as well as short-term increases in incubation temperatures by 1 °C over standard during the last 4 days of incubation had no negative effect on hatchability, chick quality and post-hatching performance. Only short-term warm stimulation ('temperature training') improved hatchability by more than 1.5% and induced a significantly higher percentage of hatched male chicks in all experimental series. At slaughter the male broilers incubated under short-term warm stimulation reached the highest final live weight and a significantly better feed conversion in comparison with males from the control as well as males from the chronic warm incubated group. These results suggest that 'temperature training' with short-term temperature variation during the last days of incubation can be applied to improve poultry performance (applied for European Patent EP2105048, published September 30, 2009).

Zusammenfassung

Das Brutklima beeinflusst signifikant das Schlupfergebnis, die Kükenqualität und die spätere Leistung des Geflügels. Einer der bedeutendsten Klimafaktoren ist die Bruttemperatur. Für die praktische Anwendung eines pränatalen 'Temperatur-trainings' sind u.a. folgende Fragen zu beantworten: (1) Welche Form der Temperaturvariation ist optimal? (2) Treten Unterschiede hinsichtlich des Effekts eines pränatalen 'Temperaturtrainings' zwischen verschiedenen Geflügelarten, Zuchtlinien sowie männlichen und weiblichen Tieren auf? (3) Welchen Einfluss üben Faktoren wie das Alter der Elterntiere und die Dauer der Lagerung der Bruteier auf den Erfolg des pränatalen 'Temperaturtrainings' aus? In einer Beispielsstudie an männlichen und weiblichen Broilern (ROSS 308) wurden der Einfluss einer milden (1°C über dem Standard) kurzzeitigen (täglich 2 h) sowie einer chronischen (über 4 Tage) Erhöhung der Bruttemperatur am Ende der Brutphase (Tag 18 bis Schlupf) auf die Schlupfrate, das Geschlechtsverhältnis der geschlüpften Küken (sekundäres Geschlechtsverhältnis), die Kükenqualität und die Leistung bis zum Schlachtalter untersucht. Chronische und kurzzeitige Erhöhung der Bruttemperatur in der Endphase der Inkubation beeinflussten das Schlupfergebnis, die Kükenqualität und die spätere Leistung der Tiere nicht negativ. Jedoch nur die kurzzeitige Warmstimulierung ('Temperaturtraining') führte zu einer Verbesserung der Schlupfrate um mehr als 1,5% und in allen Versuchsserien zu einer signifikanten Erhöhung des Anteils geschlüpfter männlicher Küken. Die pränatal kurzzeitig warmstimulierten männlichen Broiler erreichten im Schlachtalter ein signifikant höheres Schlachtgewicht und eine signifikant verbesserte Futterverwertung im Vergleich zu den männlichen Tieren der Kontrolle und der chronisch warm erbrüteten Gruppe. Die Ergebnisse zeigen, dass ein Bruttemperaturprofil mit kurzzeitiger Temperaturvariation ('Temperaturtraining') in den letzten Bruttagen die Leistung des Geflügels deutlich verbessern kann (eingereicht zum Europäischen Patent 2105048, publiziert 30. September 2009).

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