Final Scientific Report

Cover Page

BARD Project Number: IS-4198-09

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Project Title: Development of strategic pre-natal cycling thermal treatments to improve livability and productivity of heavy broilers

Investigators	**Institutions**
Principal Investigator (PI): Shlomo Yahav	**ARO the Volcani Center Israel**
Co-Principal Investigator (Co-PI): john brake	**NC State University USA**
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Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.
Angiogenesis, Breast muscle, Chorioallantoic Factor VIII, Membrane, Performance, Thermotolerance, Thermal challenge,

Abbreviations commonly used in the report, in alphabetical order:

BDNF, BW, CAM, FCR, FGF2, HIF-1α, MMP2, PO/AH, SHL, T_b, TM, VEGF-α2, VEGFR,

Budget: IS: $ 130,000 US: $ 115,000 Total: $ 245,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Appendix G6a

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	2			2
Submitted, in review, in preparation	3			3
Invited review papers				
Book chapters	1			1
Books				
Master theses		1		1
Ph.D. theses			1	1
Abstracts	9		9	18
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	2	1		3
Longer Visits (Sabbaticals)		1		1

Description Cooperation:

Details of cooperation: During the first year Shlomo Yahav visited John Brake's laboratory in order to coordinate techniques used in both countries. During the second year, John Brake visited Israel, taking part in the BARD conference entitled "Affecting early life poultry development to improve domestic fowl livestock" where scientists from several countries shared their scientific ideas and results. During this visit Brake visited Yahav's facilities and laboratory and discussed different subjects with the students. During the second year Amy Lin, a MSc student, visited Yahav's laboratory to conduct the CAM and yolk sac membrane blood vessel quantification with the CAM-era, version 1.0 computer software. During the third year, Shlomo Yahav spent a one-year sabbatical leave in John Brake's laboratory where they both conducted several experiments to evaluate the improvement of thermotolerance and carcass quality of broilers on a semi-commercial scale, based on our earlier findings (Manuscript 2). The third year was also spent finalizing the studies on several other objectives of the BARD project.

Abstract: The necessity to improve broiler thermotolerance and live performance led to the following hypothesis: Appropriate comprehensive incubation treatments that include significant temperature management changes will promote angiogenesis and will improve acquisition of thermotolerance and carcass quality of heavy broilers through epigenetic adaptation. It was based on the following questions: **1.** Can TM during embryogenesis of broilers induce a longer-lasting thermoregulatory memory (up to marketing age of 10 wk) that will improve acquisition of thermotolerance as well as increased breast meat yield in heavy broilers? **2.** The improved sensible heat loss (SHL) suggests an improved peripheral vasodilation process. Does elevated temperature during incubation affect vasculogenesis and angiogenesis processes in the chick embryo? Will such create subsequent advantages for heavy broilers coping with adverse hot conditions? **3.** What are the changes that occur in the PO/AH that induce the changes in the threshold response for heat production/heat loss based on the concept of epigenetic temperature adaptation?

The original objectives of this study were as follow: **a.** to assess the improvement of thermotolerance efficiency and carcass quality of heavy broilers (~4 kg); **b.** to improve peripheral vascularization and angiogenesis that improve sensible heat loss (SHL); **c.** to study the changes in the PO/AH thermoregulatory response for heat production/loss caused by modulating incubation temperature. To reach the goals: ***a.*** the effect of TM on performance and thermotolerance of broilers reared to 10 wk of age was studied. ***b.*** the effect of preincubation heating with an elevated temperature during the 1st 3 to 5 d of incubation in the presence of modified fresh air flow coupled with changes in turning frequency was elucidated; ***c.*** the effect of elevated temperature on vasculogenesis and angiogenesis was determined using *in ovo* and whole embryo chick culture as well as HIF-1α VEGF-α2 VEGF-R, FGF-2, and Gelatinase A (MMP2) gene expression. The effects on peripheral blood system of post-hatch chicks was determined with an infrared thermal imaging technique; ***c.*** the expression of BDNF was determined during the development of the thermal control set-point in the preoptic anterior hypothalamus (PO/AH).

Background to the topic: Rapid growth rate has presented broiler chickens with serious difficulties when called upon to efficiently thermoregulate in hot environmental conditions. Being homeotherms, birds are able to maintain their body temperature (T_b) within a narrow range. An increase in T_b above the regulated range, as a result of exposure to environmental conditions and/or excessive metabolic heat production that often characterize broiler chickens, may lead to a potentially lethal cascade of irreversible thermoregulatory events. Exposure to temperature fluctuations during the perinatal period has been shown to lead to epigenetic temperature adaptation. The mechanism for this adaptation was based on the assumption that environmental factors, especially ambient temperature, have a strong influence on the determination of the "set-point" for physiological control systems during "critical developmental phases." Recently, Piestun *et al.* (2008) demonstrated for the first time that TM (an elevated incubation temperature of 39.5°C for 12 h/d from E7 to E16) during the development/maturation of the hypothalamic-hypophyseal-thyroid axis (thermoregulation)

and the hypothalamic-hypophyseal-adrenal axis (stress) significantly improved the thermotolerance and performance of broilers at 35 d of age. These phenomena raised two questions that were addressed in this project: **1.** was it possible to detect changes leading to the determination of the "set point"; **2.** Did TM have a similar long lasting effect (up to 70 d of age)? **3.** Did other TM combinations (pre-heating and heating during the 1st 3 to 5 d of incubation) coupled with changes in turning frequency have any performance effect? The improved thermotolerance resulted mainly from an efficient capacity to reduce heat production and the level of stress that coincided with an increase in SHL (Piestun *et al.,* 2008; 2009). The increase in SHL (Piestun *et al.,* 2009) suggested an additional positive effect of TM on vasculogenesis and angiogensis. **4.** In order to sustain or even improve broiler performance, TM during the period of the chorioallantoic membrane development was thought to increase vasculogenesis and angiogenesis providing better vasodilatation and by that SHL post-hatch.

Major conclusions: To address the first question: was it possible to detect changes leading to the determination of the "set point", application of intermittent TM (39.5°C for 12 h/day) during embryogenesis when the thyroid and adrenal axis was developing and maturing (E7 to E16 inclusive) caused the neurotrophic factor BDNF, which was previously (Katz and Meiri, 2006) reported to be involved in post natal thermotolerance acquisition, to exhibit a significant or numerical increase. The increase was evident from E12 to E16. These results demonstrated that BDNF was critically involved in thermal-experience-dependent development and may help explain the improved thermotolerance acquisition of broilers that experienced TM during embryogenesis (See results in appendix A).

These TM experiments further demonstrated that a long lasting thermoregulatory effect (up to 10 wk of age) improved thermotolerance by lowering the broiler functional T_b set point, thus lowering metabolic rate and producing improved relative breast muscle yield as well (see attached 1st paper – accepted for publication in Poultry Science).

Assessing the following question: Do other TM combinations (pre-heating and increased temperature during the 1st 5 d of incubation) have any performance effects? The experiments came to the conclusions that thermal treatments pre-incubation or during the sex determination period of incubation had, in general, a positive effect on hatchability, growth performance, and secondary sexual characteristics of broiler males and females most probably caused by the increase of plasma testosterone concentration in both genders (see attached 2nd paper – accepted for publication in Poultry Science).

TM (39.5°C; 65% RH) for 12 h / day between E5-E12 of incubation was applied to determine the effect during CAM development on the CAM vasculature as a model for the embryo vascular system, on the chick/chicken vasomotor response, and on thermotolerance acquisition of broilers. Blood vessel density, within a specific area of the CAM, exhibited a significant increase in the TM embryos. The expression of some relevant angiogenic genes (VEGFα2, FGF-2, HIF1α, and MMP-2), using real-time PCR, indicated that TM increased

the expression of those genes by 2 to 11 fold on E7, E9, and E12. The TM hatched chicks exhibited significantly lower T_b. Thermal challenge at 35 d of age demonstrated an improved capability of the TM chickens to maintain T_b that resulted in significantly lower mortality. However, no significant differences were found in SHL, which raised the optional conclusion that such differences may appear only in hot acclimated broilers (see results in Appendix B).

To sustain the effect of elevated temperature combined with changes in egg turning frequency, the following study was conducted. The effect of increasing incubation temperature to 38.1 °C during the 1st 3 or 5 d of incubation, coupled with daily turning frequency of 96 times up to E14, E15, or E18 of incubation, was compared to a tuning frequency of 24 times in order to study its effect on embryo's development and hatching. This study suggested that while early incubation temperature may control metabolic rate and growth of the embryonic vasculature, turning may interact and modulate early development. It was further suggested that there may be a relationship between embryo growth, as represented by chick length, and absolute amount of turning prior to a change of turning conditions. The combination of 37.5°C incubation temperature and turning 24 n/d required a greater turning length to achieve maximum chick length but turning at 96 n/d stimulated embryo development in such a manner as to achieve maximum chick length at an earlier stage of development (See results in Appendix C).

Scientific and agriculture implications: This study proved for the first time **that TM during broiler embryogenesis had** the ability to establish: **a.** a long-lasting alteration in the thermoregulatory threshold response which, no doubt, improved thermotolerance of broilers; **b.** the capacity to improve broiler performance by using TM; **c.** thermal treatments pre-incubation or during the sex determination period of incubation had, in general, a positive effect on hatchability, growth performance, and secondary sexual characteristics of broiler males and females most probably caused by the increase of plasma testosterone concentration in both genders; **d.** TM within the period of development of the CAM (E5 to E12) increased the expression of the main angiogenesis genes in the CAM leading to the enlargement of the CAM blood vessel density; **e.** TM within the development period of the CAM improved thermotolerance acquisition post hatch most probably by elevating vasodilatation response and by increasing the number of blood vessels in the periphery, but with no significant effect on SHL; and, **f.** turning frequency coupled with changes of incubation temperature has an effect on the embryo metabolic rate and the embryonic vasculature, and may interact and modulate early development.

The full agriculture implication lies within the rate of adoption of these procedures in commercial hatcheries.

Achievements and innovations: This study demonstrated that utilizing the embryogenesis (E) period of broilers, in particularly E7 to E16 as the "critical phase" for TM of chick embryos, significantly enhanced

thermotolerance using intermittent (12 h/day – an optimal treatment) TM at 39.5°C and 65% RH during this "critical period". Hatchability and male BW were not significantly affected by TM but TM females demonstrated a significantly lower BW during the entire 70 d post-hatching study. However, following embryonic TM, both sexes exhibited significantly lower feed intake and body temperature accompanied by improved FCR and greater breast muscle weight. In light of this study and previous once, it was concluded that intermittent TM during broiler embryonic development had a long-lasting effect on energy balance that led to improved FCR and breast muscle yield. To achieve the goals of this study, an increased machine ventilation rate was required during TM so as to supply oxygen required for the periods of increased embryonic development (Manuscript 1).

Based on the data presented in this study a simple calculation was made in order to demonstrate the overall economic implications of using TM between E7 to E16 of broiler embryogenesis. It was assumed that TM was successfully applied to all broiler eggs incubated in the USA. The value of improved thermotolerance as a result of TM, a critical economic parameter to 70 d of age, was not considered in these calculations. To be conservative, it was assumed that there was an improvement in FCR of 10 points, which means saving 225 g of feed per broiler with an annual savings of approximately (using 8.6 billion broilers) $553 million. It was further assumed that the 4.5 kg broilers with a 1% increase in relative breast muscle represent an average increase of 39 g of breast muscle per broiler. This resulted in further income of approximately $1.07 billion while losing 3% hatchability will cause a loss of $53.75 million in terms of lost hatching egg cost and 310 million chicks that would not be available to be grown to market age. Obviously, if hatchability was not affected, and appropriate incubation equipment was available, TM would be quite attractive in appropriate markets (Manuscript 1).

The ability to induce long-lasting physiological memory by epigenetic temperature adaptation was studied as well, investigating the changes of the expression levels of the neurotrophic factor BDNF in the PO/AH, the brain centers that may control changes in threshold response for heat production/heat loss. It was previously shown that this gene was involved in post natal thermotolerance acquisition (Katz and Meiri, 2006). It was found that indeed TM affected the expression levels of BDNF. The largest effect was at E12 and E13 where the expression levels of BDNF in heat treated embryos was about 50% higher that the expression in age matched non treated embryos (Appendix 2).

The significance and importance of the pre-incubation and incubation temperatures for broiler chickens has been elucidated by altering normal incubation conditions to study the effects on embryo development and on its influence on biased sex ratio of avian. This study elucidated the effects of temperature prior to and/or during the sex determination period of incubation (E0 to E5 inclusive) on hatchability, apparent sex ratio, growth and development post-hatching, and secondary sexual phenotypic characteristics. These thermal treatments positively affected hatchability, increased body weight of both sexes at 35 d of age that was

followed by increased relative breast muscle weight and secondary sexual characteristics (comb, wattles, testes in males), which may be attributed to the finding that thermal treatments resulted in numerically or significantly increased plasma testosterone concentration in both sexes. Furthermore, some treatments had positive effects such that a biased sex ratio of 55:45 males and females, respectively, was produced. Therefore, it was concluded that thermal treatments pre-incubation or during the sex determination period of incubation had, in general, a positive effect on hatchability, biased sex ratio, growth performance, and secondary sexual characteristics of broiler males and females most probably caused by the increase of plasma testosterone concentration in both sexes (Manuscript 2).

To further study optimal incubation conditions that enhanced embryo development, a combination of different turning frequency with increasing incubation temperature during the first 3 to 5 d of incubation was conducted (Appendix 3). The main results suggested that while early incubation temperature may control metabolic rate and growth of the embryonic vasculature, turning may interact and modulate early development. There was also some evidence that turning eggs 96 times daily increased the variance in the growth of the tissues measured, which suggested that turning eggs 24 times daily may limit the growth of certain embryos and prevent complete genetic expression.

Answering the following two questions: does elevated temperature during incubation affect vasculogenesis and angiogenesis processes in the chick embryo, and will such create subsequent advantages for heavy broilers coping with adverse hot conditions?; required the following experiments: 1. increasing incubation temperature during the development of the chorioallantoic membrane (CAM) on E5 to E12 (39.5 °C and 65% RH, 12 h/day); 2. rearing hatched chicks from the previous experiment to marketing age while employing thermal challenge experiences. Increasing incubation temperature during the development of the CAM significantly enhanced the vascular area of the yolk sac, and from E8.5 had a positive and significant effect on the CAM vascular area of the TM treated eggs. To explain the dynamics of the CAM, primary gene expression involved in vasculogenesis and angiogenesis (HIF1α, VEGFα2, VEGFR2, FGF-2, and MMP2) was evaluated. The dynamics were evaluated on E5, E7, E9, and E12 for 360 min. In the case of most of the genes, on each day of incubation, an elevation of the expression was detected after 30 and 180 min from the initiation of TM. This kind of elevation was not observed on E5 for FGF-2 and MMP2 (Appendix 2).

It was concluded that the main angiogenesis genes in the CAM were expressed and responded to elevated temperature between E5 and E12, which helped explain the enlargement of the CAM blood vessel density.

To study the effect of TM during CAM development on heat resistance at marketing age, thermal challenge (35°C for several hours) of broilers was employed at 35 and 42 d of age. In both thermal challenges, TM broilers were able to maintain much lower body temperature. This coincided with much lower mortality rate in males. Molecular markers (factor VIII and VEGF-R2) were present at greater levels in the skin of TM

chickens, suggesting better blood flow (vasodilation) and more blood vessels, despite lack of effect on SHL (Appendix 2).

Overall conclusions: **1.** TM had a long-lasting effect on the metabolic rate and on broiler performance (FCR, breast muscle relative weight), the changes in metabolic rate were supported by BDNF gene expression in the PO/AH; **2.** Pre-incubation coupled with increased incubation temperature during the period of sex determination had in general, a positive effect on hatchability, biased sex ratio, growth performance, and secondary sexual characteristics of broiler males and females most probably caused by the increase of plasma testosterone concentration in both sexes; **3.** Early incubation temperature appeared to control metabolic rate and growth of the embryonic vasculature while rate of egg turning may interact and modulate early development. There was also some evidence that turning eggs 96 times daily increased the variance in the growth of the tissues measured. This suggested that turning eggs 24 times daily may limit the growth of certain embryos and prevent complete genetic expression; **4.** TM during CAM development enhanced CAM vasculature as a result of a significant increase of expression of genes that were related to vasculogenesis and angiogenesis. **5.** TM during CAM development led to better heat resistance of the TM chickens coupled with better blood flow to the periphery.

The main innovation of this study lies in accumulating evidence showing that in broilers, the epigenetic adaptation approach, associated with changes in the incubation environment with emphasis on fine-tuning can elicit efficient and beneficial long-lasting thermoregulatory and performance adaptations.

Agriculture and or economic impacts of the research findings: Mortality and morbidity among commercial broilers have typically significantly increased from late spring to autumn as a result of heat spells. TM in commercial hatcheries can be used for two main purposes: **1.** to improve thermotolerance acquisition and by that to reduce mortality from heat spells, mainly during the latter period of growth. Mortality rate during this period has always been the highest, and the main cause for economic losses, and **2.** to improve broiler carcass quality (breast muscle, abdominal fat pad). Improving relative breast muscle weight by approximately 1% may contribute million(s) of US$ to the industry in both countries.

Details of cooperation: During the 1st year Shlomo Yahav visited John Brake's lab, to combine techniques used in both countries.

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on a semi-commercial scale, based on the findings included in this report. The third year was also spent finalizing studies on several other objectives of the BARD project.

List of publications:

Piestun, Y., Druyan, S., Brake, J. and Yahav, S. (2013). Thermal treatments prior to and during the beginning of incubation affect phenotypic characteristics of broiler chickens post hatching. *Poult. Sci.* (in press).

Piestun, Y., Druyan, S., Brake, J. and Yahav, S. (2013). Thermal manipulations during broiler incubation alter performance of broilers to 70 days of age. *Poult. Sci.* (in press).

Yahav, S. and Brake, J. (2013). Chick Embryogenesis: A Unique Platform to Study the Effects of Environmental Factors on Embryo Development. NovaScience publications Inc. NY USA (in press).