The 4th Workshop
on Fundamental Physiology
and Perinatal Development
in Poultry

PROGRAM & ABSTRACTS

September 10 - 12, 2009

Congress Centre Družba
Comenius University Bratislava
Botanická 25, Bratislava
Slovak Republic

Organised by

Department of Animal Physiology
Faculty of Natural Sciences
Comenius University Bratislava
Slovakia

Institute of Animal Biochemistry
and Genetics
Slovak Academy of Sciences
Ivanka pri Dunaji

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of The World’s Poultry Science Association
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members of the Department of Animal Physiology and Ethology, Comenius University Bratislava

Proceeding of The 4th Workshop on Fundamental Physiology and Perinatal Development in Poultry held September 10-12, 2009 in Bratislava, Slovak Republic

Edited by Iveta Herichová and Michal Zeman
Universitas Comeniana Bratislava, 2009
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Preface

Dear colleagues,

Sixteen years ago, in November 1993, the first Workshop on Perinatal Adaptation was organized in Berlin by Martin Nichelmann and 15 talks were presented there. Since that time the tradition to organize International Workshops on Perinatal Adaptation and Development in birds has continued and the event has evolved into the serious international meeting, held mostly in Berlin, either at the Humboldt University (Barbara Tzschentke) or the Free University of Berlin (Heike Tönhardt). Only twice this event was organized outside Germany, in 1998 and now, in 2009. In both cases it was organized in Slovakia, the first time at Ivanka pri Dunaji (a small village close to Bratislava where the Institute of Animal Genetics and Biochemistry is located) and now in Bratislava, at the Comenius University Bratislava, Faculty of Natural Sciences. We are very glad that we can see here some colleagues who took part in the first meeting in 1993. It means, that they consider useful to come, it is worth for them to visit Slovakia and its capital Bratislava, that is rather small but we think it’s nice. We hope that after your stay here you will agree with us. Enjoy your stay in Bratislava but especially enjoy this most interesting scientific meeting.

During its history the workshop evolved into the serious international meeting focused on exiting subject that has offered challenging problems and applications. Since 2003 this is clear especially after merging the Workshop on Perinatal Adaptation with the activities of the Working Group 12 (Physiology) of the European Branches of the World’s Poultry Science Association represented by Shlomo Yahav, Barbara Tzschentke and Eddy Decuypere. This European Working Group 12 was inaugurated 2003 in Berlin during the first combined Workshop on Fundamental Physiology and Perinatal Development in Poultry.

Our workshop grown up and this year more than 60 participants from 18 counties from all around the world have registered for this event. We are especially pleased that many young scientists take part in the workshop and we will try to keep the tradition and engage at least some of them as chairmen or chairwomen of sessions, together with experienced and recognized scientists.

We hope that the workshop will fulfill your expectations from both the scientific and social point of view. The local organizing committee and members of the Department of Animal Physiology and Ethology have done their best to make you feel like home.

We wish you a pleasant stay in Bratislava and successful meeting with new ideas, contacts and future cooperation.

Michal Zeman and Barbara Tzschentke
PROGRAM

Friday, September 11, 2009

10:00  10:15  Opening ceremony

Keynote Lecture I
Chairman: Eddy Decuypere and Floriane Guibert

10:15  11:15  Ton G. G. Groothuis  The Netherlands
Was Lamarck right? Causes and consequences of variation in maternal hormones in avian eggs

Maternal influences

11:15  11:35  Monika Okuliarová  Slovakia
Divergent selection of Japanese quail for yolk testosterone concentrations

11:35  11:55  Michal Zeman  Slovakia
Selection for high egg testosterone content increases growth rate more in male than female Japanese quail

11:55  12:15  Henry van den Brand  The Netherlands
Effect of early feeding on the transfer of maternal antibodies and development of immune competence in the broiler chicken

12:15  12:35  Floriane Guibert  France
Yolk testosterone content and chicks' behaviour are influenced by maternal age in Japanese quail (Coturnix c. japonica)

12:35  13:50  Lunch

Early development of body functions
Chairman: Heike Tönhardt and Zehava Uni

13:50  14:10  Zehava Uni  Israel
The digestive systems of the developing broiler embryo

14:10  14:30  Liran Yadgary  Israel
Yolk nutrient uptake by the broiler embryo towards hatch

14:30  14:50  Shelly Druyan  Israel
The effect of genetic line (broilers vs. layers) and parent flock age on embryonic development

14:50  15:10  Iveta Herichová  Slovakia
Ontogeny of clock gene expression in central and peripheral circadian oscillators in chicken.

15:10  15:30  Eva Verhoelst  Belgium
Structural analysis of the vascular network of the chorioallantoic membrane

15:30  16:00  Coffee break

Epigenetic effects
Chairman: Noam Meiri and Ingrid Halle

16:00  16:20  Noam Meiri  Israel
Epigenetic regulation of thermotolerance acquisition and memory
16:20 16:40  **Irene Walstra**  
The Netherlands  
Thermal conditioning during layer chick embryogenesis

16:40 17:00  **Marcin Lis**  
Poland  
Malformations of chick embryos exposed to high doses of *in ovo* injected ascorbic acid

### Models of human diseases

**Chairman:**

17:00 17:20  **Nadia Everaert**  
Belgium  
The chick embryo as a model to study persistent effects of embryonic protein-undernutrition on postnatal growth and metabolism

17:20 17:40  **Jean Eduardo de Oliveira**  
Belgium  
Development of an in vivo model to screen for antioxidant potential of compounds utilizing chick embryos: finding the best method to induce mild oxidative stress

17:40 18:20  **Poster session**

**Chairman:**  
**Henry van den Brand and Boris Bilčík**

19:00  **Social evening**

### Saturday, September 12, 2009

**Keynote Lecture II**

**Chairman:**  
**Shlomo Yahav and Marina Nechaeva**

9:00 10:00  **Wolfgang J. Schneider**  
Austria  
Mechanistic insights into chicken oocyte growth

**New incubation developments**

10:00 10:20  **Inge Reijrink**  
The Netherlands  
Gas concentrations during storage do not affect hatchability and chick quality

10:15 10:30  **Lotte van de Ven**  
The Netherlands  
Effects of hatching time and hatching system on broiler physiology

10:30 11:00  **Coffee break**

**Cardiovascular system**

**Chairman:**  
**Jordi Altimiras and Krystyna Skwarlo-Sońta**

11:00 11:20  **Heike Tönhardt**  
Germany  
Possible mechanisms of catecholamines for protecting the embryonic heart against cold and heat

11:20 11:40  **Jordi Altimiras**  
Sweden  
Developmental programming of beta-adrenergic receptors in the heart of broiler chickens

11:40 12:00  **Marina Nechaeva**  
Russia  
Effect of acute hypoxia on the motor activity and heart rate of the 10- and 14-day chick embryo
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Investigation of development and heart rate using image processing in early stages of chick embryos

R. Akiyama¹, T. Komoro¹ and K. Moriya²

¹Muroran Institute of Technology, Japan, ²Hakodate National College of Technology, Japan

Heart rate (HR) was detected continuously by ICG in early stage (from 3 days to 7 days) of chick embryos in the previous study, the spontaneous instantaneous heart rate (IHR) was not occurred and mean heart rate (MHR) was increased with embryonic development. We attempted to develop a computer-aided video system, which captured continuously the inside of the egg using a CCD camera, and record HR of early stage of chick embryos. The camera simultaneously functioned as a lid to close the window so that the embryos could survive several days or longer. Before beginning of incubation, a CCD camera was inserted into the attachment using plastic tube and styrene board. On day 1 of incubation, when the certification of heart beat, the camera focused to the embryonic heart, which was illuminated by high luminance green LED (super-high intensity; 9000mcd, wave-length; 525nm). The video image from the camera captured into the computer at 30 frames per sec.

The rectangle range of the diastolic heart was determined on first frame of the original video image. In this rectangle range, the area of heart was abstracted by the color filter, which had bandwidth of pixels values, and then the number of pixels in the area of heart was counted every frame in a video image. The HR was calculated form peak-to-peak time interval of pixels data, which was detected by digital filter and interpolation from waveform regeneration. The body of embryo and CAM were abstracted by the color filter and division of object group. The embryonic and CAM development were decided by count of number of pixels from several object in every frames of video image.

Keywords: heart rate; CCD camera; early development; chick embryo; image processing

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Developmental programming of β-adrenergic receptors in the heart of broiler chickens

J. Altimiras and I. Lindgren

Division of Zoology, IFM Biology, Linköping University, Linköping, Sweden

β-adrenoceptors (βARs) are essential for regulating the development of the heart and its function. Adverse events during embryonic development may have deleterious effects on the adult phenotype and may predispose for pathologies such as cardiac failure and hypertension.

We have studied cardiac the abundance of βAR and its downstream signaling in broiler chickens (15 and 19 day of fetal development out of a 21 day incubation and 14 and 35 days post hatching, F19, F15, P14 and P35, respectively) developed in normoxia (21% O2, N) or chronic hypoxia (14% O2, H) to investigate whether low fetal oxygen conditions have a ‘programming’ effect on the adult.

Apart from evaluating the total βAR density (Bmax) and affinity (Kd), we distinguished between βAR subtype 1 (βAR1) and 2 (βAR2) in P14 and P35 hearts to assess the composition of the βAR population.

To obtain Bmax and Kd we incubated ventricular micropunches with a tritiated β-antagonist ([3H]CGP-12177). To discriminate between receptor subtypes micropunches were incubated with β1AR and β2AR specific antagonists (CGP20712A and ICI-11855 respectively) in competition with [3H]CGP-12177. EC50 was obtained from concentration-response curves recorded from paced ventricular strips treated with increasing adrenaline concentrations.

Bmax did not differ between treatments in F15 but F19H had a significantly lower Bmax than F19N (5.0±2.1 vs. 8.1±2.7 fmol μg protein−1). While the overall density was significantly increased (>50%), hypoxia had no effect on βARtot post hatching. P14N and P35H showed higher numbers of β2AR than β1AR, while the composition in P14H and P35N was 50/50. The increase in Bmax in P14N and P35N was accompanied by a significant decrease in EC50. Hypoxia did not show an effect in P14, but significantly increased the EC50 in P35 (0.096±0.07 and 0.31±0.28 μM, N vs H respectively). Despite lower βAR density with hypoxia, the contractile response to adrenaline was unaltered in F19H, while hypoxia significantly shifted the adrenaline concentration-response curve to the left in F15.

Conclusions: 1) cardiac βARs in the chicken go through a maturation process from the fetus to the adult with a decrease of affinity that is functionally overridden by increased receptor numbers and thus, a higher sensitivity to agonist stimulation, 2) hypoxia has a transient effect in the late fetus with a lowered number of βARs, but with a preserved or elevated sensitivity to agonist which indicates a receptor hypersensitization, 3) there is a possible programming effect of fetal hypoxia resulting in a lowered sensitivity to agonist related to a downregulation of βAR1 (despite maintained βARtot) in the P35 chickens. In vivo studies are needed to assess whether the hypoxia-induced sensitivity alteration has an, possibly deleterious, effect on cardiac function in the adult chicken.

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Effects of embryo thermal conditioning on thermotolerance, parameters of meat quality and muscle energy metabolism in a heavy line of chicken

L. Bedrani¹, C. Berri¹, S. Grasteau¹, Y. Jégo², S. Yahav³, N. Everaert¹, M. Jlali¹, R. Joubert¹, S. Métayer Coustard¹, C. Praud¹, S. Temim⁵, S. Tesseraud¹ and A. Collin¹

¹INRA, UR83 Recherches Avicoles, F-37380 Nouzilly, France. ²Hubbard, F-35220 Châteaubourg, France. ³ARO The Volcani Center, Institute of Animal Sciences, Bet-Dagan, P.O. Box 6, Israel. ⁴Laboratory of Livestock Physiology, Immunology and Genetics, Department of Biosystems, K.U. Leuven, Kasteelpark Arenberg 30, 3001 Leuven, Belgium. ⁵Ecole Nationale Supérieure Vétérinaire BP. 161 Hacène Badi El Harrach, Alger, Algérie

Meat-type chickens are particularly sensitive to heat stress. The aim of this study was therefore to investigate the effects of thermal manipulations (TM) during embryogenesis (12-hour-daily exposure of eggs at 39.5°C between days 7 and 16 of incubation) on different breeding parameters and the capacity to withstand an acute heat exposure at 42 days of age in a Hubbard heavy line of chickens. Gene expression of several factors regulating mitochondrial metabolism was also measured in Pectoralis major muscle by real-time PCR.

Compared to a control group incubated in standard conditions, the hatchability of the TM group was significantly reduced (65.4% vs. 72.9%). Body weight recorded at day 38 of age was also decreased in TM chickens, whereas feed conversion ratios on the whole period were not different between groups. Body temperature (Tb) measured two hours after hatching was significantly reduced in TM chicks (38.5°C vs. 39.1°C) and was maintained lower than in control chickens at days 4, 11, 28 and 42 of age. When exposed to an acute heat stress (5h at 35°C on day 42), TM male chickens exhibited a 43% reduced mortality than control male chickens. Embryo heat conditioning also significantly affected the messenger expressions of factors involved in muscle mitochondrial function (avian uncoupling protein, peroxisome-proliferator-activated-receptor γ-coactivator 1α…).

After slaughter on day 43, no difference due to TM treatment was observed on breast or abdominal fat yields, and on breast muscle ultimate pH, red color index and drip loss. Nevertheless, the lightness and yellow color index of pectoral muscle were significantly higher in TM than in control chickens.

Altogether these findings suggest that heat conditioning during embryogenesis affected the regulation of muscular energy metabolism and thermotolerance without affecting significantly meat quality traits in heavy chickens.

Keywords: embryo heat conditioning, thermotolerance, meat quality, mitochondria

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Anxiolytic effect of Diazepam on two lines of Japanese quail selected for different levels of maternal testosterone: a pilot study

B. Bilčík¹, A. Kovaríková², I. Košťál¹ and M. Zeman¹,³

¹Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, Ivanka pri Dunaji, Slovakia, ²Faculty of Pharmacy, Comenius University, Bratislava, Slovakia, ³Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic

Deposition of maternal androgens into the egg yolk can influence early development as well as behaviour of adults. Higher levels of maternal testosterone affect behavioural phenotype and shift it towards more proactive, dominant behaviour. This seems to be age dependent, since in very young birds the effect of testosterone is inhibitory.

The aim of our study was: (a) to validate the use of automatic activity recorder Conducta for the use with quail, (b) to compare behavioural characteristics of two Japanese quail lines selected divergently for low and high testosterone content, (c) to study the effect of anxiolytic (Diazepam) on behaviour under different testing conditions.

Two lines of Japanese quail (Coturnix japonica) selected for high (HET) or low (LET) levels of testosterone deposited into the egg yolk were used. Both HET and LET were divided into the two groups. One received 0.5 mg/kg of Diazepam i.m. 20 minutes before the test, while control group received the same volume of saline. Birds were tested at the age of 4 to 6 days in either open field test or social interaction test (similar to open field test, however, birds were tested in pairs). Behaviour was video recorded in both tests and for open field also additional automatic activity recording was performed (Conducta). Behaviours recorded in the open field were latency to move, locomotion, jumps, pecks at objects and defecation. In social interaction test focal bird was marked with white tape. Behaviours recorded were pecks at objects, pecks at other animal and inter-individual distance.

Results from line comparison and diazepam administration will be presented at the workshop.

Acknowledgement: This study was supported by the VEGA 2/0151/08.

Keywords: Japanese quail, testosterone, diazepam, behaviour, open field

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Neuronal glucose sensitivity of the *Nucleus infundibuli hypothalami* in chickens

S. Bogatyrev, J. Klein, M. Nassar¹, J. Olt and B. Tzschentke

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In mammals including man gestational diabetes may results in increased later risk of becoming overweight and developing diabetes in the offsprings. During ‘critical periods’ of neuronal development perinatal hyperglycaemia, leading to perinatal hyperinsulinism and elevated leptin concentration may cause a malprogramming of the regulatory centre of body weight and metabolism, especially in the hypothalamus. The basically mechanisms of these malprogramming are still unknown. The bird embryo seems to be a suitable model, because of its development isolated from the mother organism, that allows to study prenatal influences of various factors on the embryo directly and standardised. Further, central regulation of metabolism and body weight in birds and mammals are similar to a higher extent. Equivalent with the *Nucleus acuatus hypothalami* in mammals, in birds the *Nucleus infundibuli hypothalami* (NI) is the primary regulatory centre. However, in birds no information on sensitivity of NI-neurons to changes in glucose concentration is available. Based on this fact, the aim of this study was to investigate the effect of different glucose concentrations on activity of single NI-neurons.

The experiments were carried out in brain slices (400 µm) of 20- to 30 days old chickens of both sexes. Using extracellular recording neuronal activity of NI-neurons was measured under superfusion with artificial cerebrospinal fluid (ACSF), which contained glucose concentrations of 1, 5 or 30 mmol/l, with 11 mmol/l as control (identical with the usual used ACSF).

Altogether 115 NI-neurons were determined. Under glucose concentration of 1mmol/l 18 neurons were glucose sensitive (n= 33), under 5 mmol/l 28 (n= 53) and under 30 mmol/l 21 (n=29), respectively. Similar with mammals, ‘high glucose sensitive neurons’, which increased or decreased the neuronal activity if glucose concentration is increasing from the control level and ‘low glucose sensitive neurons’, which increased or decreased the neuronal activity if glucose concentration is decreasing from the control level up to 1 mmol/l were found.

Further experiments in normal incubated chickens as well as under the influence of prenatal hyperglycaemia are scheduled.

Keywords: chicken, hypothalamus, glucose sensitivity, neuronal activity, extracellular recordings

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Neural recovery in songbirds

E. Bosíková¹, Ľ. Košťál¹, E.D. Jarvis² and L. Niederová-Kubíková¹

¹Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, Ivanka pri Dunaji, Slovakia; ²Duke University, Durham, NC, USA

Songbirds represent one of the three bird lineages (including parrots and hummingbirds) in which vocal learning has evolved over evolutionary history. Zebra finch (Taeniopygia guttata), in which similarly to other birds only males sing, represents a popular model for neurobiological studies of birdsong. Specific vocal areas located in forebrain are required for vocal acquisition and song production. These nuclei increase in size and add new neurons mostly during postnatal development until adulthood, but neuronal incorporation persists also in adulthood. Adult neurogenesis in both birds and mammals was proven only in 80’s. The new neurons are born in subventricular zone in avian brain and migrate to the whole forebrain, primarily to vocal nuclei HVC and Area X of the striatum.

The vocal region Area X is necessary for vocal learning in juveniles, and probably also for the song maintenance in adulthood. Our preliminary studies showed that Area X after bilateral neurotoxic lesion recovers. The aim of the presented experiments was to find the mechanism of this regeneration. Our hypothesis was that the regeneration occurs either (1) via migration of neurons from adjacent area to the lesioned place or (2) via neurogenesis and intense incorporation of new neurons. The other aim of the study was to determine time course of this recovery and compare neuronal organisation of the recovered area with intact brain.

Four to twelve months old zebra finch males were used. Ibotenic acid (1%) was used for bilateral neurotoxic lesions in Area X. In order to identify the newborn neurons, the cell division marker 5-bromo-2’-deoxyuridine (BrdU, concentration 10mg/ml, ~ 0.08mg/g body weight) was injected i.m. for 7 days twice a day one month before sacrifice. Double and triple immunohistochemical labelling with specific antibodies as markers of various cell types were used to localize and count the neurons, newborn neurons, and types of neurons.

We found that one month after lesioning substantial part of the striatal region is recovered, i.e. the cells expressed neuronal marker. Further we found that many of these cells expressed DARPP-32 which is a marker for striatal medium spiny neurons. The identity of the rest of the neurons is being analysed.

Keywords: striatum, vocal nuclei, zebra finch, neurogenesis

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Effect of early feeding on the transfer of maternal antibodies and development of immune competence in the broiler chicken

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We investigated effects of early feeding on the development of maternal antibodies in yolk and plasma and the consequences on growth and immune responses in later life.

Broiler chickens (Ross 308) were immediately fed after hatching or withheld from feed and water for 24, 48 or 72 h. Both fed and unfed chickens were sacrificed immediately after hatch or 24, 48 or 72 h after hatch and IgY contents of the residual yolk and plasma were analyzed. Another group of fed and held chickens was raised in floor housing till d 19 and were intra-tracheally challenged with a lipopolysaccharide (LPS) – Human Serum Albumen (HuSA) cocktail as a model for lung infection. Nitric oxide (NO) production and plasma antibody titers against LPS and HuSA were determined on d 0, 1, 3, 7, 14 and 21 post-challenge.

Body weight showed a faster increase in the fed than in the held chickens, ending up in a body weight at d 40 of 2,269 and 1,994 g, respectively. Absorption of residual yolk was not affected by treatment, but results demonstrated that IgY in the yolk is not utilized as fast as the rest of the residual yolk, possibly due to dehydration in the held chickens or a kind of saturation in the IgY transport system in the fed chickens. No differences between treatments were found for NO production or LPS/HuSA antibody titers after the challenge. However, held chickens showed a significant decrease in growth following the LPS/HuSA challenge at d 19, whereas this drop in growth was less severe in the fed chickens.

We concluded that early feeding in broiler chickens results in faster growth in later life, but also that fed chickens are better able to cope with an immunological challenge in later life, although this is not reflected in different antibody titers.

Keywords: early feeding, immune response, maternal antibodies, residual yolk

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Influence of external hypercapnia under normoxic and hypoxic conditions during the first 10 days of incubation on the course of embryonic heart development of two broiler strains differing in growth rate

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Recent studies show that higher incubator CO₂ levels change the course of embryonic development and hatching features. High CO₂ levels in the first half of incubation can result in enhanced embryonic growth, early hatching and increased hatchability.

Our studies focus on the effect of external hypercapnia in normoxic and non-ventilation-conditions during the first 10 days of incubation on two broiler strains differing in growth rate (Isa/Ross). Group 1, incubated with rising CO₂ levels and 21% O₂ until D 10, will be compared to a group incubated in an airtight non-ventilated incubator. Controls are incubated under standard conditions. The effect of CO₂ on metabolism and morphological parameters of the embryonic chicken in general and especially on the heart development will be determined.

The embryonic bodyweight (EBW), heart weight and morphological properties such as heart length from base to apex and heart diameter were measured and the growth efficiency was determined on D 12, 15, 18. Under hypercapnic/normoxic conditions EBW seems to be influenced differently in both strains. Whereas the Isa-strain shows decreased EBW (D 13: 7,04 g vs. 9,16 g) the Ross-strain shows an increase in EBW (D 13: 8,16 g vs. 8,85 g) compared to the control. Relative heart weights of both of the CO₂ groups are higher than in the control from D 10-11 (1,12 vs. 1,45 Isa; 1,15 vs. 1,73 Ross). Aforementioned parameters seem to align towards the end of incubation. Heart length is increased in CO₂-Isa towards the end of incubation and increased in CO₂-Ross compared to control.

These results will be completed by further results of the non-ventilated group, blood/amnion-examinations and expression rates of selected parameters. Findings so far suggest that CO₂ influences both strains differently. By enhancing the metabolism especially in the Ross strain it may act as a stressor to the myocardial development.

Keywords: external hypercapnia, broiler, perinatal development, heart

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The effect of genetic line (broilers vs. layers) and parent flock age on embryonic development.

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The last decades were characterized by genetic selection for broiler and layer chickens for different purposes. While broilers have been selected for improved growth rate and meat yield, layers have been selected for intensify egg production. It is to be expected that the genetic selection for diverse trait also influence embryonic development and growth patterns affecting metabolic rate. The objective of the present study was to examine the effect of broiler and layer genetic strains in 2 parent flock age (31 and 38wk) on embryonic development, heart rate, O_2 consumption, and blood parameters.

Two incubation settings with eggs from one layer (Lohmann) and two broilers (Cobb and Ross) lines were studied. 500 eggs per line were used from breeder flocks age 31 and 38wk at the 1st and 2nd incubation, respectively. Layer embryos showed a slower and different developmental pattern. They hatched one day later than the broiler lines. At hatch their relative embryonic weight was significantly lower although yolk relative weight was similar, probably as a result of the excessive period till hatch. O_2 consumption of layer embryos was found to be relatively lower compare to both broiler lines, from E10 till hatch, or till E15 for 31 and 38wk parent flocks, respectively. In addition layer embryos had a significantly higher heart rate than that of the Cobb embryos, however, compared to the Ross embryos it was significantly higher only on a few singular days. Plasma T_3 concentration, hematocrit, and hemoglobin concentration measured for layer embryos were found to be lower compare to the broiler lines during embryogenesis. Comparison between the Ross and Cobb embryos exhibited for the first time differences in embryonic development between the 2 lines. O_2 consumption of Ross embryos was slightly higher than in Cobb. Heart rate and plasma T_3 concentration were significantly higher for Ross embryos compare to Cobb's. These differences suggest that although the genetic selection for rapid growth rate in the two broiler lines did not cause differences in embryonic growth patterns, it did however affect their metabolic rate.

The results of this study suggest that changing the incubation parameters like temperature or gases concentration, in order to create epigenetic effect, should be done by considering the line, and adapting the environmental changes to the developmental patterns and metabolic needs.

Keywords: broiler, laying hen, embryogenesis, metabolic rate

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Short-term cold influence stimulates neuronal NO-synthase activity in the anterior hypothalamus of Muscovy duck embryos

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NO is prominently involved in the regulation of various physiological functions like the regulation of body temperature, metabolism and body weight. In the brain it acts as a messenger molecule in differentiation, synaptic plasticity and neurotoxicity. NO is produced by activation of nitric oxide synthase (NOS). The marker for NOS-positive neurons is nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d). The aim of the study was to investigate the influence of short-term temperature stimulation on neuronal NOS activity of the anterior hypothalamus in Muscovy duck embryos during second half of incubation.

Experiments were carried out under normal incubation temperature (37.5°C) in Muscovy duck embryos on E20, E23, E28 and E33. Three experimental series were performed; without acute temperature stimulation (control), with 3 h warm (39°C) or cold stimulation (34°C) on the respective experimental day. In the temperature-stimulated groups the brains were immediately extracted after the 3 h of temperature influence. Activation of neuronal NOS was investigated in all experimental groups using histochemistry for identification of the NADPH-d. For analysis, NADPH-d positive neurons were counted in a defined area of the anterior hypothalamus. In the control and warm stimulated group NADPH-d positive neurons could be first detected on E23. Acute cold load induced NOS activation during an earlier embryonic age. In the cold stimulated group NADPH-d positive hypothalamic neurons were found already on E20. Also in the other age groups investigated, the activation of hypothalamic neuronal NOS was significantly increased after cold stimulation. In birds the development of body functions starts early during embryogenesis. Environmental stimulation could improve this process (‘training effect’, Nichelmann and Tzschentke, 2002, Comp. Biochem. Physiol. A 131:751-763). Both, the activation of neuronal NOS on an earlier stage of development and the significant increase in activation of NOS exclusively after cold load in all other age groups investigated leads to the hypothesis, that probably in bird embryos NO acts as a modulator of the neuronal cold and energy uptake pathway in the anterior hypothalamus.

Keywords: Muscovy duck, NO-synthase, prenatal ontogenesis, hypothalamus

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The chick embryo as a model to study persistent effects of embryonic protein-undernutrition on postnatal growth and metabolism

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The aim of this study was to investigate embryonic protein-undernutrition, by the removal of albumen, and its persistent effect on postnatal growth and protein metabolism until one week post-hatch. The main difference compared to maternal protein undernutrition during pregnancy in mammals, is that there is no influence of an altered maternal hormonal status on the embryo, due to the independent development of the chicken embryo in it’s egg.

Before the start of incubation, 10% of albumen, based on measurements of albumen weight/egg weight ratio, was removed from the sharp end of the egg with a needle and taped thereafter (albumen group, A). The sham group (S) constituted of eggs with a hole at the sharp end, which was taped thereafter. Another control group (C) was not manipulated before the start of incubation. Eggs were incubated under standard conditions.

At embryonic day (ED) 18 and hatch, chick weight of the A group was significantly lower compared to the S and C group (8% difference). The water content of the A-chicks was also significantly lower although not to the same extent (2.7% decrease). Body weight remained significantly lower until day (D) 7. There were no significant changes in plasma glucose, triglyceride and uric acid concentrations at hatch, D3, and D7. The level of phosphorylation of several proteins that regulate mRNA translation (p70S6K, S6, eif2α, 4EBP1) in breast muscle did not differ between the experimental groups (Western blots at hatch and D7). However, the capacity for protein synthesis (ribosomal capacity, i.e. RNA/protein) showed a trend to a higher level at ED18 and hatch in the A group compared to the S and C groups, compatible with the delayed development of the A-chicks. Current analyses (real-time PCR) aim to investigate possible changes of protein degradation, myosin expression and energy metabolism between treatments.

**Keywords:** embryonic undernutrition, adaptation, metabolism, growth, protein

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Effect of riboflavin in ovo on chosen blood biochemical parameters in the chick embryo

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Riboflavin (lactoflavin, vitamin B\textsubscript{2}) plays a pivotal role in the organism since it is required for a wide variety of cellular processes. In chicken the deficiency of this compound inhibits a body gain, and causes general emaciation and muscular atrophy of limbs. The content of B\textsubscript{2} in the chick egg is c.a. 60 µg and its deficiency causes hypoglycemia and rapid death of embryos between 10\textsuperscript{th} and 13\textsuperscript{th} day of incubation (E10-E13). In the available literature there is lack of data describing excess of riboflavin on embryonic metabolism, therefore, the aim of the present study was to examine the consequence of \textit{in ovo} injection of riboflavin on 6\textsuperscript{th} day of embryogenesis (E6) on chick embryo development..

Eggs originated from the same broiler breeder flock of Ross 308 line were used. The eggs (n=450 in each group) were incubated at temperature 37.8±0.1°C and relative humidity 50 ± 2%. On E6, they were injected \textit{in ovo} with 0, 60, 600 µg B\textsubscript{2}/egg dissolved in 50 µl of 0.7% NaCl. Blood samples from 10 chicks of each group were collected at E12, E14 E18 and E20. Glucose (enzymatic method), protein (Lowry method) and total immunoglobulin (PIP-test) concentrations were determined in plasma samples.

In control group, at E12 the levels of glucose, protein and total immunoglobulins in blood of the chicken embryos were 4.27±1.83 mmol/l, 1.26±0.38 g/dl and 0.89±0.31 g/dl, respectively. During embryogenesis these parameters gradually increased reaching maximal values (12.30±1.65 mmol/l, 1.78±0.38 and 0.94±0.30 g/dl, respectively) at E20 (P<0.01). There were no significant differences in glucose, protein and total immunoglobulin concentrations in blood of B\textsubscript{2}-treated embryos in comparison with the control group.

Results obtained suggest that exposition of the chicken embryo to riboflavin does not affect glucose and protein metabolism and has no effect on immunological system. Further studies are needed to explain the role of vitamin B\textsubscript{2} in chicken embryogenesis.

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\textbf{Keywords:} chick embryo, riboflavin, glucose, protein, immunoglobulins

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Was Lamarck right?
Causes and consequences of variation in maternal hormones in avian eggs

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Eggs of all avian species studied so far contain substantial amounts of steroid hormones from maternal origin. These include progesterone, the androgens androstenedione, testosterone and dihydrotestosterone, and to a lesser extend estradiol, whereas the presence of substantial levels of corticosterone could not yet be confirmed, in contrast to earlier reports. The high levels of gonadal steroids in the egg yolk have their origin in the steroigenic cell layers of the follicle wall and probably accumulate during the rapid yolking phase. Concentrations of these hormones in egg yolk vary systematically at 5 levels: 1: between species, among others in relation to species-specific variation in the timing of embryo development; 2: among females of the same species, suggesting heritability for this trait; 3: among clutches of the same female, in relation to environmental cues such as food availability and social factors; 4: within clutches, in relation to the position of the chick in the hatching sequence, affecting sibling competition; 5: within the egg depending on the yolk layer. Hormone injections in the egg have shown that these maternal hormones have pleiotrophic effects on a wide array of traits in the offspring, both in the short and long term, both on morphology, physiology and behaviour. These hormone-mediated maternal effects may therefore serve as a maternal tool to adjust offspring development to prevailing conditions, depending on how the mother perceives her environment during egg production. I will discuss among other things 1: Yolk hormone effects on development; 2: To what extend hormone concentrations in the maternal circulation is related to yolk concentrations of these hormones, or can be regulated by the mother independently of each other, providing the mother the possibility to influence her offspring without affecting her own physiology; 3: The fate of these hormones during embryo development and whether the embryo may play an active role in this.

Keywords: yolk hormones, androgens, prenatal maternal effects, adaptation

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Yolk testosterone content and chicks' behaviour are influenced by maternal age in Japanese quail (*Coturnix c. japonica*)

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The behavioural phenotype of an individual is not only determined by his genes and his immediate environment but also by epigenetic mechanisms such as maternal effects. These maternal effects can intervene after birth but also before. In birds, this prenatal maternal influence is mediated by the hormonal content of the females’ egg. Indeed, the environment experienced by the laying female can modify the level of steroid hormones in her eggs and, by this way, modify chicks’ characteristics. In our study, we wanted to know if intrinsic characteristics of the female, such as her age, could also influence this hormonal content and thus the behaviour of the chicks. We studied a group a Japanese quail females at two distinct ages: at the beginning of breeding and six months later. For each age, we measured hormonal composition of steroids in eggs’ yolk of the females and studied their chicks in several behavioural tests. We found a decrease in yolk testosterone content with female age and also a different behavioural profile of chicks depending on the female age. Thus we showed, for the first time in birds, a maternal age effect on the hormonal content of eggs and on the behavioural phenotype of chicks.

**Keywords**: prenatal effects; maternal age; yolk hormones; behavioural development; Japanese quail

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Influence of temperature stimulation during the last 4 days of incubation on performance of broiler chickens

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Incubation temperature has a great influence on the later performance in poultry. The objective of this study was to determine the effect of variation in incubation temperature from day 18 to day 21 on performance of broiler chickens.

Six Incubation Trials and three Growing Trial were carried out. Eggs (ROSS 308) were incubated from day 1 to 18 under normal incubation conditions and then sorted in three (Trials 1 and 2) or in two hatch incubators (Trial 3–6). In the first hatch incubator the temperature was 37.2 – 37.4 °C (Control). The temperature was continuously increased by 1 °C over standard in the 2\(^{nd}\) hatch incubator and 1 °C over standard for 2 hours daily in the 3\(^{rd}\) hatch incubator. The 1-day chickens from the Incubation Trials 1, 2 and 3 were used for a following 35-day growing trial. In the Growing Trial 1 and 2 chickens were kept under chronic warm temperature regime (32 °C), in the Growing Trial 3 birds were kept according to the temperature-regime of the breeder. Feed and water was provided for \textit{ad libitum} consumption.

In the Growing Trial 1 and 2 feed intake and therefore body weight gain of broilers (particularly male chicks) from permanently warm incubated group was significantly lower during the fattening period compare to the other male and female chickens. In the Growing Trial 3 the mean daily weight gain of 64.8 g for the prenatally short-term warm stimulated male broiler chicks was higher than for all other groups. The feed conversion of the short-term warm stimulated male broilers was statistically secured to be lower in comparison to the male and female animals of the control and chronic warm incubated groups. In conclusion, incubation temperature profile, which includes short-term temperature variation, can be of high relevance for improving poultry performance (European patent, pending since March 2008).

\textbf{Keywords}: broiler chickens, incubation temperature, feed intake, daily weight gain

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Ontogeny of clock gene expression in central and peripheral circadian oscillators in chicken

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The circadian system is a complex structure regulating biological rhythms with a period close to 24h. The central part of the circadian system consists from the pineal gland, retina and suprachiasmatic nuclei in birds. Peripheral oscillators are localised in most tissues of the body like the heart, liver etc. Molecular mechanisms of circadian oscillators function relay on rhythmic clock gene expression that can inhibit their own transcription. Our aim was to elucidate ontogeny of clock gene expression in the central and peripheral oscillators, therefore we analysed expression of per2, bmal1 and e4bp4 in the pineal gland, heart and liver under synchronized and constant condition. Hatching eggs of broiler breeders were incubated in a forced draught incubator under a light (L) dark (D) cycle 12:12. On embryonic day 19 and postembryonic day 4, samples of the heart, liver and pineal glands were taken in 4h intervals during the 24h cycle. During the dark phase, embryos and chicks were taken from the incubator in complete darkness and decapitation occurred within 10s in the adjacent neighbouring room under very low intensity red light. Samples of tissues were immediately frozen in liquid nitrogen and stored under -80°C until RNA extraction. After cDNA synthesis clock gene expression was measured by real time PCR. Expression of per2, bmal1 and e4bp4 in the pineal gland exerted an expected rhythmic pattern in 4-day old chicks similar to that observed in adult birds. Expression of per2 and e4bp4 was rhythmic in the pineal gland under LD conditions, but the rhythm of e4bp4 mRNA did not persist in constant conditions. The amplitude of per2 mRNA expression was reduced in constant darkness in the embryonic pineal gland. Expression of per2 and bmal1 was rhythmic in the heart and liver of 4-day old chicks and more pronounced rhythmic expression was observed in the liver. Expression of per2 and bmal1 did not show a rhythmic pattern in the liver and the heart of embryos either in constant or in rhythmic conditions. According to our data the circadian control of per2 expression in the pineal gland is detectable before circadian rhythm in e4bp4 and bmal1 emerges. We suppose that rhythm in per2 expression in the pineal gland is a consequence of synchronizing effect of a LD cycle, which is sensed by the embryo. Absence of per2 rhythmicity in peripheral tissues of embryos can reflect missing synchronizing cues during incubation, because expression of clock genes in the heart and liver was proved. This implicates an advanced stage of development of peripheral oscillators during the last third of incubation and explains the prompt functioning of circadian system after hatching. This study was supported by grants APVV-0214-07, VEGA 1/4328/07, VEGA 1/4343/07.

Keywords: per2, bmal1, e4bp4, pineal gland, heart, liver

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Influence of early inoculation (in ovo vaccination) against Marek's disease virus and reovirus on hatchability, chick degree and performance, physiological and immunological responses in broiler chicks

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A total number of 990 embryonated (fertile) chicken eggs (Arbor Acer) were used in the current study. This study was aimed to evaluate the influence of early inoculation of Marek's disease virus (MDV) and Reovirus during late embryonic development on Hatchability, Chick degree, Chick performance, and Physiological and Immunological responses in Broiler chicks. The embryonated eggs were obtained from a commercial Parent Stock farm (Cairo Poultry Company) at 53 weeks of age. The parent hens were Specific Pathogen Free (SPF) for common diseases. The eggs were weighted before setting (Zero time) and randomly distributed into equal six experimental groups. At 18 days of incubation, when routinely the eggs transfer from Setter into Hatcher, the eggs were injected by EMBREX INOVOJECT machine. The 1st group didn't receive any treatment and served as sham control. The 2nd group drilled only without injection and served as holed control (drilled). The 3rd group drilled and injected with distilled water and served as injected control. The 4th group drilled and injected with MDV vaccine (Strain H.V.T.) suspended in strilled water. The 5th group drilled and injected MDV vaccine (Respine) suspended in strilled water. The 6th group drilled and injected with Reovirus vaccine suspended in strilled water.

The obtained results could be summarized as following: 1) IN OVO vaccination didn't affect hatchability and embryonic mortality. 2) The hatchability percentages were in the normal range, 88.4 % to 96.4 % according the total setting eggs. 3) Total embryonic mortality rates were ranged between 4.27 % and 4.85 % for MDV and Reovirus injected eggs, respectively. 4) Early embryonic injection with MDV and Reovirus provide an early protection and improve the chick growth performance. Also, activate the immune system than post hatch inoculation. 5) No significant effects were found for early inoculation on plasma constituents such as total protein, albumin, glucose, AST and ALT. 6) IN OVO vaccination with MDV and Reovirus vaccines enhanced the immune response Sheep Red Blood Cells (SRBC's) at the 10th day post immunization. 7) Early administration with MDV and Reovirus vaccines improved (increased) the humoral immune response against Newcastle disease, and this effect started at 3rd week of age.

Keywords: in ovo administration, Marek's disease vaccine, reovirus, broiler chick, embryonated eggs, performance, immune response.

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Immunological parameters and response to lipopolysaccharide administration in lines of Japanese quail bi-directionally selected for yolk testosterone concentrations

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Maternal hormones contribute to the epigenetic mechanisms by means of which mothers influence the phenotypes of their offspring and prepare them for the environment they will live in. Our research in this field has focussed on bidirectional selection of Japanese quail for high (HET) and low (LET) egg testosterone content. (Okuliarova et al., this issue). The aim of this study was to analyze basic parameters of the immune system and response of birds to an immune challenge in the third generation of HET and LET line of Japanese quail.

In our experiment we used 20 HET and 20 LET young quail. At the age of 10 days the half of the experimental animals was administered by lipopolysaccharide (LPS, Sigma, USA) in amount 1.5 μg of LPS per 1g of body weight in 20 μl PBS and the control group received 20 μl of PBS only. Birds were killed by quick decapitation 24 hours after administration of LPS. Weight of lymphoid organs (the bursa of Fabricius and spleen) and hematocrit values were determined and differential number of leucocytes was counted on blood smears. The results were statistically evaluated by a three-way analysis of variance with the line, sex and treatment as fixed factors. The statistical analysis revealed a significant effect of line on weight of the bursa of Fabricius that was higher in the LET than HET line. Significant differences between both sexes were found in the total number of lymphocytes. Females of both lines had a higher number of lymphocytes than males whereas the number of heterophils exhibited an opposite tendency that was evident also in the heterophil/lymphocyte (H/L) ratio. Administration of LPS decreased hematocrit values in females but not in males and did not influence the H/L ratio between sexes and lines. Obtained results suggest that selection of quail for low and high yolk testosterone concentrations can influence an immune competence of young hatched from eggs differing in testosterone content. This effect can be sex specific and understanding of exact mechanisms requires further studies. Acknowledgement: The study was supported by VEGA 1/4343/07.

Keywords: immune system, lipopolysaccharide, yolk testosterone, Japanese quail

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The Research of qualitative and quantitative industrial cross 60 days a life chickens red blood changes in conditions of changed erythropoiesis

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The goal of research – studying of qualitative and quantitative industrial cross chickens’ red blood changes in conditions of changed erythropoiesis.

Introduction – the system of blood is the major component of the internal environment and plays the central role in realization homeostatic processes in organism, in definition of its reactance and resistency. The matter is that biological action of the majority of stressful factors and pathogenesis of many diseases are connected with changes of oxygen mode in fabrics, the special role in maintenance of adaptive and compensatory processes is occupied by the gas-transport function of blood.

Studying of qualitative and quantitative industrial cross chickens’ red blood presumes to find opportunities of planning preventive actions for adaptation of young growth by creation of the conditions meeting needs of bird’s organism.

Materials and methods – as the object of research 60 days a life chickens of meat-egg and meat cross «Lohmann Brown» and «Smena 4» were used.

Hypoxic hypoxia was spent in a pulse mode by placing chickens to a pressure chamber with a forced-air and exhaust ventilation at discharging 40,98 kPa (that corresponds to rise on 7000 meters above sea level) for 6 o’clock daily within 6 nights and days. The estimation of red blood parameters was carried out before the influence and right after each session of hypoxia.

In order to research of heterogeneous system of hemoglobin the method of electrophoresis in polyacrylamidic gel has been applied to.

Marrowy punctate was researched in the control and over every 6 hour session of hypoxia. Total quantity of myelocaryocyte was defined.

Results of research - the analysis of hemoglobical structure of chickens research results has shown, that as a result of hypoxic hypoxia influence on an organism, the most strongly expressed changes in the ratio isophorm occur for the 5th day.

Hemoglobinical structure of 60 days a life cross chickens «Lohmann Brown» and «Smena 4» in conditions of chronic hypoxical hypoxia for 6 sessions (%) (M±m)

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*-distinctions are authentic in relation to the control (p<0.05).

**-distinctions are authentic in relation to cross «Lohmann Brown» (p<0.05).

The maintenance in blood HbD increases for 5 day of experience, in comparison with the control, over chickens abstract truncated...

Keywords: Changed erythropoiesis, Chickens, Hypoxic hypoxia, Technological stresses, Preventive actions, HbA and HbD

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Can apomorphine challenge reveal chicken personality?

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Individuals differ in a non-random manner in a way they deal with stressors, novelties, manage their social relations or control their aggressive behaviour. Temperament, personalities, copying styles and other related phenomena, i.e. stable inter-individual differences in behaviour and physiology, were documented in a wide range of animal species from invertebrates to mammals. Major theories of personality hypothesize that variation in dopaminergic activity underlie personality traits such as novelty seeking or extraversion. The drug challenge paradigm allowing measurement of responsiveness of a neurotransmitter system in vivo enables to study the relationship between personality and functioning of these systems.

We have used apomorphine (mixed D1/D2 dopamine receptor agonist) challenge to characterize inter-individual differences in behaviour of chickens shortly after hatching. Behavioural response of 2-6 days old Hisex Brown chicks (n=159) to apomorphine (0.5 mg/kg i.m.) was observed for 30 min in presence of other, untreated chick and the results were subjected to principal component analysis (PCA). Apomorphine treatment caused increased motor and pecking activities with large individual variation. PCA identified three clusters of behaviours, that correlated with 1/ sociability (pecking and pulling of other chicks and own foot), 2/ aggression (pecking of other chick’s head and body) and 3/ exploration (object pecking, locomotion and head shaking).

The results of the apomorphine challenge test were correlated with feather pecking behaviour (behavioural disturbance representing serious welfare and economic problem) of hens as adult (n=84). We found some links between response to apomorphine shortly after hatching and feather pecking behaviour of adult laying hens. Factor score for Factor 2 (aggression) correlated positively with the number of gentle pecks given in adult hens (r=0.24, p=0.028) and pecking and pulling of other chick’s foot in apomorphine challenge test correlated negatively with the received severe feather pecks of adult hens (r=-0.23, p=0.032; r=-0.22, p=0.047). Acknowledgement: This study was supported by the VEGA 2/0103/08.

Keywords: domestic chicken, apomorphine, dopaminergic system, drug challenge paradigm, animal personality, feather pecking

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Malformations of chick embryos exposed to high doses of \textit{in ovo} injected ascorbic acid

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Ascorbic acid (AA) is not present in the hen’s egg, however, it is synthesized by the chick embryo from the 6$^{th}$ day of embryogenesis (E6). The aim of the present study was to examine the consequence of \textit{in ovo} injection of AA in the first week of embryogenesis on chick embryo development.

The eggs derived from the same broiler breeder’s flock of Ross 308 line were used. Eggs ($n = 300$ in each group, weighing $62 \pm 5$ g), were incubated in the Petersime setter S576 (temp. $37.8 \pm 0.1^\circ$C and relative humidity: $50 \pm 2\%$) and the hatcher H192 ($37.2 \pm 0.1^\circ$C, RH=55-70\%). The \textit{in ovo} injections of AA in doses: 0, 2.5, 5 and 10 mg in 50 µl of 0.7% NaCl per egg were carried out on E6. The embryopathological analyzes of unhatched eggs were made and the developmental stage, malformations and malpositions of the embryos in the egg were estimated.

The hatchabilities in groups injected \textit{in ovo} with 0, 2.5, 5.0 and 10.0 mg of AA in E6 were 83.2, 68.0, 59.0 and 59.5\%, respectively. Frequencies of strongly malformed embryos (very often alive) in culled eggs were 0.0, 5.6, 10.0 and 14.3\% in groups treated with 0, 2.5, 5.0 and 10.0 mg of AA, respectively. The most frequent malformations (percentage of all deformed embryos cases), i.e.: eyelids absent (ablepharon) and/or atrophy of one or both eyes (microphthalmus) -82\%, reduced or completely absent limbs (wings and legs) – 79\%, the extremely cerebral hernia (encephalocel) -70\%, abdominal and pectoral hernia - 47\%, absence of cerebral cranium bones (acrania)-29\%, and, moreover, the cases of adhesion of a visceral cranium and a chest as well as demineralization of bones, were observed.

Results obtained showed that ascorbic acid affects the chicken embryo development. Further studies are needed to explain the observed changes in chicken embryogenesis following ascorbic acid treatment. Supported by Grant of Polish Ministry of Science No N N311 1351 33.

Keywords: chick embryo, malformations, ascorbic acid.

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Investigations of body movements in early stages of chick embryos using image processing

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We developed a computer-aided video system which captured continuously the inside of the egg using a CCD camera, and succeeded to observe development of the heart and blood vessels and the CAM in the early stages of chick embryos (Akiyama et. al., 2006). Additionally, in this study, we attempted to investigate the embryonic body movement which may be involved in normal development of the chick embryos.

On Day 2 of incubation, a CCD camera was inserted into the attachment. The camera focused to the embryonic body, which was illuminated by high luminance green LED (super-high intensity; 6300-9500mcd, wave-length; 525). Eight LEDs installed outside about 15mm from the eggshell and at height of middle of the egg. The video image from the CCD camera captured every 1/30 sec. The camera simultaneously functioned as a lid to close the window so that the embryos could survive several days or longer.

The embryonic eye was extracted from other parts of image using image processing, and then time series data of eye’s position was as embryonic movement. At first of image processing, the area of eye was abstracted by the color filter, which had bandwidth of pixels values every frame of video image. Then two circles, which were filled and non-filled, were determined as same as eye’s size. When these two circles fitted to eye, the center of fitted circles was set to position of eye every frame. The time-series data of these positions indicated the embryonic movement. The time-series data was separated to horizontal and vertical movement, and was investigated in detail.

Though the long-time measurements such as 1 week were difficult by several reasons (e.g. focus of camera, out of range, embryonic death), we attempt to record more data and to elucidate embryonic body movements.

Keywords: embryonic movement; CCD camera; early development; chick embryo; image processing

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Epigenetic regulation of thermotolerance acquisition and memory

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As with other sensory mechanisms, determination of thermal-control set point is refined during a critical period of development by alterations in cellular properties in the frontal hypothalamus. These alterations in hypothalamic plasticity are achieved by renewal of the protein repertoire via activation or silencing of gene transcription, both of which are regulated by chromatin remodeling.

Here we demonstrate increase of global histone H3 di-methylation at lysine 27 during thermal-control establishment in general and at the initiation of the BDNF coding region in particular. Furthermore, antisense “knockdown” of the H3K27-specific methyltransferase, EZH2, which was induced in correlation with the methylation of H3K27, disrupted the thermal set-point and inhibited Bdnf mRNA expression.

In a second stage of epigenetic regulation of thermal control establishment we demonstrate alteration in the pattern of CpG methylation in the promoter area of BDNF. During heat conditioning there was a transient induction of methylation of two CpG positions (The first and the third CpG location downstream from the BDNF-ATG) and a reduction of methylation of one CpG position (1000bp downstream from the BDNF-ATG), while the other CpG positions did not show significant change. Furthermore, our data demonstrate a significant difference between the levels of methylation at the Bdnf promoter of conditioned compared to non-conditioned chicks during a thermal challenge a week after conditioning, indicating long-term epigenetic regulation.

Taken together, these results correlate epigenetic chromatin methylation with thermal-adaptation-related hypothalamic plasticity. Supported by ISF

Keywords; heat conditioning, Brain derived neurotrophic factor, histone methylation, DNA Methylation.

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The effect of eggshell temperature on malpositions, protein energy and heart weight in broiler embryos

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In practice, high incubation temperatures at the end of incubation are often observed due to cooling or air velocity problems in the incubator. An experiment was conducted to evaluate the consequences of high eggshell temperatures on viability, heart weight and protein use in broiler embryos. Embryos were incubated at normal (37.8°C) or high (38.9°C) eggshell temperature (EST) after 7 days of incubation and hatchlings were measured at 12 h after emerging from the eggshell.

Death embryos in the high EST had 3.1% more ‘head over wing’ and 1.7% more ‘head between legs’ malpositions than the normal EST. The reason for these malpositions is unknown, but might be related to weakness of the embryo or lower muscle activity.

Embryos in the high EST had a lower body development at hatch in terms of a lower yolk free body, a larger residual yolk and a shorter chick length. In the high EST, less protein energy was deposited in the yolk free body and more remained in the residual yolk than in the normal EST. Protein energy was used because it determines largely yolk free body mass. Efficiency in protein transfer from the egg to the hatchling was decreased in the high EST and protein energy was lost or maybe used for other purposes than body synthesis. Incubation time was 8 h shorter in the high EST and decreased the time to develop. Relative heart weight was lower in the high EST (0.60%) than in the normal EST (0.77%). This decrease in heart development might increase health problems in later life such as ascites and sudden death syndrome.

In conclusion, high EST decrease heart and body development at hatch due to a lower protein synthesis and efficiency during incubation.

Keywords: incubation; eggshell temperature; malposition; protein energy; heart weight

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The influence of hypercapnic incubation on exclusive parameters in blood and amniotic fluid in two different broiler-strains

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Various studies show that higher levels of CO$_2$ change the course of embryonic development. It can result in faster embryonic growth, faster hatching and ascites-sensitive broilers are less susceptible in receiving ascites.

This study was performed to analyze the effect of high CO$_2$ levels in combination with normoxic or hypoxic conditions during the first ten days of incubation on acid-base balance, electrolyte contents, metabolites and haemoglobin in chicken embryos. We studied the changes in the amniotic fluid and the blood in the chorioallantoic membrane, because the composition of the embryonic liquids and maintenance of the homeostasis are essential for an optimal development of the embryo.

Experiments were performed on two broiler strains differing in their susceptibility for ascites syndrome (Ross/Isa). The eggs were exposed to a gradual increase in CO$_2$ levels up to 1,5% with maintenance of 21% O$_2$. The other group was not ventilated. Blood samples from the chorioallantoic vein and amniotic fluid were examined on D 10-18.

So far results from the hypercapnic/normoxic incubation are presented. The Ross strain had more haemoglobin than the Isa strain. The electrolyte-content in the amniotic fluid of the Ross strain differed between the test and the control group on D 13 (K$^+$: 39,06mmol/l vs. 26,61mmol/l; Na$^+$: 105,25mmol/l vs. 114,45mmol/l; Cl$: 100,625mmol/l vs. 120,16mmol/l). During incubation the Ca$^+$-concentration in the amniotic fluid of the CO$_2$-Ross was higher than the concentration in the control group (D 12: 1,13mmol/l vs. 0,898mmol/l, D 15: 2,14mmol/l vs. 1,68mmol/l).

All in all hypercapnia had a greater impact on the Ross strain. Due to the changes on D 13 it seems that the seroamniotic plate ruptures earlier in the CO$_2$-Ross than in the control group. We discuss the role of oxygen in combination with hypercapnia and the reason for the benefits due to hypercapnia.

Keywords: broiler, hypercapnia, perinatal development, blood, amnion

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Effect of acute hypoxia on the motor activity and heart rate of the 10- and 14 day chick embryo.

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Embryonic motility represents an important component of development and its disturbance leads to severe malformations and functional disorders. It is known that embryonic motility in chick is periodic and consists of the activity phase (AP) and inactivity phase (IP). It depends on the environmental conditions, but the effect of environmental influences on development, via the alteration of embryonic activity, is little investigated. The goal of our study was to examine the effect of acute hypoxia on embryonic motility in chick on the incubation days 10 (D10) and D14 and to compare these data with those on heart rate (HR).

Chick eggs on D10 or D14 were placed into a thermoregulated chamber and opened at the air cell side. A force transducer connected with the limb of the embryo was used to record embryonic movements inside the egg. Force recording was synchronized with the video recording of the HR. During the experiment the recording was performed continuously in normoxia (30 min) then during acute hypoxia (10% O₂ for 10 min) and after that again in normoxia (30 min).

There were no significant changes both of the AP and IP in response to hypoxia on D10, and HR decreased by 10% of the control value. On D14, an inhibitory effect of hypoxia on embryo motility due to the increasing of IP duration was found: IP started to increase since the 1-2 cycle (0.5-1.5 min), reached its maximum exceeded the control value by 4.5 times, and then recovered partly during the hypoxic exposure. Simultaneously, HR decreased in about 2 min by 30-40% of the control and recovered partly during hypoxia. These data suggest that recovery of HR may, at least in part, contribute to the recovery of embryo motility under hypoxia in the late embryos. This work was supported by RFBR grant 08-04-01063.

**Keywords:** hypoxia, chick embryo, motor activity, heart rate

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Dopamine receptors in zebra finch brain

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Songbirds possess the rare trait of vocal learning which is speech in humans. There are many similarities between the bird song and human speech, including similar aspects of vocal learning, brain areas controlling this type of behaviour, or genes involved. However, it is difficult to study the neural mechanisms of speech and therefore songbirds are the most popular model. One of the key neurotransmitters implicated in the control of singing behaviour is dopamine. There are higher levels of dopamine released in striatal vocal nucleus Area X during singing than in silence and the midbrain areas, where most of the dopaminergic projections arise from, show higher electrophysiological activity during singing in comparison with silence. Although there are several binding studies in birds mapping the dopamine receptor localization, so far the distribution has been known only for the main dopamine receptor families, D1-like and D2-like. However, there are three types of D1-like receptors, D1A, D1B, and D1D, and three D2-like receptors, D2, D3, and D4. In songbirds only the distribution of D1A and D1B receptor mRNA has been described.

In this study we cloned five dopamine receptor subtypes, D1A, D1B, D1D, D2, and D3, and found the distribution of their mRNAs in brains of adult male and female songbird species zebra finch (Taeniopygia guttata) as well as in juvenile birds at various developmental stages. We found that while the D1A, D1B, and D2 receptors are highly expressed in striatum, the D1D and D3 receptors are mostly in pallium. Moreover, there are specialized expression patterns in the telencephalic song nuclei. We found this specialized expression for some receptors in some song nuclei already at 15 days of age, at the beginning or before the sensory phase of vocal learning. Further, there were changes in these expression patterns during development and they coincided with stages of vocal learning. These data together suggest that dopaminergic system plays an important role both for song acquisition as well as during adult singing.

Keywords: striatum, song nuclei, striatum, D1-like, D2-like, speech

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Divergent selection of Japanese quail for yolk testosterone concentrations

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The adaptive potential of maternal androgens in the egg yolk in relation to their effects on phenotypic development (growth, physiology and behaviour) of the chicks has been extensively studied. Experimental approaches to study yolk hormones include either the direct manipulation of hormonal content in avian eggs or the manipulation of environment experienced by female during egg laying. Although, the transfer of maternal hormones to the egg yolk is highly plastic, some studies have shown consistency of maternal hormone deposition among individual females, suggesting heritability for this trait. Genetic heritability is a requirement for Darwinian selection to act and so for the many adaptive hypotheses, but this heritability has not yet been demonstrated. Therefore, we adopted a genetic approach and performed divergent selection on yolk testosterone (T) concentrations of Japanese quail. In addition, we measured yolk androstenedione (A4), progesterone (P4) and estradiol (E2) concentrations to determine the effect of selection on a complete steroidogenic pathway. Quail laying eggs with extremely high and extremely low yolk T levels were selected from a random-bred population. Over two generations, we obtained 2 lines of Japanese quail with high (HET) and low (LET) egg T content. Concentrations of yolk T significantly differed between these lines in F1 as well as in F2 generation. In addition, in both generations eggs of the HET line contained higher concentrations of yolk A4 than those of the LET line. Opposite differences between lines were found in yolk E2 levels whereas the selection did not influence concentrations of yolk P4. The proportion of phenotypic variability in yolk T deposition caused by genetic effects was estimated as the regression of cumulative response to selection on cumulative selection differential and the realised heritability was 0.35. Our results demonstrate for the first time a significant genetic contribution to deposition of maternal hormones to the egg yolk in birds reared under stable environmental and social conditions with potentially important implication for both proximate and ultimate approaches to the study of hormone mediated maternal effects in birds.

Acknowledgement: The study was supported by VEGA 1/4343/07

Keywords: divergent selection, yolk testosterone, Japanese quail, heritability

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Development of an in vivo model to screen for antioxidant potential of compounds utilizing chick embryos: finding the best method to induce mild oxidative stress

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Many human and animal illness have been correlated to oxidative imbalance. There is a lot of interest from research groups to find compounds able to reduce oxidative stress and its consequences. Oxidative stress is usually accumulative and difficult to reproduce consistently in in vivo studies to screen antioxidants. It is our hypothesis that a chick embryo model may facilitate rapid screening of antioxidants. The chick embryo is known as easy to manipulate, fast responding and low cost in vivo model. New techniques make it possible to introduce substances into different egg compartments with little damage to the developing embryo. The objective of this study was to identify the best method to create a controlled oxidative challenge in chick embryos, so that they can be utilized to test potential antioxidants in ovo. Promising compounds will be identified based on their capacity to reverse some of the deleterious effects of oxidative stress. A total of 4 trials were performed testing different oxidative stress inducing methods: 1) heat-shock, 2) injecting glucose into the air-cell at E14, 3) injecting H₂O₂ into the air-cell at E14, and 4) physically blocking part of eggshell pores with tape (hypoxia) between E10 and E18. Eggs where sampled at different time points between E14 and E18. Parameters analyzed included embryonic survival, embryo and liver size, air-chamber gas composition, and hepatic ferric reducing antioxidant power (FRAP). From the tested methods, injection of glucose or H₂O₂ were discarded due to high embryonic mortality. Challenging embryos with heat-shock changed many parameters but the effects disappeared with time. Therefore, the most consistent results were found by partially blocking eggshell pores. This was demonstrated by increased CO₂ to O₂ ratio in the air-cell chamber, reduced embryonic size and increased liver FRAP. Based on these findings we concluded that, from all tested methods, partially blocking eggshell pores during late-term development is the best way to induce mild oxidative stress in chicken embryos. This method now needs to be refined so it can be applied as a challenge model to test potential antioxidant compounds.

Keywords: chicken embryo, hypoxia, oxidative stress, in ovo challenge

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Effect of photoperiod on pre and postnatal growth and early stress responses of broiler chicks

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The present study was conducted to investigate the effect of a 16 h light and 8 h darkness (16L:8D) lighting schedule on pre-and postnatal growth, and early stress responses of chicks to regular handling in the hatchery. For this purpose, broiler breeder hatching eggs were divided into 3 groups and exposed to 16L:8D daily either from first day of incubation to 21 (IL_0-21) or from 14 to 21 d of incubation (IL_14-21). Darkness during the incubation as control (ID) was also provided. Embryo weights on d 13 and 18 of incubation and chick weight at hatch were measured. Plasma corticosterone (CORT) levels of chicks were measured at hatch and after regular handling stress in the hatchery (sexing from wing feathers, wing band attachment, weighing and creating in transport boxes with no access to feed and water for 8 h). A group of newly hatched chicks from both IL were reared for 6 days either under 16L:8D (RL_16) or 24L:0D (RL_24).

IL_0-21 embryos showed an increase in growth at embryonic d of 13 and 18 compared with the ID (P≤0.05) but weight of the embryos from IL_14-21 was similar to those from ID. Hatching weight did not differ among incubation treatments. However, body weight of chicks from IL treatments was significantly higher than those from ID at 6 d (P≤0.05). The RL_24 treatment resulted in heavier weights on d 6, regardless of IL (P≤0.05).

IL did not significantly affect CORT at hatch and after regular handling. However, CORT levels significantly increased in (IL_14-21) and ID after handling stress. This result would indicate that IL_0-21 chicks had a lower stress response to environment i.e. regular handling in this experiment. Further research may be appropriate to clarify whether lighting environment during incubation may have a potential to modify birds’ growth and welfare.

Keywords: prenatal and postnatal lighting, growth, broiler chicks

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Effect of acetylsalicylic acid injection by „in ovo” method on chicken embryo hearts in hyperthermia conditions

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This trial was aimed to determine the influence of acetylsalicylic acid on embryo’s heart in hyperthermic condition in the hatcher.

Previous studies indicated the loss of embryo’s heart mass and substantial increase of heartbeat rate when incubated in higher then optimal temperature. Material used in this experiment was divided into 4 sets of 50 eggs. Embryos from first (control) and second group were incubated in standard condition and those from group 3 and 4 were placed in hyperthermic environment (1°C above optimal temperature) after 18 days of incubation in optimal temperature. Before moving the eggs from incubator into the hather, all embryos from group 3 and 4 was injected with 5 mg of acetylsalicylic acid diluted in 50 ml of 0.9% NaCl. Heartbeat rate of the embryos was registered with balistocardiograph, starting from the first day of incubation. Ten eggs from each group were sampled on twentieth day of incubation. All embryos were euthanized and each heart mass was determined. Also histological slides were prepared and analyzed with Micro Scan Basic software. All embryos from group 4, injected with 5 mg acetylsalicylic acid, didn’t show significant loss of heart mass and thickening of the left chamber wall when compare to control group. Data obtain from balistocardiograph showed increased heartbeat rate in embryos incubated in hyperthermic condition when compare to the control group. In group 3 (not injected with acetylsalicylic acid) significant increase of heartbeat rate was observed especially when compared to samples from group 4 (injected with acetylsalicylic acid), when notable increase in heartbeat rate wasn’t demonstrated.

Hatchability was also considerably improved when eggs incubated in hypothermic conditions were treated with acetylsalicylic acid.

Our findings might then support the hypothesis of beneficial effect of acetylsalicylic acid on the heart of the chicken embryo.

Keywords: chick embryo, balistocardiography, acetylsalicylic acid

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Early postnatal development of experimental peritonitis in chickens in relation to the season and pineal gland function


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Circadian pattern of melatonin biosynthesis in the chicken pineal gland is established already towards the end of embryogenesis, but after hatch it is clearly influenced by the season. As endogenous melatonin seems to be involved in immunomodulation, the aim of present study was to evaluate the kinetics of peritonitis evoked in the early postnatal chickens in relation to the season and circadian rhythm of the pineal gland function, along with supposed influence of inflammation on the pineal gland biosynthetic activity. Experiments were performed on Hi-line male chickens hatched in summer and in winter and kept from hatch in LD 12:12. Experimental peritonitis was evoked by a single injection of thioglycollate (TG) to 2-, 9- and 16-day-old chickens. The development of peritonitis (measured by the number of leukocytes, PTLs) was correlated with the diurnal rhythm of pineal gland function, measured by the activity of N-Acetyltransferase (AA-NAT), a key enzyme in the melatonin biosynthetic pathway. In summer, AA-NAT activity increased in the dark phase, reaching maximal values 3–4 hr after the beginning of darkness. In winter, the nocturnal peak in AA-NAT activity appeared later, 5–6 hr after the beginning of the dark phase. Age-related changes in the diurnal rhythm of AA-NAT activity consisted in the increase of the nocturnal peak amplitude between 2- and 9-day of age. As it was impossible to retrieve the PTLs from the peritoneum of 2-day-old chickens, the development of inflammation was compared in 9- and 16-day-old birds only. TG-evoked peritonitis developed in the age- and season-dependent way, suggesting that in ‘summer’ chickens it develops earlier and in a better pronounced extent than in ‘winter’ ones. The existence of bidirectional communication between the pineal gland and activated immune system was supported by a decreased activity of pineal AA-NAT in chickens with peritonitis compared with control birds. Supported by the Polish MSHE grant N N303 3177 33.

Keywords: chicken, pineal gland, inflammation, N-acetyltransferase, peritonitis

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Preparing the broiler embryo for post hatch hot environmental conditions

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During the last decade the incubation period of broilers has gotten more attention. It can be related to the recognition that during this period various manipulations may induce long-lasting-physiological-memory caused by epigenetic adaptation. Thermal (heat) manipulations during the period of the hypothalamus-pituitary-thyroid or adrenal axes development and maturation have demonstrated a significant improvement in thermotolerance acquisition of broiler chickens up to marketing age. However, the effect of these manipulations on the embryos' thermoregulation is still not clear. This study aimed at elucidating the effect of continuously or intermittently (12 h/day) thermal manipulations (TMs) at 39.5°C between days 7 to 16 (included) of embryogenesis on egg shell temperature, oxygen consumption and heart rate of the embryos, as well as on the plasma concentration of the thyroid and corticosterone hormones. During the ectothermic period of incubation, egg shell temperature and oxygen consumption increased along with the elevation of incubation temperature. However, heart rate was relatively constant and plasma concentrations of thyroxine, triiodothyronine and corticosterone were significantly lower in comparison with the control. During the endothermic period of incubation, on days 18 onwards, all parameters were significantly lower than that of control, suggesting a significant decline in the embryo's metabolic rate which lasted along the post-hatch growth period of the chickens. It can be concluded that TM during incubation at periods critical for the hypothalamus-pituitary-thyroid development, induces a long-lasting-thermoregulatory response characterized by significant decline in metabolic rate on pre- (from day 18 of incubation) and post-hatch periods.

**Keywords:** broilers, embryogenesis, thermal manipulation, long lasting physiological memory

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Gas concentrations during storage do not affect hatchability and chick quality

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Egg storage longer than 7 d is associated with a delay in hatch time, and a decline in hatchability and chick quality. During egg storage, embryo viability declines due to an increase in cell death. Albumen quality, which affects the micro environment of the embryo, declines as well due to a rise in albumen pH and a decline in albumen viscosity. Whether the change in albumen quality affects embryo viability is unknown. An experiment was conducted to investigate the effect of high CO₂ concentrations or high N₂ concentration during storage on albumen quality, hatchability and chick quality. A high CO₂ concentration reduces CO₂ loss from the egg and therefore maintains albumen quality. A high N₂ concentration reduces the availability of oxygen. Eggs were stored for 14 d in 4 different storage conditions: normal air (control), 0.75% CO₂, 1.5% CO₂, and 95.8% N₂. Storage temperature was 16°C and relative humidity was 75%. During incubation, eggshell temperature was maintained at 37.8°C, relative humidity varied between 45-60%, and carbon dioxide level was held below 0.35%. Albumen quality was measured at oviposition and at the end of storage. Hatchability of fertile eggs was calculated and chick quality was measured 12 h after the chick emerged from the eggshell. At the end of storage, the control and N₂ groups had a higher albumen pH (9.43 and 9.59, respectively) than the 0.75% and 1.5% CO₂ groups (8.70 and 8.48, respectively; P<0.001). Both CO₂ groups had a higher albumen height than the control and N₂ groups (P<0.001). Hatchability and chick quality did not differ among the treatment groups. Although high CO₂ concentrations during storage affect albumen quality and a high N₂ concentration during storage affects oxygen availability, hatchability and chick quality are not affected by these storage conditions when storage time is 14 days.

Key words: storage conditions; albumen quality; hatchability; chick quality

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Infectious encephalomyelitis at broilers

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In epizootic conditions on infectious encephalomyelitis in Sverdlovsk area, considering transovarial defeats of chickens, it is possible to predict economic losses at a bookmark of the egg received from hens during decrease their production on incubation for formation of industrial flocks. Thus it is necessary to note, that the percent of a conclusion decreased on 25-30 %, at dissection observed muscular dystrophy.

At dissection embryos for 20 day the cutting of an operational material and preparation of histologic cuts was spent. In cortical and brain layers of kidneys observed interstitial hypostasis, nodal lymphocytic seepage intestitium framework. In a pericardium of heart - focal congestion of lymphocytes. In a myocardium - interstitial a hypostasis, dystrophic changes cardiac histiocyte, karyopincnosis kernels, erased cross-section banding. In endocardium - the moderate hypostasis, segmentary swelling, desquamation epithem. In a liver of all opened embryos - piecemeal necrosis of hepatic cells and lymphocytic infiltration in sinusoids. In cytoplasm hepatic have defined large granules of a brown pigment – lipofuscin.

At chickens from this party in the age of 6 days clinical attributes of defeat of the central nervous system in the form of paralyses of finitenesses, a tremor of a head, shaky gait were shown. In the age of 15-17 days such attributes, as are fixed: trembling of muscles of a trunk, more mass character had position on one side paws upwards. Such attributes at chickens were within 25 hours, then disappeared without the residual phenomena.

At chickens with spasms the body temperature was below on 2-2,5 degrees. At dissection any pathological changes of internal bodies it was not revealed. The gastroenteric path was absolutely empty. At chickens the muscular tone has been raised, had wax character, as consequence of uniform simultaneous increase of a tone extensor and flexor. Observed trembling of muscles at chickens (tremor) was to constants, spasmocidal and widespread (covered all body).

In a myocardium at the chickens had clinical spasms, the centers of weak perception cardiac myocytes painting eosin, with a smoothness transversal banding took place, that can be caused by decrease glycogen in sarcomplasm cells. It is marked furnace proliferation myoblast.

At dissection chickens found out the phenomena of a softening of the brain, similar with hypovitaminosis E.

At transovariation ways of transfer of an infection on the first place, on a degree of defeat, leave fabrics of a brain where observed heavy dystrophic and necrobiotic changes neurons. There were typical attributes encephalomyelitis - pervascular cellular couplings took place and have been presented by cells microglia, located around of vessels of small diameter.

At a horizontal way of transfer of a virus among chickens-broilers, the virus encephalomyelitis showed organotropic action on a way of distribution. In fabrics of bodies of chickens observed lymphoid congestions, in a digestive tube - lymphoblast proliferation in a wall of a stomach, an intestines and in a pancreas.

Confirming to histologic researches we cite the data received at use of method PSI with whom in a brain fabric has been established by a gene "field" strain a virus encephalomyelitis birds.

Method IFA in wheys of blood of sick 3 daily chickens had been certain average credits in high and non-uniform concentration from 1:42 up to 1:5468.

In yolks chicken incubatory fine and large eggs credits were high: 1:3206 and 1:3238 accordingly.

All this specified circulation "field" strain a virus encephalomyelitis in breeding poultry farm, making incubatory eggs for sale. So, at chickens-broilers in the first week of the life, the breeding hens deduced from an hens-layer, positive credits in high diagnostic values (1:174 - 1:6627) with the big heterogeneity were defined. With the years chickens-broilers in whey of blood there was a significant decrease in parent antibodies up to minimal diagnostic negative values: at 30- daily chickens the credit was 1:39; at 48- daily allowances a credit in 80% - 1:82 though at separate individuals credits were high - 1:2863 and 1:572.

After statement of the diagnosis on clinical, serological, epizootic to data the decision on carrying out of regular vaccination against encephalomyelitis birds in poultry farm Sverdlovsk area was accepted.

Hens and a breeding livestock of hens were exposed to active immunization. For this purpose vaccine Nobilis AE-1143 which protected from infectious encephalomyelitis hens during oviposition has been chosen and provided a sufficient level of antibodies at chickens.

Keywords: Infectious encephalomyelitis, Chickens, Pathological changes, Method PSI, IFA

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Receptor-mediated mechanisms in ovarian follicular development

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The oocyte’s yolk is the major source of nutrients for the chicken embryo, but details of mechanisms for the transport of hepatically synthesized yolk precursors from their site of synthesis to the oocyte situated in the center of the follicle are largely unknown. Before oocytes can perform massive receptor-mediated uptake of yolk precursors, the macromolecules must pass through several barriers in the follicular tissue surrounding the oocyte. However, very little is known about the components facilitating this massive transfer. First insight into the multistep transfer process was provided by our finding that the heparansulfate proteoglycan perlecan serves as intermediary storage site for yolk precursor lipoproteins in the acellular matrices of the follicular wall (JBC 279:23486, 2004). We have suggested a similar function for a novel collagenous, chondroitin-sulfated protein in the membrane surrounding ovarian granulosa cells, which we termed ggBM1 (JBC 282:8011,2007). We now have obtained evidence for the involvement of a third extracellular component localized adjacent to the oocyte’s plasma membrane. Originally, we had described this protein (LRP380) as an oocyte-specific soluble Very Low Density Lipoprotein- and vitellogenin-binding LDL receptor relative. Our new data suggest that it acts as a coreceptor for the oocyte yolk precursor transport receptors characterized in our laboratory. These findings allow for the first time to propose a comprehensive model for mechanisms of yolk formation and oocyte growth in the context of follicular architecture.

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Embryonic Development of Broiler and Layer Chickens - Morphological Characteristics of Skeletal Musculature

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In this paper, morphological characteristics of embryonic development of skeletal musculature of broiler and layer chickens were analyzed.

During the experimental part of this study, the eggs of two types of chicken hybrids – broiler (Ross 308) and layer (Hy-Line Brown) were incubated. Samples of muscle tissue were taken on days 9, 12, 15, 18 and 20 of incubation, as well as on day 1 after the chicks were hatched. From tissue samples that were taken, the histological preparations were made. On those preparations the morphological characteristics of skeletal musculature – muscle of the hind extremity (m. biceps femoris) and the pectoral muscle (m. pectoralis profundus) were examined. The following parameters were observed in the analysis of histological preparations of skeletal muscles: the diameter of muscle cells, the volume density of connective tissue inside the muscles and nucleocytoplasmic ratio of muscle cells.

Comparing the diameter of muscle cells of the muscle of the hind extremity and the pectoral muscle between broiler and layer chicken, it is established that layer chicken had larger diameter of examined muscle cells. During the embryonic period of development and first day after the chicks were hatched, there were found no differences in volume density of connective tissue in m. biceps femoris and m. pectoralis profundus between broiler and layer chicken. In the muscle of the hind extremity and the pectoral muscle, broiler had the greater nucleocytoplasmic ratio than layer chicken.

These results are explained by enhanced proliferation of myoblasts and delayed differentiation of muscle cells of broiler chickens, which leads to a larger development of skeletal musculature of broiler chickens in the postnatal period.

The results of this experiment present a contribution for further researches of embryonic development of skeletal musculature of broiler and layer chickens, as well as the influence of embryonic characteristics on the postnatal muscle development of an individual.

Keywords: chicken, embryonic development, musculature

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Effect of light pulses on clock gene expression in the pineal gland, heart and liver of chicken during ontogeny.

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One of the most potent synchronizing cues of circadian rhythms is the 24h light (L) dark (D) cycle derived from the rotation of Earth around its axis. Light pulses can reset the circadian system and force oscillations with a new acrophase. Therefore, the sensitivity and responsiveness of the circadian system to light pulses is essential for its functioning. Molecular basis of the circadian oscillations is rhythmic clock gene expression that can inhibit their own transcription. Expression of per2 and e4bp4 is light sensitive and can transform information about the light pulse to the circadian feedback loop. To elucidate mechanism how responsiveness of the circadian system develops embryonic clock gene expression in the central oscillator (pineal gland) and peripheral oscillators (heart, liver) was analysed. Hatching eggs of broiler breeders were incubated in a forced draught incubator under a light (L) dark (D) cycle 12:12. On embryonic day 19 and postembryonic day 4 one hour and three hours lasting light pulses were applied during the dark and subjective light phase. Samples of heart, liver and pineal glands were taken, immediately frozen in a liquid nitrogen and stored under -80°C until RNA extraction. After cDNA synthesis clock gene expression was measured by real time PCR. Expression of per2 and e4bp4 was light sensitive in the pineal gland of 4-day old chicks. In 19-day old chick embryos only per2 expression was inducible by light and this effect of light pulse was observed only during the subjective day. The light pulse induced a significant increase in per2 expression in the liver of 4-day old chicks but this treatment was ineffective in embryos. The way how information about light pulse is delivered to the liver is unknown but direct effects of feeding are excluded because birds had no access to food. The weak responsiveness of the avian circadian system during embryonic life can be assigned to immature receptoric or oscillating component of the circadian oscillator. This study was supported by grants APVV-0214-07, VEGA 1/4328/07, VEGA 1/4343/07, UK/198/2009.

Keywords: per2, bmal1, e4bp4, pineal gland, heart, liver

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Possible mechanisms of catecholamines for protecting the embryonic heart against cold and heat

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The starting point for this investigation was the observation that, up to day (D) 20 of incubation in the chick embryo is a decreasing heart frequency, and a contradictory increase in noradrenaline (NA) concentration in the blood plasma. What is the reason for this contradictory behaviours of heart frequency and NA at the cellular level? How does temperature influence this process? What is the biological meaning of this process?

White leghorn chicken embryos were incubated at different temperatures. Blood was taken on D 18 and 20. On D 20 heart cells was stimulated by Isoprenalin and the increase of cAMP was measured.

The NA content in the blood plasma showed an age dependent increase between days 18 and 20 (12 to 38 ng/ml). The absolute cAMP content in the embryonic cardiac muscle cells dropped from D 18 to D 20. A stimulation of the cardiac muscle cells with Isoprenalin led to a higher percentage of cAMP on D18 (120%) than on D 20 (55%). The cardiac muscle cells of the cold group showed a higher proportional increase in cAMP (60%) after stimulation with Isoprenalin than the cardiac muscle cells of the warm group (20%).

Increasing NA values are combined with decreasing heart frequency and cAMP values on D 20 of incubation. With decreasing heart frequency the duration of diastole is longer compared with the duration of systole.

In the warm group we measured high NA concentrations on D 20, but only a little stimulation of cAMP (20%). Highest cAMP increases (but lowest NA values) are achieved in the cold group on D 20. This effect of sympathetic stimulation in the warm group, compared with the cold group, protects the heart against heat (diminishing sensitiveness the NA receptors) and activates the heart against cold (increasing sensitiveness the NA receptors).

Keywords: chicken embryo, blood plasma, heart cells, noradrenaline, cAMP, temperature

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Influence of temperature stimulation during the last 4 days of incubation on secondary sex ratio, hatching results and chick quality in broiler chickens

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Different incubation programs for broiler chicks do not include daily variations of incubation temperature, which might stimulate the development of various body functions and may increase the adaptability of the animals to changes in environmental factors. The most suitable time-window for application of temperature variation seems to be the end of incubation, because peripheral and central nervous thermoregulatory mechanisms, as well as other body functions, are well developed. The aim of the study was to investigate the influence of a mild short-term, as well as chronic, increase in incubation temperature from Day 18 until hatching on hatchability, sex ratio of the hatched chicks (secondary sex ratio) and chick quality of a high yielding broiler breed.

In 6 Incubation Trials a total of 9883 eggs (ROSS 308) were incubated from Day 1 to 18 under normal incubation conditions (37.2 – 37.4 °C) and then sorted into three hatch incubators (37.2 – 37.4 °C: control; 38.2 – 38.4 °C, 24 h daily: chronic warm incubation; 38.2 – 38.4 °C, 2 hours daily: short-term warm stimulation) in Incubation Trial 1 and 2 or two hatch incubators (control; short-term warm stimulation) in Trial 3-6. The 1-day old chickens were selected by sex and the chick quality was analysed in a random sampling with the Pasgar©score.

Neither chronic nor short-term increase in incubation temperature had a negative effect on hatchability and chick quality. But only short-term warm stimulation could improve hatchability by more than 1.5%. Further, under these incubation conditions, a significantly higher percentage of hatched male chicks were observed in all incubation trials.

Related to this fact, our hypothesis is, that prenatal ‘temperature training’ with short-term warm loads induced an improvement in vitality, especially in the male late-term embryos, and at least for the male hatchlings. In conclusion, incubation temperature profile, which includes short-term temperature variation, can be of high relevance for improving hatching results in poultry (European patent, pending since March 2008).

Keywords: broiler chicken, incubation temperature, hatchability, secondary sex ratio, chick quality

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Yolk sac membrane and small intestine: the two digestive systems of the developing embryo

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The developing embryo and the hatched chick are completely dependent for their growth and development on the nutrient deposits in the egg. Consequently, the physiological status of the chick at hatch is influenced by the nutrition of the breeder hen, which will influence chick size, vigor and the immune system. While there is a wealth of literature that demonstrates that the breeder diet influences egg composition and ultimately chick quality and performance, there is minor information on the mechanisms and systems involved in nutrients uptake from the yolk by the developing embryo.

Current research in our lab regarding the nutrition of the developing embryo identified three ways that nutrients are absorbed from the yolk sac. One way is absorption by endocytosis through the YSM directly into the blood. Second way is digestion and absorption by yolk sac membrane (YSM) endothelial cells, and the third way is the entrance of yolk content into the small intestine thorough the yolk stalk

Results, utilizing real time RT-PCR method, showed that YSM expresses many of the digestive enzymes and transporter genes normally associated with the small intestine. Among them are the sucrase-isomaltase and amino-peptidase digestive enzymes and CAT-1, PepT1, SGLT1, EAAT3 nutrient transporters. This demonstrates that the YSM serves as an important site for nutrient assimilation from E 11 until E 18. From E 17 until day of hatch (DOH), the small intestine expressed these genes in an elevated pattern and contributes to the abilities of digestion and absorption of the developing small intestine. The temporal patterns of expression of these enzymes and transporters in the both YSM and small intestine tissues, during E 11 until DOH imply to their role in nutrient uptake changes during incubation period. Results point toward a shifting dominants between the YSM and small intestine tissues during the last 10 days of incubation and improve our understanding of the digestive physiology of the embryo.

Keywords: yolk sac membrane; incubation; small intestine; gene expression, poultry

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Effects of hatching time and hatching system on broiler physiology

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A multi-level housing system, named Patio, was developed for broilers, in which the hatching and brooding phase are combined. In Patio, climate conditions differ from those provided in hatcher cabinets. In this study, we compared conditions in a hatcher and in Patio, and evaluated effects of hatching time on broiler physiology.

Broiler eggs obtained from one single parental flock were simultaneously incubated in one incubator for 440 h and subsequently placed in a hatcher cabinet or in the Patio, until the end of incubation. In each system, climate conditions were registered at egg level. At 3 moments during hatch: 465 (Early), 480 (Midterm) and 493 (Late) h of incubation, 24 newly hatched chicks were sacrificed for blood plasma collection and analyses of organ weights.

Mean temperature and relative humidity were 38.1°C and 50.8% in the hatcher and 35.2 °C and 29.7% in the Patio. Plasma glucose was higher in Late than in Midterm and Early chicks (P<0.01), whereas lactic acid was higher in Early than in Late chicks (P<0.01), with Midterm chicks intermediate. For uric acid and thyroid hormones, significant system*hatching period interactions were observed (P<0.01). In hatchery chicks, uric acid was higher in Late than in Midterm and Early chicks (P<0.01), but no differences were observed in Patio chicks. For organ weights, no system effects were observed. Whereas yolk weights were lower, weights of the yolk-free-body, intestines, stomach, liver and hearts were higher in Late than in Early and Midterm chicks (P<0.01). No differences in organ weights were found between Early and Midterm chicks except for yolk weights, which were heavier in Early chicks (P=0.01).

In conclusion, hatching time affects plasma hormone concentrations and organ weights in broiler chicks, and some of these effects were influenced by hatching system.

Keywords: hatcher, Patio, broiler, organ weight, hormone

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Structural Analysis of the Vascular Network of the Chorioallantoic Membrane

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In the poultry sector, one of the major problems concerning health of the adult broilers is ascites, a cardiovascular syndrome with negative impacts on production and animal welfare. The syndrome is associated with a discrepancy between oxygen need and oxygen supply. One of the hypotheses postulates that the onset of the disease might already be set at embryonic stage due to stress, such as hypoxic (i.e. reduced oxygen) conditions that occur in the late embryonic phase of the chicken development. Therefore, the hypothesis of the research is that due to O₂/CO₂ stress changes occur in the angiogenesis in the chorioallantoic membrane (CAM), the embryonic organ for gas exchange in chicken. In order to investigate this potential link, a method must be developed to quantify the effect of O₂ and CO₂ on the angiogenesis in the CAM. The existing method of manually counting the vessel density on pictures of the CAM is subjective and time-consuming. In this presentation, an objective, quantitative methodology to assess vascular development in the CAM of chicken embryos will be presented. First a method was developed to quantify the angiogenesis in the CAM. Samples of the CAM had to be taken with minimal disturbance and a measuring configuration had to be developed to determine the angiogenesis of the CAM. The latter was done by taking pictures of the samples and analyzing them in order to calculate two structural parameters of the vascular network of the CAM, namely the vascular fraction (i.e. a measure of the density of the blood vessels) and the fractal dimension (i.e. a measure of the branching degree of the vascular network). Second, two experiments were performed to analyze the differences in angiogenesis in the CAM under normal, hypoxic and hypercapnic conditions.

Keywords: ascites; angiogenesis; chorioallantoic membrane; hypoxia; hypercapnia

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Effect of exogenous leptin on angiogenesis in quail chorioallantoic membrane

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Leptin, the product of the ob gene, besides of its key role in the control of body weight and food consumption, may play role also in control of embryonic development. Our previous studies showed that leptin administration in ovo accelerated the embryonic and postembryonic development in Japanese quail. Although the mechanisms of leptin growth and development acceleration are not clear, stimulation of angiogenesis represents one of the plausible explanations. Therefore, the aim of the present study was to investigate the pro-angiogenic effect of leptin in vivo using the quail chorioallantoic membrane (CAM). The recombinant murine leptin (10, 100, and 1000 ng) was applied either ex ovo, on the CAM surface of the ex ovo incubated embryos at embryonic day 7 (ED7), or in ovo, into the egg albumen at ED5. Changes in blood vessels were quantified by the fractal analysis providing the fractal dimension (Df) estimate.

We found significant dose dependent effect of leptin on CAM angiogenesis in both in vivo experimental models. In the ex ovo model the dose 10 ng of leptin administered onto the CAM surface did not influence the vessel density but the doses 100 and 1000 ng significantly stimulated angiogenesis. In the in ovo approach, already 10 ng of leptin administered directly into the egg albumen significantly stimulated angiogenesis. A dose dependent increase in the stimulatory effect of leptin was observed using both treatments. Comparison of effects of both experimental treatments on CAM demonstrates that administration of the hormone into the egg albumen was more effective with respect to angiogenesis stimulation. Our study confirmed that exogenously applied leptin was able to stimulate angiogenesis in CAM. Leptin mediated stimulation of angiogenesis may improve nutrient utilization from the yolk and explain at least partially accelerating effect of leptin on avian embryo growth and development.

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Keywords: angiogenesis, leptin, chorioallantoic membrane, Japanese quail, fractal dimension

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Thermal conditioning during layer chick embryogenesis

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Thermal conditioning during embryonic development has demonstrated to be important for the acquisition of thermo-tolerance in broiler chickens. However, little is known about the effects in laying hens. In the current study, laying hen embryos were conditioned at d 14-18 of incubation by exposure to 40°C egg shell temperature (EST) for 4 h/day (n=96). Control embryos (n=96) were incubated at 37.8°C EST throughout incubation. After hatch, chicks from each treatment were divided into 3 subgroups (n=32 per group) and subjected to a heat challenge at d 2 (40°C), d 8 (40°C) or d 34 (35°C). One day prior to the heat challenge, the preferred ambient temperature of the chicks was determined with a temperature preference test. Blood samples were taken before and after the heat challenge at d 34 to investigate plasma T3, T4 and corticosterone levels. Thermal conditioning decreased the incubation time with 7 h (p<0.001) and additionally decreased rectal temperature at hatch (p=0.02). Conditioned chicks preferred a lower ambient temperature in the temperature preference test (p<0.05) and showed a higher rectal temperature response after the heat challenge at d 2 and d 8 (p<0.05) but not on d 34. No differences in plasma T3, T4 and corticosterone were found. These results suggest that thermal conditioning in laying hens might act through a different adaptive mechanism than in broilers and that the effects of thermal conditioning are not lasting.

Keywords: thermal conditioning; laying hens; heat challenge; temperature preference

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Thermal manipulations of broiler embryos during late incubation and their immediate and long-lasting effects

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Imprinting of a physiological control system early posthatch and/or even during the latter part of pre-hatch development, has become a popular research topic in recent years. Regarding thermal conditioning, most of the studies focused mainly on intermittent temperature manipulations ranging from 3 to 12 hours per day. However, little has been described about continuous temperature manipulations during a certain period of incubation. In addition, the focus of most studies lies on higher than standard incubation temperatures. Therefore, two identical studies were performed to elucidate the effects of a continuously lowered and raised incubation temperature (Δ of 3°C) during the end phase of ontogeny of hypothalamus-pituitary-thyroid axis and hypothalamus-pituitary-adrenal axis (embryonic dag (ED) 16 – ED 18.5) on embryonic development and metabolism as well as on posthatch growth and heat tolerance of broilers.

Embryonic growth was significantly retarded with decreased yolk consumption in the heat treated group and both air chamber and blood gas composition were altered. In addition, plasma thyroid hormones concentrations were significantly lower from ED 17. An increased blood glucose level, decreased plasma triglyceride level and decreased liver glycogen concentration clearly showed an altered carbohydrate and lipid metabolism of the embryos exposed to heat during late incubation. Continuous cold exposure only affected the hatching process by significantly delaying the start of hatch. Also, long-lasting effects of heat and cold exposure during incubation were found in a reduced posthatch growth. The results showed clear differences in embryonic development during temperature treatment as well as persistent differences during the hatching process and posthatch performance. Moreover, this study clearly shows that broiler embryos are more sensitive to heat exposure during the latter part of incubation than to cold exposure, as immediate and long lasting effects on development are more severe in the former.

Keywords: incubation temperature, embryonic development, metabolism, epigenetic adaptation, heat challenge

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Yolk nutrient uptake by the broiler embryo towards hatch

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The yolk is an important part of the chicken embryo's nutrition. Yolk sac contents are high in protein (~20%), in fat (~30%) and very low in carbohydrates (~1%). In this study yolk protein and fat content was examined during broiler embryo development towards hatch. In addition, the effect of broiler breeder age on yolk nutrient uptake by the embryo was studied. Fertile eggs were collected from a 30 wk and a 50 wk old broiler breeder flock (Cobb). Yolk sac weight and content (fat, cholesterol and protein) were examined in the fresh egg and during embryonic development at days E11, E13, E15, E17, E19, E20, E21, and the uptake of these nutrients was calculated for each day.

Results showed that from day E0 (fresh egg) to E17 almost 50% of the protein was absorbed by the embryo from the yolk (3 gr. protein at E0 to 1.35 gr. protein at E17). However, increase in protein content was observed between E17-E20. This implies a possible transfer of protein into the yolk sac from one of the other egg components (amnion, allantoic fluid). The majority of fat (2.5 gr. which composed 65% of the yolk fat content) was absorbed in a linear manner from the yolk between E11 to E17 and then only a small amount of fat was absorbed. However, at day of hatch, more than 0.5 gram of fat, which composed 15% of fat content, was absorbed during just 24 h. Cholesterol uptake exhibited the same pattern. At E11 and E13, no significant differences in weight and yolk absorption were found between embryos of different breeder ages. From E15 until hatch, embryos of the 50 wk old flock were heavier than embryos of the younger flock - a 1 gram difference at E15, 6 grams difference at E21. Despite the different total nutrient uptake between the breeder flocks, yolk nutrients uptake was similar when calculated per 1 gram of embryo. This suggests that the nutritional demand of the embryo (i.e. the building of tissues) might influence yolk uptake.

Results demonstrate that there is a differential uptake of the yolk nutrients in the different phases of the broiler’s embryonic development.

Keywords: yolk, embryo, fat, protein, prenatal nutrition

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Influence of Heat Acclimation During Incubation on Antioxidant Status of Chicks

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Glutathione peroxidase (GSH-px) catalyzes the reduction of hydrogen peroxidase using glutathione (GSH) as a reducing agent and protect cell membranes against deleterious effects of reactive oxygen species. In broilers, heat stress impairs the balance between prooxidant and antioxidant systems and results oxidative stress. Heat acclimation during incubation by exposing embryos to higher incubation temperatures could improve thermotolerance of broilers. Because protecting cells against oxidative stress is essential during embryonic development, the present study aimed to determine the changes in GSH and GSH-px levels in chicks acclimated during incubation. Malondialdehyde (MDA) level which is one of indicators of lipid peroxidation was also determined. Fertiled eggs were incubated either at 37.8°C from d 1 to 18 of incubation (Control) or were heat acclimated at 39.5°C for 6 h daily from d 10 to 18 of incubation. From d 18 to hatch, the temperature was 37.5°C for both groups. On d hatch, 5 and 10, 12 chicks from each group were sampled and jejunum were dissected, cleaned of content, washed and frozen at -80 ºC for GSH, GSH-px, and MDA analyses.

There was no effect of incubation temperature on GSH-px activity, GSH and MDA levels of chicks on d hatch. Higher GSH-px activities were obtained for acclimated chicks at 5 and 10 d. From hatch to 10 d, GSH and MDA levels were similar for control and acclimated chicks. The results also showed that GSH-px activity and GSH level increased by age while MDA level decreased from 21.17 on d hatch to 12.02 nmol/g on d 5 and increased to 18.79 nmol/g on d 10. In conclusion, heat acclimation during incubation resulted greater GSH-px activity in jejunum of chicks during postnatal stage suggesting that heat acclimation during incubation influenced antioxidant protection of chicks and those chicks might have experienced lower oxidative stress.

Keywords: acclimation, incubation, chicks, antioxidant status

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Selection for high egg testosterone content increases growth rate more in male than female Japanese quail

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Maternal androgens deposited in the egg yolk can modify postnatal growth and behaviour of offspring and these traits are often attributed to predict survival and fitness of young. Growth rate in altricial birds is determined by complex interactions between quality and behaviour of parents and physiological traits and begging behaviour of offspring. Therefore, we studied the effect of maternal androgens on postnatal growth in precocial Japanese quail kept under stable social and environmental conditions. We analysed growth rate in two lines of quail divergently selected for high (HET) and low (LET) egg testosterone content (Okuliarova et al., this issue). Body weight and tarsus length were measured once a week from hatch until 6 weeks of age. The data were calculated using repeated measures analysis of variance with age as a repeated factor, line and gender as fixed factors and the effect of mother nested within line. There were no differences between lines in body weight and tarsus length of hatchlings. At the age of two weeks, quail from the HET line were heavier than those from the LET line and these differences persisted until sexual maturity. Moreover, from one week of age onwards HET quail had longer tarsus compared with birds from the LET line. Further analysis revealed that the effects of line on body weight and tarsus length were more pronounced in males than in females. The results demonstrated a positive effect of yolk testosterone naturally deposited by mother on growth rate in precocial Japanese quail.

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Keywords: growth, body weight, yolk testosterone, Japanese quail,

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