Europ.Poult.Sci., **81.** 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Proceedings of the Combined Meeting of the Incubation and Fertility Research Group (IFRG/WPSA Working Group 6) and the Perinatal Development and Fundamental Physiology Group (PDP/WPSA Working Group 12), Hof van Wageningen, the Netherlands, 30 August – 1 September, 2017

Preface

B. Tzschentke[1] and **M. Boerjan**[2]

[1] Humboldt-Universität zu Berlin, Faculty of Life Science, Institute of Biology, Berlin, Germany

Correspondence: Barbara.tzschentke@rz.hu-berlin.de

[2] PasReform Hatchery Technology, Zeddam, the Netherlands

Correspondence: boerjan@pasreform.com and IFRG@pasreform.com

The 2017 IFRG- and PDP meeting in Wageningen was the fourth combined meeting of both WPSA working groups 6 (Incubation and Fertility) and 12 (Physiology). Altogether, 56 delegates from Australia, North America, Africa and Eastern and Western Europe were present during two and a half conference days. The delegates, speakers, and poster presenters came from different scientific disciplines and companies. The latest developments in basic and applied poultry physiology, focused on poultry incubation and long-lasting parental and environmental effects on poultry welfare and performance, as well as on incubation practice and technology, were presented under the following topics:

- Influence of breeder on chick health and performance
- Egg storage and handling
- Impact of parental effects
- Adaptation and epigenetic alteration in birds
- Incubation parameters and chick vitality
- Control and programming of energy balance

With the support of the WPSA Speakers Bureau we were able to invite two early-career scientists as keynote speakers. Mylene Mariette, postdoctoral fellow at the Centre of Integrative Ecology, Deakin University, Victoria, Australia, gave an impressive talk on avian adaptation to heat by prenatal acoustic stimulation. Bence Lácár, Phd student from the Doctoral School of Animal Husbandry Science, Szent István University, Gödöllö, Hungary, presented an excellent lecture on the combined effect of parental thermal stress and thermal treatment of the offsprings on early embryonic development and on primordial germ cells. Further, we were very pleased to convince Prof. Dr. A.G.G. (Ton) Groothuis from the Department of Behavioural Biology, Centre for Behaviour and Neuroscience, University of Groningen, the Netherlands, a cutting-edge researcher on hormone mediated maternal effects using the bird as important model, to give a keynote lecture on avian yolk hormones and the state of the art of understanding the mechanisms and their functions.

The importance of parental effects and early environmental stimulation on the development of body functions and its long-lasting effects on health, welfare and finally performance and production efficiency in poultry is accepted to a large extent. However, the underlying key-mechanisms and their extensive interactions, as well as the influences of poultry production factors such as breeder age and management are not well understood and need further basic and applied research. For a long time, both working groups deal with the research, dissemination and regular exchange and praxis transfer of knowledge in this field. In this regard, interdisciplinary collaboration adds significant enrichment. To learn from the disciplines of biology, medicine and psychology is of paramount importance, for

instance, to improving animal welfare from incubation. On the other hand, the poultry embryo provides an excellent animal model for parental, environmental pre- and perinatal effects in other animal species and humans.

At the end of the meeting delegates agreed that the 2019 combined meeting of IFRG working group 6 and the PDP working group 12 will be organized in Tours (France).

The proceedings of the combined IFRG- and PDP meeting provide an abstract collection of oral and poster presentations related to the respective session.

Acknowledgement

We wish to thank the members of the organizing committee Anne Collin-Chenot, INRA Nouzilly, France, and Glenn Baggott, Birkbeck, University of London, London, UK, for their contributions in reviewing abstract and website communication.

Our special thanks are directed to the sponsors (Figure 1), the European Branches of WPSA, the Foundation for Promoting Poultry Science, and the companies Lohmann Tierzucht GmbH, HatchTech, Petersime and PasReform Hatchery Technology.

Figure 1. Sponsor-Logos

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Abstracts of oral presentation

Session 1: Influence of breeder on chick health and performance

Breeder age, fertility and chick vitality

M. Boerjan[1]

[1] Pas Reform, Hatchery Technology, Zeddam, the Netherlands

Correspondence: Boerjan@pasreform.com

It is generally accepted that reproductive efficiency decreases as breeder hens age. When the breeder ages, both fertility and hatching egg quality decreases. In older flocks the daily hatching egg production is lower and egg composition differs from those eggs produced by peak production flocks. Although breeder management can influence the impact of ageing to a certain extent, it is impossible to keep the reproductive fitness of a 60 week old flock at a level similar to a 35 week old flock because physiological control systems adapt to age of the animal. In the hen the physiological function of the ovary is changing as the hen ages resulting in bigger eggs containing a larger proportion of yolk, and a thinner egg shell than that of eggs produced by younger flocks. Flock age also negatively influences the early and late proportions of embryonic mortality and increases the impact of egg storage on chick vitality as the breeder ages.

In this presentation I focus mainly on the formation and development of the ovary during egg formation and growth of the hen towards sexual maturity. In the day-old chick the ovary is loaded with almost half a million of primordial follicles containing a 'primitive oocyte' in each. In the primitive oocyte the chromosomes have been organised such that meiosis can only be finalized after penetration of the sperm cell during fertilization and the formation of the zygote.

When the ovary develops to sexual maturity during the rearing phase an enormous selection process is initiated among follicles in the premature ovary such that the number of follicles in the mature hen has decreased to less than fifteen thousand at the time of sexual maturity. Each of these follicles contains an oocyte in which the 'meiotic' chromosomes are waiting for fertilization. The structure of these 'meiotic' chromosomes is very sensitive to the changing physiology of the ageing female. Consequently, the number of chromosomal abnormalities increases as the female age which produces increased infertility and increased embryonic mortality. Also the number of abnormal embryos may increase. I will show examples from mammalian ageing since this process has mainly been studied in mammals.

Key words

Age, breeder, management, physiology, reproduction

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Effects of breeder age and oxygen concentration during incubation on embryonic heat production and development, and post-hatch chick performance

R. Molenaar[1], **A. Nangsuay**[1], **R. Meijerhof**[2], **I. van den Anker**[1], **M.J.W. Heetkamp**[1], **B. Kemp**[1] and **H. van den Brand**[1]

[1] Adaptation Physiology Group, Wageningen University, Wageningen, the Netherlands

[2] Poultry Performance Plus, Voorst, the Netherlands

Correspondence: roos.molenaar@wur.nl

Nutrients in the egg and oxygen diffused through the eggshell and membranes are two important factors affecting the metabolism and growth of avian embryos (WILSON, 1997; WANGENSTEEN and RAHN, 1970). The composition of the egg is influenced by breeder age (Marion et al., 1960) and similar sized eggs of an old compared to young breeder flock showed a higher yolk size and consequently higher energy content (NANGSUAY et al., 2013). To optimise nutrient use and development during the incubation and post-hatch period, embryos originating from different breeder ages that differ in their initial nutrient availability might therefore require different oxygen concentrations during incubation.

To test this hypothesis, the current study investigated effects of breeder age and oxygen concentration during incubation on embryonic metabolism and development, and post-hatch chick performance. Similar sized eggs of a young (29 weeks) or old (53 weeks) Cobb 500 breeder flock were incubated at 3 oxygen concentrations (17%, 21% or 25%) from day 7 of incubation until 6 hrs after emergence from the eggshell. Egg composition at set, embryonic weight and heat production during incubation and post-hatch performance until 7 days of age was evaluated.

Results showed that eggs of the old compared to the young flock were 1.2 g heavier and had 3.9 g more yolk ($P < 0.01$). No interactions between breeder age and oxygen concentration were found for the characteristics described below. Embryonic heat production was significantly higher from ED14 until ED18 with a higher oxygen concentration ($P < 0.05$). Yolk-free body mass at 6 hours after hatch was only affected by oxygen concentration, where the 21 and 25% treatment showed a higher weight than the 17% treatment ($P < 0.01$). This difference disappeared at 7 days of age ($P > 0.10$). Body weight and relative intestine weight at 7 days of age was higher in chickens of the old compared to the young breeder flock ($P < 0.05$). Other relative organ weights were not affected by the treatments ($P > 0.05$). FCR was affected by the oxygen concentration during incubation; the 17% treatment had the highest FCR and the 21% treatment had the lowest FCR, whereas the 25% treatment was intermediate ($P < 0.05$).

Although the higher yolk weight of the eggs of the old compared to the young flock probably increased the energy content and nutrient availability for the embryo, this did not result in a higher metabolic rate or development when oxygen concentration was increased during incubation. This suggests that other factors such as the development and functionality of the yolk sac membrane and/or CAM may limit embryonic metabolism and development. The higher post-hatch growth of chickens of the old compared to the young breeder flock might be related to differences in physiology or development of supply organs, such as the intestines. Embryonic development was positively influenced by a higher oxygen concentration during incubation, but this effect disappeared in the post-hatch period. A higher O_2 concentration during incubation did improve the nutrient efficiency for post-hatch growth until day 7 of age, expressed by a lower FCR.

Key words

Breeder age, broiler embryo, incubation, oxygen

References

NANGSUAY, A., R. MEIJERHOF, Y. RUNAGPANIT, B. KEMP, H. VAN DEN BRAND, 2013: Energy utilization and heat production from eggs originating from young and old broiler breeder flocks. Poult. Sci. **92,** 474-482.

MARION, W.W., A.W. NORDSKOG, H.S. TOLMAN, R.H. FORSYTHE, 1964: Egg composition as influenced by breeding, egg size, age and season. Poult. Sci. **43**, 255-264.

WANGENSTEEN, O.D., H. RAHN, 1970: Respiratory gas exchange by the avian embryo. Resp. Physiol. **11,** 31-45.

WILSON, H.R., 1997: Effects of maternal nutrition on hatchability. Poult. Sci. **76,** 134-143.

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Adipokines, feeding levels and omega-3 fatty acids supplementation in the control of fertility, steroidogenesis, laying performance and offspring development in broiler breeders hens

N. Mellouk[1], **C. Ramé**[1], **J. Delaveau**[2], **C. Rat**[2], **M. Marchand**[2], **F. Mercerand**[2], **A. Travel**[3], **P. Chartrin**[4], **L. Ma**[1], **P. Froment**[1] and **J. Dupont**[1]

[1] INRA UMR85 Nouzilly, France

[2] INRA, UEPEAT, Nouzilly, France

[3] ITAVI, Nouzilly France

[4] INRA, URA, Nouzilly, France

Correspondence: Joelle.dupont@inra.fr

In poultry, broiler breeder hens selected for their rapid growing with free access to feed develop a strong fattening and disruptions of reproductive performances. This study is aiming to demonstrate that a food restriction leading to a body fat mass reduction and/or a diet supplementation with omega polyunsaturated fatty acids (PUFA), derived from fish oil may be effective to prevent reproduction disorders in broiler breeder hen, through regulation of adipokine expression.

From birth to the 3rd week all female chicks (n = 380) received an *ad libitum* diet then they were separated into two groups. One group received a restricted diet and the second group received an *ad libitum* diet (with a quantity of food 1.7 times greater than in restricted animals). From the 9th to the 39th week, these two groups were each subdivided into 2 groups to which we supplemented the diet with or without PUFA (1%). Herein, we found that a food restriction increased circulating levels of *RARRES2* in 21 weeks old hens, delayed of one week the sexual maturity, improved eggs quality and fertility associated with higher progesterone production in response to IGF1 (or LH) in cultured granulosa cells and in egg yolk, as comparing to hens fed *ad libitum* ($p < 0.05$). The PUFA supplementation increased by two-fold progesterone production in response to IGF1 (or LH) in cultured granulosa cells and in *in vivo* in egg yolk of *ad libitum* and restricted hens ($p < 0.05$), probably due to a high incorporation of PUFA in egg yolks. These effects improved fertility (% of born chicks/incubated eggs) of *ad libitum* hens. Using RT-PCR, we detected mRNA transcripts related to *NAMPT*, *RARRES2* and *ADIPOQ* in theca and granulosa cells from pre-ovulatory follicles 1 (F1) of 39 weeks old hen of each group. We also found that mRNA *RARRES2* levels were lower in theca cells of restricted hens and mRNA *NAMPT* levels were increased by the PUFA supplementation whereas levels remained unchanged by the diet and the PUFA in granulosa cells. A significant positive correlation between *RARRES2* mRNA expression in granulosa cell and the weight of F1 pre-ovulatory follicle was observed. We also demonstrated a negative correlation of *RARRES2* plasma level with the weight and the fattening and a positive correlation with the fertility of hens. Interestingly, we also noted that the offspring from restricted hens and fed with a traditional diet during 10 days had a lower weight than offspring from hens fed *ad libitum* and the weight was lower when offspring received the PUFA supplementation. In addition, plasma *NAMPT* and *ADIPOQ* were correlated with the weight of 10 days old offspring.

In conclusion, the amount and the PUFA composition of diet affect reproductive parameters such as laying, egg quality, fertility and reproductive steroidogenesis in broiler hen probably through alterations in adipokine expression in ovarian cells. The effect of the diet and PUFA supplementation may be transmitted to the second generation.

Key words

Adipose tissue, fatty acid supplementation, hormone, metabolism, reproduction

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Adipokine expression profiles during early broiler embryo development and regulation by maternal feeding restriction and omega-3 fatty acid supplementation

N. Mellouk[1], **C. Rame**[1], **J. Delaveau**[2], **C. Rat**[2], **M. Marchand**[2], **F. Mercerand**[2], **P. Froment**[1] and **J. Dupont**[1]

[1] INRA, UMR85, Nouzilly, France

[2] INRA, UEPEAT, Nouzilly, France

Correspondence: joelle.dupont@inra.fr

Novel adipokines such as adiponectin, visfatin and chemerin are hormones mostly produced by adipose tissue. Their involvement in the regulation of energy homeostasis and growth mechanism has been well documented in mammals but much less in avian species. In chicken, adiponectin was involved in the control of metabolism and reproductive processes (YAN et al., 2014, CHABROLLE et al., 2007). Conversely to mammals, visfatin was identified mostly as a myokine than adipokine (KRZYSIK-WALKER et al., 2008) and only one study referred to the role of chemerin in reproductive functions in turkey (DIOT et al., 2015). The aim of this study was to determine the expression pattern of adiponectin, visfatin and chemerin in different metabolic tissues (subcutaneous adipose tissue, liver and muscle) during embryo development with special emphasis on their modulation by maternal food restriction supplemented or not with omega 3 polyunsaturated fatty acids (ω3 PUFA). For the first part of this study, we collected subcutaneous adipose tissue, liver and muscles of 15 (E15, n = 15), 19 (E19, n = 16) day old broiler breeder embryos and at hatching (D1, n = 19). For the second part of the study, we collected plasma, subcutaneous adipose tissue, liver and muscles of embryo (E15, E20, n = 8/group/stage) and chicks (D1, 5 days old, D5 and 10 days old, D10, n = 8/group/stage) from 4 different groups of broiler breeder hens (feeding restriction or overfeeding diet with or without ω3 PUFA). By RT-qPCR, we demonstrated that in subcutaneous adipose tissue, the expression of adiponectin and visfatin decreased whereas the expression of chemerin increased overtime. In muscle, the expression of visfatin increased while the expression of adiponectin and chemerin decreased overtime. In liver, only the expression of visfatin decreased at D1 as compared to E15. At the plasma level, visfatin and adiponectin decreased from E15 to D1 and slightly increased from D1 to D10 and chemerin decreased from E15 to D5 and remained stable. The restricted diet induced the lay of thinner eggs with a small yolk vesicle and consequently thinner embryo. Even if chicks were all fed with an overfeeding diet after hatching chicks from restricted hen were still thinner after 10 days of life and even more when hens received the ω3 PUFA ($P < 0.05$). In addition, the maternal restricted diet increased circulating visfatin levels and decreased those of adiponectin and chemerin. At D10, the weight of chicks was negatively correlated with plasma visfatin levels and positively correlated with those of adiponectin. By western blot, we have also shown at D10 that only visfatin expression was increased in muscles of chicks from the restricted supplemented with ω3 PUFA. In conclusion, the expression profile of adipokines is strongly regulated during ontogenesis. Moreover, it also depends on the maternal nutrition suggesting transmission of epigenetics marks. Additional experiments are investigating to determine the role of these hormones in embryo development.

Key words

Adipose tissue, fatty acid supplementation, hormone, ontogenesis

References

CHABROLLE, C., L. TOSCA, S. CROCHET, S. TESSERAUD, J. DUPONT, 2007: Expression of adiponectin and its receptors (AdipoR1 and AdipoR2) in chicken ovary: potential role in ovarian steroidogenesis. Domest. Anim. Endocrinol. **33**, 480-487.

DIOT, M., M. REVERCHON, C. RAME, P. FROMENT, J.P. BRILLARD, S. BRIERE, G. LEVEQUE, D. GUILLAUME, J. DUPONT, 2015: Expression of adiponectin, chemerin and visfatin in plasma and different tissues during a laying season in turkeys. Reprod. Biol. Endocrinol. **13**, 81.

KRZYSIK-WALKER, S.M., O.M. OCON-GROVE, S.R. MADDINENI, G.L. HENDRICKS, R. RAMACHANDRAN, 2008: Is visfatin an adipokine or myokine? Evidence for greater visfatin expression in skeletal muscle than visceral fat in chickens. Endocrinology **149**, 1543-1550.

YAN, J., H. YANG, L. GAN, C. SUN, 2014: Adiponectin-impaired adipocyte differentiation negatively regulates fat deposition in chicken. J. Anim. Physiol. Anim. Nutr. (Berl) **98**, 530-537.

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Session 2: Egg storage and handling

Influence of heat treatment during egg storage on the number of chicks and chick quality

A.K. Ferreira

Hatchery Support Team, Aviagen Inc., UK

Correspondence: aferreira@aviagen.com

Typically egg storage beyond a period of 7 days results in a reduction in hatchability. Prolonged egg storage affects both the dormant embryo and the incubation chamber (egg) that contains it. With increased storage the internal quality of the egg deteriorates, this affects both the albumen quality vitelline membrane integrity and typically results in increased embryonic cell death. This deterioration in quality within stored eggs results in a higher proportion of ruptured yolks and candled clears (due to increased early embryo mortality). Eggs will also take longer to hatch due to the reduced starting number of embryonic cells and typically there is an increased proportion of culled first day old chicks. Previous studies performed on eggs heated prior to storage (FASENKO et al., 2001) demonstrated improved embryo survivability when the eggs were stored for prolonged periods. Embryology assessments from this study revealed that by progressing embryonic development to a stage where hypoblast formation is completed (Stage XIII) enabled the embryos to better withstand storage. An alternative hypothesis by MEIR and AR (1998), investigated giving eggs regular, short periods of heat treatment (less than 6 hours) during storage would enable the embryos to undergo cellular repair and thus offset the impacts of cell death. Heating eggs to incubation temperatures immediately before cooling them for storage can be difficult to achieve within a commercial farming operation thus making it impractical in some cases. For this reason work continued to focus on short periods of incubation being applied during the storage period, which can be applied at a single location (the hatchery), which is already equipped with suitable equipment. This technique for applying heat treatment during egg storage became known as SPIDES. In order to investigate the potential for SPIDES to be used within the Aviagen hatching business a series of trials aimed at validating the method were performed. These trials aimed to answer the critical factors that would be fundamental to the success of the technique. This consisted at 4 replicated experiments being undertaken at Aviagen's product development unit at Albertville (USA) between July 2010 and June 2011. The key focus of these trials was to investigate 1. How many heat treatments were needed to give the best hatchability? 2. What benefit to hatch could be seen in eggs stored for various periods of time? 3. What was the best combination of treatment duration and treatment frequency? 4. Is the speed at which the eggs are heated important? A series of large scale field trials were also undertaken at various locations around the world (New Zealand, UK, USA, Turkey, Hungary, India, Russia and Sweden). Studies have also since been undertaken on layer breeds and turkey eggs. All the aforementioned studies provided evidence that SPIDES could work and was indeed capable of improving hatchability of stored eggs on a large scale.

In conclusion, the best practice for storing hatching eggs is still to set the eggs within a week of being laid. However, if longer storage is unavoidable, hatchability can be maximised by using appropriate SPIDES treatments during storage. For the best results, the eggs should be treated before hatch starts to fall, with repeat treatments every 6–7 days. While the heating speed and final temperature are both very forgiving, even cooling after treatment will help to maximise the impact. Warming the eggs too often, or for too long will limit the value of using SPIDES, and cumulative time above 32°C should not exceed 15 hours. SPIDES treatment is therefore of potential benefit to broiler and commercial layer breeds, and also to turkeys.

Key words

Chick quality, egg storage, hatchability, SPIDES

References

FASENKO, G.M., F.E. ROBINSON, A.I. WHELAN, K.M. KREMENIUK, J.A. WALKER, 2001: Prestorage incubation of long-term stored broiler breeder eggs: 1. effects on hatchability. Poult. Sci. **80**, 1406-1411.

MEIR, M., A. AR, 1998: Pre-incubation warming as a means of lengthening storage time of fertile eggs. Proc. 10th EPC, Israel 1998, 825-829.

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Hatch traits of artificially incubated ostrich eggs as affected by setting position, angle of rotation and season

Z. Brand[1,2] and **S.W.P. Cloete**[1,3]

[1] Oudtshoorn, South Africa

[2] Elsenburg, South Africa

[3] Dept. of Animal Sciences, University of Stellenbosch, Matieland, South Africa

Correspondence: zanellb@elsenburg.com

Embryonic mortality during the early and late incubation periods contributes substantially to hatching failure in artificially incubated ostrich eggs. Setting position and angle of rotation of artificially incubated ostrich eggs may affect hatching success and were considered in this study. The eggs used were collected during the 2015 – 2016 breeding seasons from the commercial, pair-bred ostrich resource flock at Oudtshoorn Research Farm, South Africa. Eggs were randomly divided into the three different treatment groups at setting, with all setters set to turn eggs automatically through either a 60° or 90° angle hourly. The treatments considered per turning angle were: eggs set in the horizontal position for the total incubation period of 6 weeks; eggs set horizontal for 3 weeks and vertically for 3 weeks and eggs set vertically for the total incubation period of 6 weeks. These treatments were repeated over a period of two years to including winter, spring and summer months. Data of between 846 and 1 549 egg records were used to derive averages for groups of eggs treated similarly and subjected to a factorial analysis in a 2 (years) × 3 (seasons) × 3 (setting position) × 2 (angle of rotation) analysis.

Year affected incubation traits of ostrich eggs but was not discussed. Early embryonic mortalities were independent of the effects studied (Table 1). Late embryonic mortalities (LEM) was improved in eggs set in trollies to turn through an angle of 90° compared to eggs set in trollies turning through a 60° angle regardless of season and setting position. LEM was also improved in eggs set vertically in comparison to eggs set horizontally for 3 weeks and vertically for 3 weeks. The effect of turning angle on LEM was carried over to total embryonic mortalities.

Table 1. Means depicting the effects of position during incubation and turning angle on embryonic mortalities (EM) expressed as proportions

Effect and level	Early EM	Late EM	Overall EM
Setting position	0.25	*	0.09
Horizontal	0.06 ± 0.01	0.22 ± 0.03	0.28 ± 0.03
Horizontal/Vertical	0.03 ± 0.01	0.27 ± 0.03	0.31 ± 0.03
Vertical	0.04 ± 0.01	0.21 ± 0.03	0.25 ± 0.03
Angle of rotation	0.69	**	**
60°	0.05 ± 0.01	0.28 ± 0.02	0.33 ± 0.03
90°	0.04 ± 0.01	0.16 ± 0.02	0.20 ± 0.03

* – P < 0.05; ** – P < 0.01; actual significance for P > 0.05

Water loss, pipping time and day-old chick weight were largely independent of setting position and turning angle. The latter effects were affected by season though, with water loss decreasing from winter to spring to summer. In contrast, pipping time became later and day-old chick weight heavier from winter to autumn to summer.

In conclusion, data from this study suggested that season affected water loss, pipping time and day-old chick weight more than setting position or angle of rotation. The preferred way of setting ostrich eggs will be in the vertical position in a trolley that turn through an angle of 90° on an hourly basis with the air cell up in order to utilize incubator space optimally.

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Key words

Embryonic mortality, ostrich, rotation angle, season, setting position

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Handle with care – controlling loss of hatching eggs and hatchability of broiler breeders due to mechanical impact

T. Torma and **K.G. Kovácsné**

Széchenyi István University, Faculty of Agricultural and Food Sciences, Institute of Animal Sciences, Hungary

Correspondence: info@iknoweverythingabouthatcheries.com

This study evaluates the effects of field and modelled mechanical impact on the hatchability of broiler breeder hatching eggs originating from different lines and investigates if low level and controlled vibration can be used as an alternative for turning during storage A further question is if Short Period of Incubation (SPIDES) could compensate for the hatching loss caused by mechanical impacts prior to or during incubation (18^{th} day). This latter has high importance due to the spreading phenomenon of on-farm hatching systems.

Aims of this trial: 1.) determine the threshold of mechanical impact on loss of hatching eggs and hatchability, 2.) test if moderate level of controlled vibration would decrease the level of early embryo mortality due sticking to the shell membrane, 3.) test if SPIDES could alleviate the negative effect of mechanical impact, 4.) monitor the effect and mechanical impact of transportation at day 18 under field conditions.

In total, 8100 broiler breeder hatching eggs originating from the same young female and male lines were divided into factorial design groups (VIBRATED, SPIDES, TURNING, 18^{th} DAY TRANSPORT, LINE). VIBRATED groups were placed on a vibration machine with a two dimensional vibration plate and treated for 5 minutes either on 10, 20 or 30 Hz and data was on the motion was collected by a HOBO® accelerometer. RSS were calculated (obtained from square root of the sum of the squares of the resultant acceleration -RMS; m/s^2) for each direction. The SPIDES groups received heat treatment for 2 hours above effective (95°F) eggshell temperature. In the TURNING treatment, eggs were turned twice a day through 45^{o} during egg storage. The 18^{th} DAY TRANSPORT groups were transported for 3 hours. SPSS software was used to analyse the data. Significant differences were assumed if $P \leq 0.05$.

Using MANOVA the corrected model -which reflects the variation in the dependent attributed to other effects in the model, after corrected by the mean – showed significant effect on hatchability ($R^2 = 0.729$), level of early ($R^2 = 0.701$) and late embryo mortality ($R^2 = 0.613$), proportion of cracked eggs ($R^2 = 0.828$) and malposition. Among independent factors, VIBRATED had the most significant correlations (in the same attributes as in the corrected model). VIBRATED × LINE interaction was significant for hatchability, cracked eggs and level of early and late dead embryos, while SPIDES × LINE, LINE × TURNING, SPIDES × LINE were significant for late dead embryos. VIBRATED × 18^{th} DAY TRANSPORT only affected embryo malpositions. Embryos that stuck to the membrane showed significant correlation with TURNING, LINE × TURNING and LINE × 18^{th} DAY TRANSPORT, but their calculated R^2 was below 0.5.

For linear stepwise regression the following equations were calculated:

Cracked eggs (%) = 0.146 × VIBRATED + 0.124 ($R^2 = 0.64$)

Hatchability as fertile (%) = −0.452 × VIBRATED −3.573 × LINE + 91. 94 ($R^2 = 0.46$)

Hatchability as set (%) = −0.460 × VIBRATED +3.701 × SPIDES+ 72.12 ($R^2 = 0.46$)

Although the equations for the hatchability explain less than 50% of the variance the tendency shows different sensitivity of lines on mechanical impact and on SPIDES alleviating effect. Performing a Tukey test ($P < 0.05$) it appears that up to 20 Hz (RRS = 10.8 m/s^2) there is no significant effect on the level of cracked eggs, and up to 30 Hz (RRS = 13.7 m/s^2) there is no effect on hatchability in the current trial. The mechanical impact (RSS = 9.75 m/s^2) used in this trial during the 18^{th} day transport has no negative effect on hatchability or chick quality.

In this trial there was no evidence that low level of shaking could be used as an alternative of turning during storage.

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Key words

Hatchability, logger, mechanical impact, SPIDES, vibration

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Effects of broiler breeder age and egg storage duration on hatchability rate, chick quality and later life performance in hot climates

H. Nasri[1], **T. Najjar**[1], **H. van den Brand**[2] and **T. Masmoudi**[3]

[1] INAT University, Tunisia

[2] Wageningen University, Wageningen, the Netherlands

[3] DICK POULINA

Correspondence: nasri.hedia@gmail.com

The objective of this study was to investigate effects of storage duration and breeder age on hatchability, chick quality and later life performance

Four storage durations (5, 9, 16 and 23 days) and three breeder ages (31, 56 and 60 weeks) on hatchability, chick quality and later life performance were used. Evaluated parameters were hatchability rate, hatchability, percentage of unsalable chicks, chick weight and organs weights (heart, liver, yolk, intestine and proventriculus). Chicks hatching after the storage duration of 16 days were reared for all 3 breeder ages. During the rearing period, body weight, mortality rate, average weight gain (AWG), feed intake (FCR) and performance index (PI) were determined.

Results showed a decrease in hatchability and an increase of unsalable chicks rate with the increase of storage duration (Δ= −52.5%; Δ= +14.2%) and breeder age (Δ= −16.8%, Δ= +4.5%). Chick weight decreased with the increase of storage duration (Δ= −1.99gr) and increased with the increase of breeder age (Δ= +7.7gr). The heart (Δ= −0.1%), liver (Δ= −0.16%) and intestine (Δ= −0.64%) percentage decreased with the increase of the storage duration. The heart (Δ= −0.04%), intestine (Δ= −0.95%) and proventriculus (Δ= −0.7%) percentage decreased and the yolk (Δ= +2.2%) percentage increased with the increase of breeder age.

The AWG (Δ= +64.4gr), body weight (Δ= +99.7%) and PI (Δ= +21.2%) increased with the increase of breeder age. The FCR (Δ= −0.037%) decreased with the increase of breeder age.

It can be concluded that hatching results (hatchability and salable chicks rate) deteriorate with the increase of storage duration and the breeder age. We note also a decrease of the weight of chicks and of the majority of organ percentage with the increase of storage duration. However, in 16 days stored eggs, later life performance was improved with the increase of the breeder age.

Key words

Breeder age, chick quality, egg storage duration, hatchability, performance

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Session 3: Impact of parental effects

Examination the combined effect of parental thermal stress and thermal treatment of the offspring on early embryonic development and on primordial germ cells

B. Lázár[1,2], **Á. Drobnyák**[2], **É. Váradi**[2], **J. Barna**[2], **E. Patakiné Várkonyi**[2], **E. Gócza**[1] and **K. Liptói**[2]

[1] Animal Biotechnology Department, NARIC, ABC, Gödöllö, Hungary

[2] Research Centre for Farm Animal Gene Conservation, Gödöllö, Hungary

Correspondence: lazar.bence@abc.naik.hu

High environmentaltemperature can be a stress factor for poultry, thus it can cause reduced productivity traits. The animals can be protected from such negative effects with increased adaptation capabilities through the usage of special keeping technologies. Therefore, during this study our aim was to investigate the impact of heat treatment and heat shock on chicken embryos and on chicken PGCs by using in vitro culture system. As a heat treatment, the 2-day old chicks were kept at 38.5°C for 12 hours. Later, in case of the heat stress, animals were kept at 30°C for 12 weeks. Experimental animals were measured for spermatological traits, while embryological parameters were collected from the progeny of the experimental group.

We performed our experiments on the Transylvanian Naked Neck breed. Three different types of experimental groups were used: heat treated and heat stressed (HTHS), non-heat treated, but heat stressed (HS) and non-treated, non-stressed (C). The main embryological, spermatological and productivity parameters were measured regularly. PGC cultures were derived from blood which was isolated from 2.5-day (HH16) embryos, and then samples were collected after 23, 30 and 50 days of culturing. DNA and RNA were isolated at each time points and samples were prepared for immunostaining.

In the heat-treated group significantly less abnormal embryonic development was observed when the parents were heat stressed. The heat treatment also caused significant growth in the egg production. Difference was not found in the percentage of abnormally developed embryos. The heat treatment did not have any effect on the spermatological parameters.

In the treated group, almost twice as many eggs (46.8%) were experienced, which were appropriate for the PGC culturing, than in the non-treated group (29.8%). In HTHS and HS populations derivation rate was approximately the same (72.3% and 78.6%), however in the C group it was 60%. We could establish 39 PGC cultures from the used 56 embryos. 29 cell lines were frozen.

The expression of the pluripotency markers and the HSP70 heat shock protein gene were detectable in the cell cultures. The expression of the HSP70 was considerably higher in the gonads of HTHS and HS embryos than in the C group.

Key words

Cell culture, chicken primordial germ cells, heat shock, thermal manipulation, Transylvanian Naked Neck

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Session 4: Adaptation and epigenetic alteration in birds

Impact of embryonic thermal manipulation on quail transcriptome and methylome

A. V. Carvalho[1], **N. Couroussé**[1], **S. Crochet**[1], **S-A. David**[1], **T. Bordeau**[1], **E. Godet**[1], **B. Piégu**[2], **C. Hennequet-Antier**[1], **A. Brionne**[1], **C. Noirot**[3], **Y. Bigot**[2], **F. Pitel**[4], **A. Collin**[1] and **V. Coustham**[1]

[1] INRA – UR83 Recherches Avicoles, Nouzilly, France

[2] INRA – UMR85 PRC, Nouzilly, France

[3] INRA – UR875 MIAT, Castanet-Tolosan, France

[4] INRA – UMR1388 GenPhySE, Castanet-Tolosan, France

Correspondence: vincent.coustham@inra.fr

Changes in gene activity through epigenetic alterations induced by early environmental challenges during the embryogenesis are known to impact the phenotype, health and disease risk of animals. The epigenome is therefore an essential contributor to phenotypic plasticity, and learning how environmental exposures translate into persisting epigenetic changes may open new doors to improve robustness and resilience of developing animals. Birds are agronomic species of choice to directly manipulate the embryonic environment and study its consequences on the developing animal. We previously showed that the heat tolerance of male commercial chickens was improved by cyclically elevating the egg incubation temperature. This treatment named embryonic thermal manipulation (TM) was associated with changes in gene expression that persisted during the development of chickens and enhanced gene expression response in case of heat challenge at slaughter age, 35 days post-hatch.

To further explore the molecular basis of heat acclimation, we took advantage of an inbred line of Japanese quails (*Coturnix japonica*) to investigate the impact of TM on bird methylome. Among other advantages, quail generation cycle is 3–4 time faster than chicken and the use of an inbred genotype should reduce the phenotype variations associated with genetic variability. A characterization of TM on quail phenotype was performed in interaction with a post-hatch heat stress at the onset of sexual maturity. Notably, we observed an impact of the embryonic treatment on the temperature and the weight of young quails, similarly to what was reported for chicken. Concomitantly we investigated the impact of TM on quail transcriptome by RNA-seq and methylome by whole genome bisulfite sequencing (WGBS) on brain tissues sampled at hatch. Several differentially expressed gene and differential methylation regions were identified. Interestingly, the nature of some transcripts seemed to be affected by the embryonic treatment at both methylation and transcript levels.

Key words

DNA methylation, epigenetics, quail, thermal manipulation, transcriptome

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Avian adaptation to heat by prenatal acoustic stimulation: from songbirds to poultry

M. Mariette

Deakin University, Centre for Integrative Ecology, Victoria, Australia

Correspondence: m.mariette@deakin.edu.au

In many species, ranging from crocodiles and birds to humans, embryos can perceive, learn and even produce sounds. Surprisingly however, the implications of such embryonic capacities for adaptation and developmental programming have not been recognized. Here, I will present a novel function of prenatal communication, by showing that in the Australian Zebra finch, parents acoustically signals high ambient temperatures to their embryos by calling to their eggs when ambient temperature rises above 26°C and their eggs are within 5 days of hatching (MARIETTE and BUCHANAN, 2016). Using a large playback experiment, I will show that exposure of embryos to these acoustic parental cues alone adaptively alters subsequent nestling begging and growth in response to nest temperature (Mariette & Buchanan 2016, Science). Indeed, individuals exposed to parental incubation calls before hatching subsequently become lighter in hot nests, suggesting they might limit weight gain to avoid the oxidative costs of growing in the heat. Subsequently at adulthood, individuals produce more offspring if they had followed the growth strategy induced by playbacks of incubation calls (i.e. heavy in cool nests or light in hot nests), demonstrating that this response is favourable for offspring fitness. Further, I will discuss the possible underlying mechanisms of such developmental programming by presenting preliminary results in zebra finches and discussing what is known on the development of thermoregulation in birds. In particular, I will highlight some previous studies in a variety of precocial and altricial birds where embryos have been found to regulate their temperature vocally, by calling from the egg when their temperature falls below the optimal incubation temperature. Finally, given the threat caused to poultry by hot weather, but also the wealth of knowledge accumulated in chicken on the effect of early thermal manipulation on later heat resistance, I will draw the parallel between songbirds and poultry and discuss exciting research avenues. Overall, my talk seek to shed light on a novel mechanism for thermal adaptation in birds, which is particularly critical under climate change for the resilience of both wild and domestic species.

Key words

Adaptation, developmental programming, maternal effects, phenotypic plasticity, prenatal acoustic communication, thermal acclimation

References

MARIETTE, M.M., K.L. BUCHANAN, 2016: Prenatal acoustic communication programs offspring for high ambient temperatures in a songbird. Science **353**, 812-814.

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Session 5: Incubation parameters and chick vitality

Importance of incubation conditions on proper In-Ovo vaccination

M.J. Wineland[1] and **R.B. Wysocky**[2]

[1] Hatcheryconsult LLC, USA

[2] North Carolina State University, USA

Correspondence: mike@hatcheryconsult.com

Marek's vaccine has been utilized to protect against Marek's disease since the 1970's when it was injected subcutaneously on day of hatch in chickens. Embrex brought In-Ovo to the fore front when they formed back in the mid 1980's. There are many factors that can decrease viability of Marek's vaccine. This includes the addition of antibiotics to the vaccine/diluent mix, the interval of time between preparations of the vaccine, as well as how the vaccine is thawed. Additionally, if you are using In-Ovo then the pressure that the vaccine experiences when being injected can have an effect.

In-Ovo Marek's vaccination can utilize the conventional HVT vaccine or the more recent vectored vaccines with HVT in combination with either avian influenza (AI), Infectious Bursal disease (IBD), Laryngotracheitis (ILT) or Newcastle disease. The process of In-Ovo needs to place the vaccine in specific locations to be efficacious. The best location is to place the vaccine into the amnion. Secondary locations would be to have it placed sub-cutaneous or intra-muscular. Sites of deposition that are of little or no good are the yolk sac, allantois or the air cell. Since the vaccine must be placed in specific locations, timing of administration is important. Proper positioning of the embryo is critical and thus both the incubational process and the time of transfer become much more important than if the chicks were being vaccinated sub-cutaneous. Regarding the incubational process we must remember that temperature drives the rate of development. Thus, temperature and uniformity of temperature of the developing embryos in the incubator are very important. The elimination or minimization of micro climates within machine is also important. We also know that embryos from eggs that have been stored for extended times will hatch a slower rate and thus be in correct position later.

Age of breeder is also important as we know the chicks will hatch at different times and would be in proper position at different times. Typically, in a hatchery there are eggs being transferred from young, prime and old breeders on the same day and in the same order because of contamination levels found in eggs from older breeders. Additionally, the process of In-Ovo vaccination must be performed over a specific duration period of time during which the embryo is in proper position. Excessive vaccination time will not insure all embryos are properly vaccinated. The time at which the In-Ovo vaccination process begins is extremely important. Some of the timing issues we see are the time of transfer because of incubator type. In the U.S. we have primarily multi-stage incubators of 2 diverse types (Chick Master and Jamesway) which are typically transferred at different times because of temperature control of embryo. Another concern we have seen with timing of the vaccination procedure is that in some cases we have seen the transfer and vaccination occurring at the convenience of the transfer crew or upon availability of personnel after they have finished other hatchery work to better utilize workers.

Below are two graphs demonstrating the difference in vaccination location (percent of total embryos observed) in a prime flock over a twelve-hour period and an old flock (Figure 2). Young flocks will show slightly different results. It is important to remember that incubation conditions between hatcheries can influence results also.

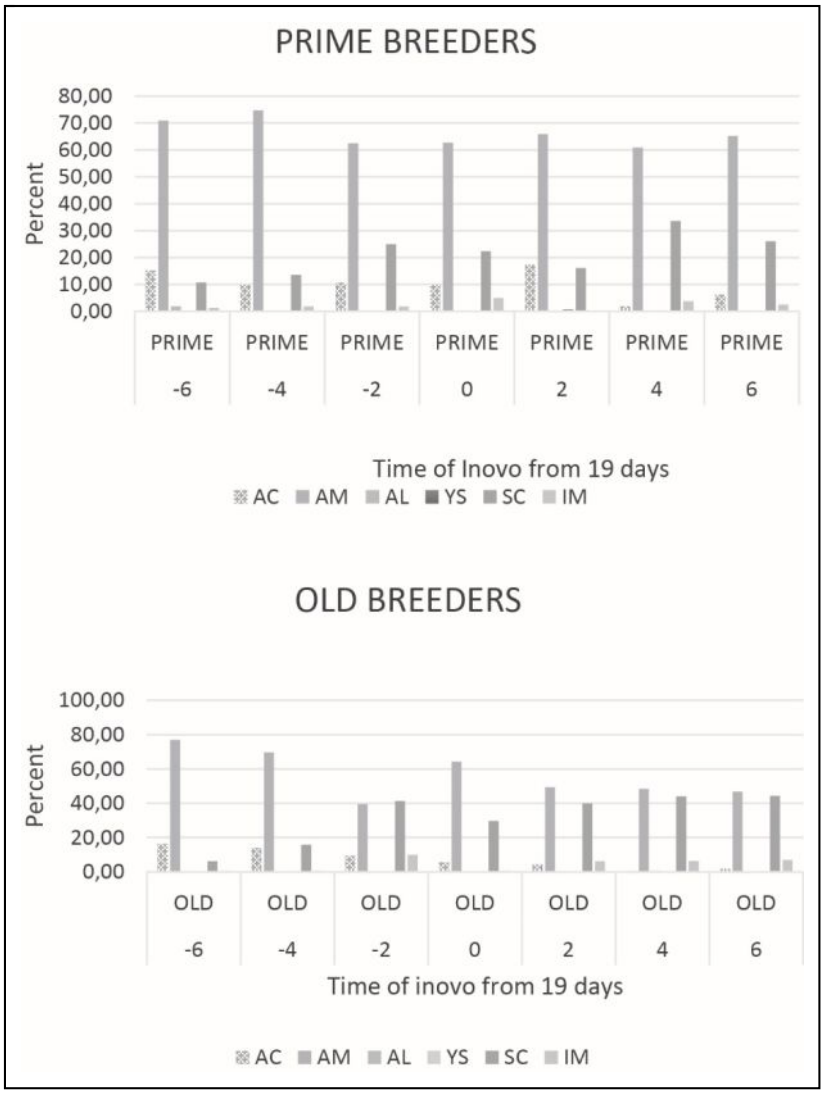

Figure 2. Difference in vaccination location (percent of total embryos observed) in a prime flock and an old flock over a twelve-hour period.

In summary, it is important to realize that making a hole in the egg is not necessarily effective vaccination. Remember that consistent and uniform control of the incubation process will influence that the embryos are all in the correct position for effective In-Ovo vaccination.

Key words
Breeder age, embryo position, In-Ovo vaccination, Marek's disease

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

How much the In-Ovo injection process can affect the contamination level and chick quality (case study)

M. Salem

National Poultry, Delmonte group, Amman, Jordan

Correspondence: mun_salem@yahoo.com

In-Ovo injection is a process to deliver a solution dose to the embryo during incubation period (mostly at transfer time on incubation day 18–19). In-Ovo injection had been proven to enhance chick quality and overall broiler performance by delivering a uniform and hygienic dose while reducing the time of vaccinations in the hatchery. In-Ovo injection can replace all or most of the conventional vaccination at lower cost and better quality. However, the hatchery performance parameters could be adversely affected by this process as well. This case study was aimed to quantify some possible effects of the In-Ovo injection process on the main hatchery performance parameters.

The study was conducted under normal industrial conditions, which could be different from the controlled laboratory conditions. This may give this study the potential to be used as reference for planning or economical evaluation of the In-Ovo injection process. The study was divided into two groups: with and without In-Ovo injection. Then nine parameters have been measured and tested: chick weight, chick quality (%), chick yield (%), hatch of fertile eggs (%), egg contamination level, cracked shell (%), culled chicks (%), late mortality (%) and fungus proliferation. The infertile eggs were not excluded at transfer for more subjective and unbiased results especially for the contamination tests. The contamination levels were tested for both the bacterial and fungal presence. The total scale of the study was 384,000 eggs. Tested eggs at transfer were 48,000 and tested unhatch eggs at hatch for breakout analysis were 24,000. The total chick sample used for chick quality assessment or scoring was 2,000 chicks. Chick quality scoring was carried out by two independent evaluators to ensure the best results accuracy.

The results showed a direct relationship between the efficiency of the used In-Ovo injection process and the hatching eggs quality and hatchery managements and/or practices. The chick weight, chick quality (%) and chick yield (%) were positively affected while the hatch of fertile eggs (%), egg contamination level, cracked shell (%), culled chicks and late mortality (%) were negatively affected. Fungus proliferation was not affected by the In-Ovo injection process.

In conclusion, to gain the maximum benefits from the In-Ovo process it is highly recommended to avoid bad quality eggs (upside down, weak shell, dirty shell, small and bad uniform egg sizes) and improve the hatchery management practices as well. For better understanding of the total effect of the In-Ovo injection process, further parameters, such as egg shell temperature (EST), yolk free body mass (YFBM), egg shell strength, and hatch window, should be tested.

Key words

chick quality, chick weight, chick yield, contamination, egg quality, fungus proliferation, In-Ovo injection, late mortality

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Changes in oxygen levels during incubation as a new way to affect tibial dyschondroplasia incidence in broiler chicks

E. Salomon[1,2], **O. Genin**[1], **Y. Cinnamon**[1] and **and S. Druyan**[1]

[1] Agricultural Research Organization, Volcani Centre, Department of Poultry and Aquaculture Science, Rishon LeTsiyon, Israel

[2] The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

Correspondence: Shelly.druyan@mail.huji.ac.il

During the past 60 years, intensive genetic selection has increased the growth rate of broiler (meat-type) chickens by more than 300%. However, these improvements in growth rate and feed efficiency are accompanied by higher incidence of skeletal abnormalities, especially leg deformities with poor walking ability and locomotion. Whether these disorders are direct effects of the growth rate or indirect effects that result from increased body weight and improper development of bone, muscles and/or tendons is still unknown.

Tibial dyschondroplasia (TD) is a bone disorder frequently observed in broilers and turkeys flocks. It is a disease of the growth plate (GP) at the ends of long bones, which is characterized by enlargement of the GP, abnormal chondrocyte differentiation, and a major decrease in blood vessels and perfusion. Based on our preliminary results, we hypothesize that increase in angiogenesis, and improved oxygen supply to the growth plate, will lead to amelioration of lameness in TD.

Improving angiogenesis can be achieved by reducing oxygen level and exposing the developing embryo to hypoxic condition. The aim of this study was to investigate the effect of hypoxic microenvironment during embryonic limb bud development (E6 to E9) on bone disorders.

Fertile Cobb strain broiler chicken eggs (n = 400) were incubated under standard incubation conditions. At E6 the eggs were randomly allocated to four treatments: control; 17% O_2, 13% O_2 and 11% O_2 concentration for 12 h/d from E6 through E9.

Our results show that 11% O_2 is the biological limit for hypoxic adaptation, resulting in embryonic mortality at the first day of exposure. GP histology and vascular quantification at time of hatch and D7 of the three remaining treatments reviled, that the 13% treatment significantly affected vascular area density of the GP and in this group the GP blood system was dense with a higher blood vessel complexity. A higher vascular density area of the GP coincided with a positive effect on TD occurrences. Following thiram administration (which serves as an agent to challenge GP formation) to the feed (40 ppm), and TD scoring, incidence of TD in the control group was found to be 78%. TD incidence in the 17% group was 57% while in the 13% TD incidence was only 57%, significantly lower compare to control.

Thus, our results suggest that hypoxic exposure to 13% oxygen concentration during the limb bud development increase angiogenesis, and improved oxygen supply to the GP, hence it reduces chicks' susceptibility to TD.

Key words

Broiler, incubation, oxygen, tibial dyschondroplasia

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

The interaction between carbon dioxide concentration and eggshell temperature during the second half of incubation in broiler chickens

H. van den Brand[1], **R. Meijerhof**[2], **M.J.W. Heetkamp**[1], **I. van den Anker**[1], **M. Ooms**[1] and **B. Kemp**[1]

[1] Adaptation Physiology Group, Wageningen University and Research, Wageningen, the Netherlands

[2] Poultry Performance Plus, Voorst, the Netherlands

Correspondence: Henry.vandenbrand@wur.nl

The interaction between carbon dioxide concentration and eggshell temperature during the second half of incubation on broiler chicken embryo development was investigated. The experiment was setup as a 2 × 3 factorial design with eggshell temperatures (EST) and CO_2 concentrations as main factors. The experiment was executed in six consecutive batches. Two EST (37.8 or 38.9°C) and 3 CO_2 concentrations (0.1, 0.4, or 0.8%) were applied from d 8 of incubation onward. Within each batch, both EST were applied, but CO_2 concentration varied among batches. CO_2 concentrations were based on the following findings: 0.1% CO_2 is a common concentration in a multistage incubator, 0.8% CO_2 is incidentally happening in single stage incubators at the end of incubation, and 0.4% CO_2 is in between, to investigate whether effects are linearly or not. Within all treatments, the O_2 concentration was remained the same at 20.95%. During incubation heat production was determined continuously and embryo and hatchling quality was determined. Cobb 500 eggs (105 per batch) were selected on an egg weight of 62–65 gram from broiler flocks of 37–45 weeks. At day 18 of incubation and within 6 hours after hatching, embryos or chickens were used for determination of embryo or chicken development (weight, length, YFBM, RY, organ weights), blood variables (glucose, lactate, uric acid) and hepatic glycogen. Data will be available during the workshop.

Key words

Chickens, chicken development, CO_2, eggshell temperature, glycogen

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

The effects of temperature during late incubation on first week broiler chicken development

C. M. Maatjens[1], **I. A. M. van Roovert-Reijrink**[3], **I. van den Anker**[2], **B. Engel**[4], **C. W. van der Pol**[2,3], **B. Kemp**[2] and **H. van den Brand**[2]

[1] WIMEX Agrarprodukte Import und Export GmbH, Baasdorf, Germany

[2] Adaptation Physiology Group, Wageningen University, Wageningen, The Netherlands

[3] HatchTech B.V., Veenendaal, the Netherlands

[4] Biometris, Wageningen University, Wageningen, the Netherlands

Correspondence: C.Maatjens@probroed.com

Little is known about applying various eggshell temperatures (EST) during the last week of incubation. In particular, the effect of an EST below 37.8°C during the last week of incubation is poorly investigated. Therefore, we investigated effects of EST of 35.6, 36.7, 37.8, or 38.9°C applied from d of incubation (E) 15, E17, or E19 on hatching pattern, embryonic organ development and first week broiler development and performance.

A total of 2,850 first grade eggs of a 43 wk old Ross 308 broiler breeder flock were incubated at an EST of 37.8°C until E15. From E15, E17, or E19 onward, eggs were incubated at an EST of 35.6, 36.7, 37.8, or 38.9°C. Moment of internal pipping (IP), external pipping (EP), and hatch were determined and organ development was measured at E15, E17, E19, IP, EP, and hatch. Chick quality was determined at placement in the broiler house and organ development was measured at d7. BW was determined at placement, d4, and d7. Feed intake (FI) was measured at d4 and d7 and G:F was calculated between placement and d4, and between d4 and d7.

A lower EST extended incubation duration compared to a higher EST. The longer incubation duration was mainly caused by the extended time until IP, whereas time between IP and hatch hardly varied between treatments.

Relative organ weight was affected by EST. Relative heart weight was affected by EST already from 2 d after start of EST treatment on E15, and effects became more pronounced at longer exposure time to various EST treatments. At hatch, the largest difference in relative heart weight was found between an EST of 35.6 and 38.9°C started at E15 (Δ=64.4%). From E17 onward, EST affected yolk-free body mass (YFBM) and relative stomach weight, where a lower EST resulted in a lower YFBM and relative stomach weight before IP and a higher YFBM and relative stomach weight after IP. From E19 onward, a lower EST resulted in a higher relative liver and spleen weight regardless start time of treatment. Yolk weight and relative intestine weight were not affected by EST before and at E19, but a higher EST resulted in a higher yolk weight and lower relative intestine weight from IP onward.

Chick quality at placement was higher at an EST of 35.6°C compared to all other EST treatments, expressed by a longer chick length and highest prevalence of closed navels. BW d7 was higher at an EST of 36.7°C compared to all other EST treatments, which was not caused by a higher FI during the first week. A higher G:F between d0 and d7 was found at an EST of 36.7°C compared to 35.6 and 38.9°C. At d7, a higher relative heart weight was found at an EST of 35.6 compared to 38.9°C.

This study indicates that an EST of 38.9°C applied from E15 onward negatively affected chick quality, based on the lower YFBM and lower relative organ growth found at hatch. In addition, an EST of 38.9°C applied from E15 onward negatively affected organ development, and G: F until d7 compared to 37.8°C. Moreover, an EST of 36.7°C had a clear positive effect on embryo development, chick quality, organ development, G:F, and growth performance until d7. An EST of 35.6°C resulted in equal or higher chick quality and organ weights compared to 36.7°C, but this was not reflected in performance parameters.

Key words

Broiler performance, chick quality, incubation, organ development, temperature

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Affecting leg bone development in broiler chickens through incubation lighting schedules

C. W. van der Pol[1,2], **I. A. M. van Roovert-Reijrink**[1], **M.H. Priester**[1,2], **H.J. Wijnen**[1,2] and **H. van den Brand**[2]

[1] HatchTech B.V., Veenendaal, the Netherlands

[2] Adaptation Physiology Group, Wageningen University, the Netherlands

Correspondence: cvdpol@hatchtech.nl

The effects of circadian lighting schedules during incubation on broiler leg bone development and leg health were investigated in two experiments. In the first experiment, applying a circadian lighting schedule of 12 hours of light, followed by 12 hours of darkness (12L:12D) resulted in higher tibial cortical area and thickness and a better distribution of bone material at hatch compared to continuous light (24L) as revealed by MicroCT scanning. It was furthermore found that 12L:12D resulted in a lower incidence of tibial dyschondroplasia at day 35 post hatch compared to 24L. Continuous darkness (24D) was mostly intermediate.

In the second experiment, 24L or 24D were compared to a circadian lighting schedule of 16 hours of light, followed by 8 hours of darkness (16L:8D). At day 35 post hatch, incidence and severity bacterial chondronecrosis with osteomyelitis and epiphyseal plate abnormalities were lowest for broilers exposed to 16L:8D throughout incubation, and tibial dyschondroplasia tended to be lower for 16L:8D than for 24D.

Another pathway that may affect embryonic bone development is activity. Between embryonic day (E)14 and E16, heart rate was found to be higher for 24D than for 24L. However, by E19, heart rate was higher for 16L:8D than for both 24D and 24L. This suggests that embryonic activity and/or metabolism were affected by lighted incubation, with effects depending on incubation phase.

The overall findings suggest that applying a light-dark rhythm during incubation may improve embryonic leg bone development and leg health at slaughter age compared to continuous light and continuous darkness, while continuous light during incubation had a detrimental effect on embryonic and early post hatch leg bone development and health.

Key words

Bone development, incubation, leg problems, lighting schedule

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Session 6: Control and programming of energy balance

Identification and characterization of missing genes in chickens including two adipokines: leptin and TNF

E. Seroussi[1], **S. Yosefi**[1], **S. Benjamini**[1], **S. Miyara**[1], **S. Bornelöv**[2,3], **F. Pitel**[4], **S. Leroux**[4], **M. Morisson**[4], **L. Andersson**[2,5,6] and **M. Friedman Einat**[1]

[1] Agricultural Research Organization, Volcani Centre, Rishon LeZion, Israel

[2] Dept. of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

[3] Current affiliation: Wellcome Trust Medical Research Cambridge Council Stem Cell Institute, University of Cambridge, Cambridge, UK

[4] Université de Toulouse, INRA, INPT, ENVT, Castanet Tolosan, France

[5] Dept. of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, USA

[6] Dept. of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden

Correspondence: miri.einat@mail.huji.ac.il

The satiety hormone leptin has a key role in the control of energy homeostasis in mammals. Leptin is produced almost exclusively by the adipose tissue and signals the amount of fat stores primarily to the hypothalamus, but also to peripheral tissues. Recently, we identified the long sought leptin (SEROUSSI et al., 2016), in the "dark side of the chicken genome", meaning extremely GC-rich regions that have been difficult to sequence and assemble. Expression profiling of the avian leptins showed a different pattern compared to mammals, surprisingly with no expression in the adipose tissue. Nevertheless, we found that leptin and leptin receptor (*LEPR*) genes are expressed in tissues that are implicated in the response to leptin signalling in mammals, and their expression pointed to an autocrine/paracrine instead of endocrine mode of action. Therefore, the role of avian leptins may be relevant to the control of reproduction, stress response and metabolism, but in a different way than in mammals, as it is not the signal of the adipose tissue.

Following the success of the identification of chicken leptin and realizing its presence in RNA-seq data rather than genomic assemblies, we asked if other missing genes in chicken could be identified using the RNA-seq approach. Extensive RNA-seq analysis of visceral fat, hypothalamus and pituitary, followed by transcript assembly and bioinformatic analysis led to the identification of 191 novel genes in chickens (BORNELOV et al., 2017), all with very high GC content (~70%). These included the tumour necrosis factor (TNF, also known as TNF-alpha) and nephrin (*NPHS1*), which were long sought after in the chicken genome. Interestingly, 25 of our novel genes were mapped to the chicken genome in the most recent genome assembly Galgal5. This mapping showed that about 70% of the genes were mapped to chicken microchromosomes, which are known to be GC-rich. The same proportion of genes were mapped in gene blocks demonstrating that the indication that missing genes arranged in gene clusters in other vertebrates cannot be taken as an indication for their evolutionary loss. Altogether, these findings suggest that other missing genes in chickens may be positioned in GC-rich gene clusters in the chicken genome.

Among our novel chicken genes, we found five genes belonging to the leptin synteny group in vertebrates: *RBM28*, *SND1*, *LRRC4*, and *FLNC*. We used this identification to map the chicken leptin gene and its syntenic genes to chicken chromosome 1p (SEROUSSI et al., 2017), thus providing the final proof for the correct identification of the chicken leptin gene.

Key words

Chicken, GC-rich, leptin (LEP), missing genes, NPHS1, RBM28, TNF

References

BORNELOV, S., E. SEROUSSI, S. YOSEFI, K. PENDAVIS, S.C. BURGESS, M. GRABHERR, M. FRIEDMAN-EINAT, L. ANDERSSON, 2017: Correspondence on Lovell et al.: identification of chicken genes previously assumed to be evolutionarily lost. Genome Biol. **18**:112.

SEROUSSI, E., Y. CINNAMON, S. YOSEFI, O. GENIN, J.G. SMITH, N. RAFATI, S. BORNELOV, L. ANDERSSON, M. FRIEDMAN-EINAT, 2016: Identification of the Long-Sought Leptin in Chicken and Duck: Expression Pattern of the Highly GC-Rich Avian leptin Fits an Autocrine/Paracrine Rather Than Endocrine Function. Endocrinology **157**:737-751.

SEROUSSI, E., F. PITEL, S. LEROUX, M. MORISSON, S. BORNELOV, S. MIYARA, S. YOSEFI, L.A. COGBURN, D.W. BURT, L. ANDERSSON, M. FRIEDMAN-EINAT, 2017: Mapping of leptin and its syntenic genes to chicken chromosome 1p. BMC Genet. **18**:77.

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Embryonic 'over'-nutrition and metabolic malprogramming in the avian primary brain area for control of energy balance, feed intake and body weight

B. Tzschentke[1], **S. Bogatyrev**[1], **K.S. Yakimova**[1,3], **R.C. Rancourt**[2], **K. Schellong**[2] and **A. Plagemann**[2]

[1] Humboldt-Universität zu Berlin, Faculty of Life Science, Institute of Biology, Berlin, Germany

[2] Charité University Medicine Berlin, Campus Virchow-Klinikum, Clinic of Obstetrics, Division of Experimental Obstetrics, Berlin, Germany

[3] Medical University Sofia, Faculty of Medicine, Department of Pharmacology, Sofia, Bulgaria

Correspondence: barbara.tzschentke@rz.hu-berlin.de

During critical prenatal periods nutrient supply programs central control of energy balance, feed intake and body weight. In mammals as well in birds, prenatal glucose oversupply, for instance, may increases the offspring risk for metabolic disorders and related diseases throughout life. We investigated the long-lasting influence of temporary *in-ovo* hyperglycemia in chickens on neuronal mechanisms of the *nucleus infundibuli hypothalami* (NI), the 'critical' centre regulation metabolism, feed intake and body weight as well as on related peripheral parameters. Experiments were carried out in Lohmann White Leghorn chickens starting in embryos in three experimental groups: one untreated control group, one *in-ovo* injection control (daily single injection of 0.5 ml NaCl-solution) and one group prenatally experienced with temporary hyperglycemia by daily single injection of 0.5 ml glucose solution (30 mmol/l) from days 14 to 17 of incubation. After three weeks post-hatching in the untreated control group for the first time the avian neuronal glucose and leptin sensitivity was investigated using extracellular recordings in brain slices. Human recombinant leptin was also tested in combination with $GABA_B$-receptor agonist and antagonist. The long-lasting effect of temporary *in-ovo* hyperglycemia was examined on neuronal glucose sensitivity, the glucose transporter GLUT1 and 3, the leptin and insulin receptor expression as well as on the total body fat and blood glucose level. Additional, in male and female chickens the body weight development was recorded in all experimental groups during the total 3-weeks growing period.

Results can be summarized as follows (RANCOURT et al., 2015; TZSCHENTKE et al., 2015; BOGATYREV et al., 2017):

1) NI-neurons show mammalian-like responsiveness after acute glucose, leptin and GABA application. $GABA_B$-mechanisms involved in GABA release play a likely important role in the leptin mediated effects on NI neurons via functional leptin receptors.

4) Temporary late prenatal hyperglycemia induces lasting hypothalamic 'glucose-resistance' via strongly decreased neuronal glucose sensitivity as well glucose transporter, insulin- and leptin receptor expression.

3) After 3 weeks post-hatching the strong changes in the neuronal mechanisms were not accompanied by changes in related peripheral metabolic parameters as well in the body weight in male and female chickens.

In conclusion, although in 3 weeks old chickens no changes in peripheral metabolic parameters and body weight development could be observed, just temporary hyperglycemia during the second half of incubation, may leading to persistent malprogramming in central control of energy balance, feed intake and body weight as predisposition for the development of metabolic disorders. It shows a risk of *in-ovo* feeding. Further, the chicken embryo provides a valuable new model for investigating early central nervous origins of 'diabesity' and related diseases.

Acknowledgement

Supported by grants of the German Research Foundation (DFG: TZ 6/17–1, PL 241/6–1).

Key words

$GABA_B$-receptor agonist and antagonist, glucose resistance, glucose transporter, insulin receptor, leptin receptor

References

BOGATYREV, S., K.S. YAKIMOVA, B. TZSCHENTKE, 2017: Influence of leptin and $GABA_B$-receptor agonist and antagonist on neurons of the hypothalamic infundibular nucleus in the chicken. J. Comp. Physiol. A **203,** 291-299.

RANCOURT, R.C., K. SCHELLONG, R. OTT, S. BOGATYREV, B. TZSCHENTKE, A. PLAGEMANN, 2015: Acquired alterations of hypothalamic gene expression (INSR, LEPR, GLUT1 and GLUT3) in prenatally high-glucose exposed postnatal chickens do not coincide with significantly altered promoter DNA methylation. PlosOne DOI: 10.1371/journal.pone.0119213.

TZSCHENTKE, B., S. BOGATYREV, K. SCHELLONG, R.C. RANCOURT, A. PLAGEMANN, 2015: Temporary prenatal hyperglycemia leads to postnatal neuronal 'glucose-resistance' in the chicken hypothalamus. Brain Res. **1618**, 231-240.

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Heat production of chicks with and without access to feed and water between hatching and pulling

I.A.M. van Roovert-Reijrink[1], **C.W. van der Pol**[1], **H. van den Brand**[2], **M.H. Priester**[1,2] and **H.J. Wijnen**[1,2]

[1] HatchTech B.V., Veenendaal, the Netherlands

[2] Adaptation Physiology Group, Wageningen University and Research, Wageningen, the Netherlands

Correspondence: ivroovert@hatchtech.nl

In current practice, chicks are provided with feed and water directly post-hatch more often. When feed and water is provided directly post-hatch, heat production of day-old chicks is expected to be increased in comparison to withheld chicks. To ensure a comfortable environment (combination of air temperature, relative humidity, and air velocity) for fed chicks post-hatch, it is crucial to know how the intake of feed and water affects heat production in the perinatal period.

The aim of this trial was to investigate effect of feed and water access directly post-hatch on heat production between hatching and pulling.

Chicks had access to feed and water (early fed) or were withheld from feed and water (withheld) between hatching and pulling. In two consecutive batches, 120 eggs with a viable embryo were set in one of two climate respiration chambers (CRCs) at embryonic day (E) 18.5 (N = 30 per batch per treatment). Eggs of a Ross 308 parent flock of 45 weeks of age were used. Between batches, treatment was switched between climate chambers to exclude the effect of CRC. The median of 5 eggshell temperatures (ESTs) sensors was maintained at 37.8°C until the first chick hatched, where after the air temperature was kept constant and EST was allowed to increase.

O_2 consumption and CO_2 production were measured per CRC with a 9 minute interval and were used to calculate heat production (mW/chick). Moment of hatch was determined through video observations. Chicks were removed from the CRC after 22 days of incubation (528 hours of incubation), and individual body weight and feed intake per CRC were measured. From O_2 consumption and CO_2 production, heat production (mW/chick) was calculated.

O_2 consumption and CO_2 production did not differ between early fed and withheld chicks until E20.3. At that moment 80% of the chicks had hatched. Heat production increased for all chicks as the hatching process progressed until E20.3. The early fed chicks continued increasing heat production, whilst the withheld chicks plateaued at approximately 340 mW/chick. Heat production of the early fed chicks was 390 mW/chick at E21, 521 mW/chick at E21.5, and 634 mW/chick at E22.

By pulling time at E22, body weight of the early fed chicks averaged 57.0 g, while it averaged 42.7 g for the withheld chicks.

In conclusion, heat production of early fed chicks increased with 86% at pulling in comparison to withheld chicks. The increased heat production of early fed chicks emphasizes that it is important to pay attention to environmental conditions in the hatcher, but also during chick handling, chick storage, transport, and after placement in the broiler house.

Key words

Body weight, broilers, early feeding, heat production

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Impact of perinatal temperature training and feed composition during growing phase on production efficiency in males of different chicken lines

I. Halle[1] and **B. Tzschentke**[2]

[1] Inst. of Animal Nutrition (FLI), Braunschweig, Germany

[2] Humboldt-Universität zu Berlin, Faculty of Life Science, Institute of Biology, Berlin, Germany

Correspondence: barbara.tzschentke@rz.hu-berlin.de

Short-term temperature training in the perinatal/hatching phase (PTT) may long-lasting improve robustness and production efficiency in poultry. Obviously, PTT programs the basic metabolism to a lower level, accompanied by more available energy for performance, adaptation, stress- and immune responses throughout life (TZSCHENTKE and HALLE, 2016). Experiments in meat type poultry have shown that particularly males benefit from PTT with significant changes in neuronal mechanisms in the 'critical' centre regulation metabolism, feed intake and body weight, as well in changes in related blood hormone levels. Further, PTT experienced birds show a better growth and feed conversion rate until slaughter age. We investigated the long-lasting effect of PTT on production efficiency in males of meat type, laying and dual chicken lines fed with different feed compositions during the growing phase (TZSCHENTKE and HALLE, 2016; HALLE and TZSCHENTKE, 2017; HALLE et al., 2017). Data were compared from male chickens of Ross 308 broiler, Lohmann Tradition, Lohman Brown and Lohmann Dual. Eggs of each chicken line were incubated under standard conditions until hatching (control) or with PTT (+1°C for a maximum of 2 hrs per day) during the last days before hatching. During the growing period of 35 (Ross 308) or 70 (laying type/dual chickens) days the birds were fed with feed supplemented with 40/80/0 mg vitamin-E/kg (broiler, n = 264), 0/1/5 mg Iodine/kg (broiler, n = 216) or with feed of high (215–220 g/12 MJ AME_N/kg) and low (200 g crude protein/11 MJ AME_N/kg) level in protein-energy concentration (laying type cockerels, n = 400; dual chicken cockerels, n = 160). Hatching and growing performance as well feed conversion rate were detected.

Results can be summarized as follows:

1) PTT improved the hatching results in favour to high quality male chickens only in broiler chickens. However, we did not observe any negative effects of PTT on hatching rate and chick quality in the layer and dual chicken lines.

2) During the growing period in males of Ross 308 and of Lohmann Dual chickens PTT may improve growing performance and feed conversion rate, which was not as pronounced in laying line cockerels (Lohmann Brown/Lohmann Tradition).

3) Feed composition may modify the performance and production efficiency in all investigated chicken lines (control/PTT), but the effects of PTT (e.g. the improved feed conversion rate) observed in broilers and dual chickens normally were persistent also if different feed compositions were applied. Obviously, a main factor is the protein-energy content in the diet. In dual chickens the effect of PTT on the feed conversion rate, for instance, was persistent until slaughter age when feed with a low protein-energy level was applied. In conclusion, production efficiency, especially the feed conversation rate, could be improved by PTT in meat type poultry and dual chickens fed with different feed compositions, but not in laying type cockerels. However, in meat type and laying type cockerel, for instance, similar PTT (temperature increase, duration, repetition rate) was applied. In comparison with layers broiler chickens are characterized by higher growth and metabolic rate already during embryonic development. Hence, for the fine-tuning of the PTT protocols especially for layer-type chicken lines, more research on its basic physiological functions is needed. Further, long-lasting effects of PTT on production efficiency as shown in broilers and dual chickens may be modified especially by the protein and energy level of the diet.

Key words

Broiler, dual-chicken, feed composition, layer, perinatal temperature training

References

HALLE, I., B. TZSCHENTKE, 2017: Combined effect of short-term temperature stimulation during the hatching phase and of protein and energy concentrations in the diets on performance of laying-type cockerels of different strains. Europ. Poult. Sci. **81**, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.174.

HALLE, I., M.A. LIEBOLDT, M. HENNING, B. TZSCHENTKE, 2017: Pre-hatching temperature training improves performance in dual and laying-type cockerels fed with different protein and energy concentrations. Europ. Poult. Sci. (accepted).

TZSCHENTKE, B., I. HALLE, 2016: Improvement of robustness and performance in meat-type chicken and ducks by short-term temperature training in the hatcher. LOHMANN Information **50**, 30-35.

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

NPY and metabolic hormones: programming by short-term temperature training during final embryonic development

S. Tatge[1], **M. Boerjan**[2], **I. Halle**[3], **W. Kloas**[4] and **B. Tzschentke**[1]

[1] Humboldt-Universität zu Berlin, Faculty of Life Science, Department of Biology, Berlin, Germany

[2] Pas Reform Hatchery Technology, Zeddam, the Netherlands

[3] Friedrich-Loeffler Institute, Federal Research Institute for Animal Health, Institute for Animal Nutrition, Braunschweig, Germany

[4] Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

Correspondence: barbara.tzschentke@rz.hu-berlin.de

In Broiler chickens short-term temperature training in the perinatal phase (PTT) improves robustness, which leads to better hatching results, performance and production efficiency. Robust chicks need only small amounts of energy for maintaining of basic body functions. The hypothesis is that chickens experienced during a critical developmental period in the course of final incubation by PTT are life-long featured by a lower basic metabolism (TZSCHENTKE and HALLE, 2016). Under stressful conditions they are able to mobilize more energy for physiological defence and adaptation processes. The following study was performed to investigate the long-lasting effect of PTT on central and peripheral metabolic markers.

Three incubation trials with ROSS 308 broiler chickens were carried out in commercial incubators with a total capacity of 115.200 eggs (setter: SmartSet®) and 6 hatcher sections for each of 19.200 eggs (SmartHatch®, Pas Reform Hatchery Technology, NL). In the control group, the eggs were incubated under the standard single stage incubation programme (36.7°C/98 F, relative humidity setpoint: 50%) until hatching. In the PTT-group the temperature was increased daily by 1°C (from 98 F to 100 F) on embryonic days (E) 17.5 for 2 hrs and on E18.5 and 19.5 for 1 h. Random samples of 120 males and 120 females from the control as well the PTT-group were used for growth trials of 35 days (D) in the experimental research station of the FLI, Institute for Animal Welfare and Husbandry in Celle, Germany. In one trial, on D 35 brains of all experimental groups were collected for immunohistochemical investigation of neuronal neuropeptide Y (NPY) expression, a physiological parameter related to central control of metabolism, feed intake and body weight. In the other two trials, during slaughtering on D 35 blood samples were collected for hormone analysis of Triiodthyronine (T3), Thyroxine (T4).

PTT had no negative effect on hatching results and performance. Rather, it may improve hatching results and performance (growing and feed conversion rate) especially in males. Hypothalamic NPY-expression was statistically significant lower in chickens of the PTT-group, which is related to a lower basic metabolism. This finding corresponds with the higher hormone mobilization (T3, T4) under acute stress and the better feed conversion rate in chickens of the PTT group. It is interesting that the long-lasting effects of PTT are sex specific (see also TZSCHENTKE and HALLE, 2009).

In conclusion, PTT obviously induced long-lasting programming of basic metabolism and can be a useful tool to improve production efficiency alongside with animal welfare in high yielding broiler lines.

Acknowlegement

We thank Kuikenbroederij Munsterhuis b.v. and Pas Reform Hatchery Technology, NL, for realization of the commercial incubation trials.

Key words

Immune response, incubation, metabolic programming, neuropeptide Y, perinatal temperature training

References

TZSCHENTKE, B., I. HALLE, 2009: Influence of temperature stimulation during the last 4 days of incubation on secondary sex ratio and later performance in male and female broiler chickens. Brit. Poult. Sci. **50**, 634-640.

TZSCHENTKE, B., I. HALLE, 2016: Improvement of robustness and performance in meat-type chicken and ducks by short-term temperature training in the hatcher. LOHMANN Information **50**, 30-35.

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Abstracts of poster presentations

The heart rate response to moderate and severe acute hypoxia in the 4-day old chicken embryo

M. Nechaeva[1,2], **T. Alekseeva**[1] and **I. Kubasov**[2]

[1] Inst. of Developmental Biology RAS, Moscow, Russia

[2] Inst. of Evolutionary Physiology and Biochemistry RAS, St. Petersburg, Russia

Correspondence: mnechaeva2003@yahoo.com

The mechanisms of the effect of hypoxia on chick embryogenesis are being intensely studied; however, they remain unclear thus far. Earlier, we showed that acute hypoxia (10% O_2 10 min) inhibited heart rate (HR) on Day 10 (D10) and D14 and found a characteristic hypoxic response: the HR initially decreased and then was partly restored while the hypoxic exposure continued (NECHAEVA et al., 2010). For detailed analysis of this phenomenon, it is necessary to determine at which stage of incubation this pattern appears, how it changes with embryonic age, whether it is expressed if the oxygen level is even lower, and how it is affected by the increase in the duration of the hypoxic exposure. The aim of this study was to describe the time-course of the HR response to acute hypoxia (10% or 5% O_2 for 10 or 20 min) at the earlier stages of chick incubation (D4) *in ovo* and *in vitro* and compare results to estimate the possible role of factors inside the egg in this hypoxic response. At this stage gas exchange occurs only by diffusion and the heart does not yet have neurohormonal regulation.

We investigated the effect of acute hypoxia on the HR using video recording of the beating embryo heart inside the egg and in isolated heart. A digital video camera mounted on a dissecting microscope was used to perform a continuous video recording with a capture rate of 30 fps and data was collected and processed with the software Danio Scope (Noldus). During the experiment, the HR was recorded sequentially in normoxia 30 min, hypoxia 10 or 20 min and then recovery in air during 30 min.

Moderate hypoxia (10% O_2) uniformly decreased HR to about 94% of the control value *in ovo*. Additionally, under severe hypoxia (5% O_2) the inhibitory effect on HR was more pronounced and the time course of the HR response was biphasic: the HR initially decreased to 60% and then was progressively restored under hypoxia to 85% of the pre-hypoxic value. During recovery in air, an overshoot in HR was observed. The similar biphasic effect during hypoxia 5% (but without an overshoot during recovery in air) was found in isolated heart *in vitro*. Consequently, it could indicate that this cardiac hypoxic response on D4 may be the result of the direct action of low oxygen on the cardiac muscle and pacemaker cells at this stage. Some factors inside the egg could be involved in the overshoot observed during recovery in air.

Acknowledgement

This work was supported by RSF Grant №15–15–20008.

Key words

Acute hypoxia, chick embryo, heart rate

References

NECHAEVA, M., I. VLADIMIROVA, T. ALEXEEVA, 2010: Effect of acute hypoxia on the motor activity and heart rate of the 10-and 14-day chick embryo. Open Ornithol. J. **3**, 127-133.

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

The effect of preincubation during egg storage on embryo development of young parent stock

M. Tesarova[1], **M. Lason**[2], **D. Hampel**[1], **M. Foltyn**[1] and **M. Lichovnikova**[1]

[1] Mendel University, Brno, Czech Republic

[2] Wrocław University of Environmental and Life Sciences, Wrocław, Poland

Correspondence: lichovmartina@gmail.com

Freshly laid eggs from parent stock of Ross 308 at the age 31 weeks were used in the experiment. They were divided into five groups; fresh eggs (WP – without preincubation), preincubated once per 4 h (PS1 – one short preincubation), preincubated twice for 4 h (PS2 – two short preincubations), preincubated once per 8 h (PL1 – one long preincubation) and preincubated twice per 8 h (PL2 – tow long preincubation). Preincubated eggs were incubated after 5 and 10 days of storage for 4 or 8 h. In fresh eggs and after each treatment 60 eggs were examined for embryo development. After distinguish the egg status (fertilized or not), blastoderm was isolated and cleaned off the egg-white and yolk, and then analyzed under the stereo microscope and staged according to Eyal-Giladi and Kochav (EGK, Roman numerals) procedure. However not all embryos were successfully isolated. The following numbers of embryos were successfully determined: WP – 23, PS1 – 25, PS2 – 10, PL1 – 20, PL2 – 8.

Because of discrete nature of data with low number of variants, nonparametric Kruskal-Wallis ANOVA with subsequent pairwise comparisons was used for evaluation. Two statistically different groups were detected; the first one was created by WP (median X) and PS1 (median XI), the second one by PL1 (median XII – XIII), PS2 (median XIII) and PL2 (median XIII). It seems that second long term (8 h) preincubation does not significantly improve embryo development. Anyway this should be confirmed by staging higher number of embryos.

Key words

Blastoderm, broilers, embryo staging

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

The effect of preincubation during egg storage on hatchability in Japanese quail

M. Lichovnikova, **J. Freislebenová**, **M. Foltyn** and **D. Hampel**

Mendel University, Brno, Czech Republic

Correspondence: lichovmartina@gmail.com

Long storage of hatching eggs has a negative effect on hatching in Japanese quail. Hatchability is significantly reduced when the eggs are stored longer than 10 days. The hypothesis was that preincubation used in broiler chickens is a method that could reduce the negative impact of long-term storage in the Japanese quail also. Four experiments were done, using one long and three short preincubations. In total one thousand two hundred and ninety eggs of Japanese quail were used. These eggs were divided into preincubated eggs and eggs without preincubation. The preincubation was long (8 h) and short; 3x1 h (including preincubation on the day of egg collection and subsequently every 5th day), 2x1 h (every 5th day of storage) and 3x 1 h (every 5th day during the 21 day storage). The preincubation time was measured from when 35°C was reached on the surface of the eggshell (37.5°C inside the incubator). The eggs were stored at 12.0°C for 14 or 21 days. Embryonal mortality was determined after hatching. Preincubation 8 h had significant negative effect ($P < 0.05$) on hatchability at storage of 14 days; the hatchability was 76.2% in the non-heated eggs and 56.5% in the preincubated eggs. The preincubation for 3x1 h and 2x1 h during the 14 day storage period had positive effect on hatchability, which was improved by 7.1% (preincubation for 3x1 h) and by 4.8% (preincubation 2x1 h), but these positive effects were not significant ($P > 0.05$). In the last experiment, preincubation was applied 3 times for 1 h during the 21 days of storage, and the positive effect of the pre-incubation, which increased the hatching by 0.9%, was observed. This positive effect was also not significant ($P > 0.05$). Long preincubation (8 h) had negative effect on hatchability, however short preincubation (1 h) regardless length of storage had positive effect on hatchability in Japanese quails ($P < 0.05$).

Key words

Egg storage, Japanese quail, preincubation

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Development of the body wall in the chick embryo

Z. Zemanová, **M. Vohníková**, **E. Maňáková** and **L. Hubičková**

Dept. of Histology and Embryology, 3rd Faculty of Medicine, Charles University, Prague, Czech Republic

Correspondence: zemanovazma@gmail.com

During studies of the embryotoxic effects of drugs on the chick embryos we met disruptions of the body wall closure. For this reason we decided to analyse this effect further and to start with getting data on the morphogenesis of the body wall (BW) by studying its histology.

From the literature we know, that the anterior BW in the chick embryo closes gradually, between embryonic days (ed) 5 and 8 (HAMILTON, 1952). It starts when lateral closing folds arise as ridges of the lateral BW, first at their anterior ends. The process of the BW closure was also described in rhodent embryos (MUNGER and MUNGER, 1991), the folds were there described as abdominal bands of mesoderm. They were associated with the development of the segmental spinal nerves and their cutaneous terminal branches. Differentiation of the epithelium was described as the important component of the process.

The aim of this study was to gather further details on the morphogenesis of the BW in the chick embryo: to describe its closure between ed 7 – 9 and to reveal the changes in its histological structure. We used ten chick embryos of this age. We weighted them and fixed them in 4% formaldehyde dissolved in the chick physiological solution (0.8% of NaCl). The trunks were embedded in paraffin, cut to 8 μm thick slides and stained with hematoxylin-eosin.

We assessed, that the BW closure in the chick embryo proceeds cranio-caudally from the sternum to the umbilicus by the union of the left and right side of the thoracic and abdominal folds. The thorax was always closed at the place of the sternum formed by the cartilage tissue. In the abdominal portion of the BW we realized, that at the point of the union the mesenchyme of both sides accumulated and visibly intermingled forming a distinct seam. We could see there at 7 – 8 ed an aggregation of the originally regularly organized layers of the connective tissue (and muscles) of both sides, and also disorganization of the epithelia in the middle of the BW. This appearance changed during ed 9 to the more organized and compact aspect.

We assessed that the process of BW closure depends on the time limited concretion of two components, mesenchymal and epithelial folds. We will study a role of their proliferation. A presumptive participation of the nervous tissue in this process will be in future also examined.

Acknowledgement

The study was supported by the grant 236083/IPUK and Progres Q 16.

Key words

Body wall morphogenesis, chick embryo, left and right abdominal folds, seam at the place of concrescence

References

HAMILTON, H.L., 1952: Lillie's Development of the Chick. H. Holt &Co, ASIN B000KOQHDK, 409-424.

MUNGER, G.T., B.L. MUNGER, 1991: Differentiation of the anterior body wall and truncal epidermis and associated co-migration of cutaneous nerves and mesenchyme. Anat. Rec. **231,** 261-274.

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

Effects of temperature variations during incubation and postnatal growth on performance, metabolism and health of meat-type chickens

A. Collin, **V. Coustham**, **N. Couroussé**, **C. Praud**, **T. Bordeau**, **E. Godet**, **E. Cailleau-Audouin**, **S. Crochet**, **P. Chartrin**, **C. Berri** and **L.A. Guilloteau**

INRA, URA, Nouzilly, France

Correspondence: Anne.Collin@inra.fr

In the context of climate change where farmers have to face the consequences of increasing frequency of extreme thermal events in Northern countries, and of chronic heat in Southern countries, the adaptive capacities of fast-growing chickens are strongly challenged and become a critical issue. One strategy for improving broiler robustness while potentially limiting energy use is the conception of new incubation strategies exposing eggs directly in incubators to environmental fluctuations. This could prepare the developing bird to sustain later thermal fluctuations on farm. However, the consequences of the combination of both heat and cold embryo stimulations on animal physiology, health status and performance remain to be determined in thermally fluctuating or standard rearing conditions. A multidisciplinary approach was applied to evaluate the effects on selected pathophysiological markers of standard conditions or temperature variations during incubation combined with standard rearing conditions or cool chick start followed by late heat exposure. Incubation condition I_1 including elevated incubation temperatures during mid-incubation and cold stimulations at the end of embryogenesis did not affect hatchability as compared to standard incubation conditions I_0. Hatched-chick BW was not different between incubation groups. Postnatal chicken BW was affected by both incubation conditions and postnatal conditions, with lower BW in I_1 group than in I_0, and lower BW in the postnatal variable conditions than in standard conditions. Feed conversion ratio (FCR) was lower in the I_1 treated chickens reared in standard conditions compared to both incubation groups reared in variable postnatal conditions ($P = 0.006$). Chickens exposed to variable conditions were less affected by diarrhea than those in standard conditions at 11 d of age. Mortality was greater in I_1 chicks than in I_0 before 11 d of age ($P = 0.049$), whereas at 35 d, there was no effect of incubation or postnatal conditions on mortality rates. At 41 d, overall mortality and morbidity rates were higher in standard conditions than in variable conditions ($P = 0.046$). Meat quality was affected by both incubation and postnatal conditions: incubation I_1 induced lower values of meat ultimate pH (pHu) measured 24 h after slaughter, but increased drip loss and meat shear force compared to I_0 incubation. Postnatal thermal variations decreased the occurrence of the meat defects white striping and wooden breast, breast yield, drip and cooking loss and yellow color of the meat, but increased abdominal fat percentage, thigh yield, pHu and curing-cooking yield of breast meat. Both factors interacted to affect the lightness of breast meat and its intramuscular lipid content that were lower in I_1 than in I_0 chickens when reared in standard conditions.

Only postnatal conditions significantly affected blood gas and ion concentrations. Exposure to postnatal variable conditions increased plasma uric acid concentrations as well as blood total antioxidant status, but decreased the 'reduced to oxidized' glutathione ratio, suggesting a disrupted redox equilibrium in these conditions as compared to control postnatal conditions.

In conclusion, in broiler chickens postnatal variable thermal conditions and, to a lesser extent, variable incubation conditions, affected health, performance and meat quality criteria. The adaptive capacities and metabolism of fast-growing chickens were strongly challenged by postnatal temperature fluctuations, which were not mitigated by incubation conditions including heat and cold stimulations. However, postnatal varying environment limiting growth reduced mortality and meat quality defects as compared to standard conditions at six weeks of age.

Acknowledgement

This work was supported by a grant from the Integrated Management of Animal Health metaprogram of INRA for the "GISA-ROBUSTCHICK" project (www.gisa.inra.fr/en).

Key words

Incubation, temperature variations, health, performance, adaptation

Europ.Poult.Sci., 81. 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.204

In vitro effects of triiodothyronine and insulin on the metabolism of muscle cells from chickens submitted or not to heat manipulation during embryogenesis

N. Courousse[1], **C. Praud**[1], **T. Loyau**[1,2], **V. Coustham**[1], **S. Tesseraud**[1], **S. Métayer-Coustard**[1] and **A. Collin**[1]

[1] INRA, URA, Nouzilly, France

[2] INRA, URZ, Petit-Bourg, Guadeloupe, France

Correspondence: anne.collin@inra.fr

Broiler chickens are particularly sensitive to heat exposure during growth, with socio-economic consequences for poultry industry. An embryonic heat acclimation technique was developed to improve chicken long-term heat tolerance (PIESTUN et al., 2008). This thermal manipulation (TM) consists of a rise in temperature during the incubation of eggs 12 h/d from day 7 to 16 of incubation. TM was notably shown to change the thyroid axis regulation in the long term. Chickens resistance to an acute heat challenge was improved at slaughter age. The glucose-insulin balance of birds was also modified (LOYAU et al., 2013). The aim of this work was to better understand *in vitro* the involvement of the thyroid hormone triiodothyronine (T_3) and of insulin in the regulation of metabolic pathways affecting *in vivo* energy and protein utilization as well as muscle growth in TM *Pectoralis major* (LOYAU et al., 2014). To identify T_3 and insulin effects in interaction to incubation condition, we measured by qRT-PCR the expression of candidate genes involved in metabolism and muscle growth, in the regulation of muscle thyroid hormone concentrations and of oxidative stress. Signaling pathways regulating protein synthesis were also studied by biochemical method. The study was carried out both at proliferative (myoblasts) and differentiation (myotubes) cell stages in cells derived from muscle *Pectoralis major* of chicks submitted to the embryonic treatment TM or not.

In our study, TM stimulated pathways that promote proteosynthesis and modified a signaling pathway controlling cellular stress (P38 mitogen activated protein kinase). The TM also affected the regulation of energy metabolism. Indeed, the expression of Peroxisome proliferator activated receptor coactivator 1α (PGC-1α) gene involved in muscle cell energy metabolism was increased in TM as compared to control myoblasts.

Apart from the observed effects of incubation conditions, direct effects of hormones were observed. T_3 hormone had contrasting effects on the AKT/S6K1 (S6 kinase 1)/S6 (ribosomal protein) pathway, likely stimulating proteosynthesis in myotubes. It also increased the expression of deiodinase 3 promoting T_3 conversion to T_2 (diiodothyronine). Insulin activated the AKT/S6K1/S6 pathway in both cell types and stimulated the expression of genes involved in the use of energy nutrient such as Citrate Synthase, key enzyme of the Krebs cycle, and β hydroxyacyl-Coa dehydrogenase controlling fatty acid oxidation. Furthermore, insulin interacted with incubation condition to stimulate mitochondrial metabolism via greater PGC-1α expression in TM myotubes only.

In conclusion, the in vitro study of TM-derived muscle cells revealed physiological and metabolic modifications promoting protein synthesis and energy use consecutive to embryo heat acclimation treatment in birds that were maintained through cell mitosis. Our results suggest programming mechanisms controlling gene expression under the effect of environment. We speculate that epigenetic alterations could be involved.

Acknowledgement

This study was performed with the financial support of the French Agence Nationale de la Recherche, «Jeunes Chercheuses et Jeunes Chercheurs» Project, ANR-09-JCJC-0015–01, THERMOCHICK.

Key words

Chicken, embryo acclimation, heat, insulin, in vitro, muscle cells, T_3 (triiodothyronine)

References

LOYAU, T., C. BERRI, L. BEDRANI, S. MÉTAYER-COUSTARD, C. PRAUD, M.J. DUCLOS, S. TESSERAUD, N. RIDEAU, N. EVERAERT, S. YAHAV, S. MIGNON-GRASTEAU, A.J. COLLIN, 2013: Thermal manipulation of the embryo modifies the physiology and body composition of broiler chickens reared in floor pens without affecting breast meat processing quality. Anim. Sci. **91**, 3674-85.

LOYAU, T., S. MÉTAYER-COUSTARD, C. BERRI, S. CROCHET, E. CAILLEAU-AUDOUIN, M. SANNIER, P. CHARTRIN, C. PRAUD, C. HENNEQUET-ANTIER, N. RIDEAU, N. COUROUSSÉ, S. MIGNON-GRASTEAU, N. EVERAERT, M.J. DUCLOS, S. YAHAV, S. TESSERAUD, A. COLLIN, 2014: Thermal manipulation during embryogenesis has long-term effects on muscle and liver metabolism in fast-growing chickens. PLoS One 9(9):e105339. doi: 10.1371/journal.pone.0105339.

PIESTUN, Y., D. SHINDER, M. RUZAL, O. HALEVY, J. BRAKE, S. YAHAV, 2008: Thermal manipulations during broiler embryogenesis: effect on the acquisition of thermotolerance. Poult. Sci. **87**, 1516-1525.