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Association between Metabolites in Egg Yolk and Hatchability Traits in Laying Hens

A. R. Sharifi[1], Y. Zhang[1], S. Weigend[2], A. Weigend[2], R. Preisinger[3], M. Schmutz[3], L. Römisch-Margl[4], R. Jonczyk[4], M. C. Gülersönmez[4], H.P. Piepho[5], W. A. Malik[5], F. Sitzenstock[6] and H. Simianer[1]

[1]Georg-August-University Goettingen, Germany, [2]Institute of Farm Animal Genetics of the Friedrich-Loeffler-Institute, Neustadt-Mariensee, Germany, [3]Lohmann Tierzucht GmbH, Cuxhaven, Germany, [4]Technical University Munich, Germany, [5]Hohenheim University, Germany, [6] Osnabrueck University of Applied Sciences, Germany

ABSTRACT: A total number of 86'899 hatching eggs were collected from 4 different lines consisting of white- and brown laying stock from selected commercial populations and experimental lines. Heritability and breeding values were estimated for early, medium, and late embryonic survival ability and hatchability. The estimated heritabilities were low and ranged from 0.029 to 0.188 for different traits. Based on estimated breeding values, 2'082 yolk sample from 732 high and low hatching commercial hens and 495 yolk samples from 171 hens from experimental lines were sampled to determine metabolite profiles using gas chromatography–mass spectrometry. A total number of 109 different metabolites known in egg yolk, including fatty acids, amino acids carbohydrates, steroids, glycerides, vitamins, and organic acids, were detected. Using association analysis, metabolites of different components were identified which have a significant influence on embryonic survival ability.

Keywords: Laying hens; Metabolites; Hatchability

Introduction

According to the Food and Agriculture Organization (FAO, (2013)) production data base, the total number of chickens for meat production in the world was 59.7 billion in 2012, and the total number of hens for egg production was estimated to be 6.8 billion. Improving hatchability by even 1 percent would result in enormous savings of biomass, costs of energy and waste disposal and also would be a major improvement from the animal welfare point of view. Therefore, fertility and hatchability are important traits in breeding programs and have a substantial economic relevance for franchise hatcheries as well. Infertility and embryonic mortality play an even more important role in the broiler industry because of their higher demand for day–old chicks. Furthermore, a high reproductive ability of chicken leads to higher genetic progress of desirable traits in the nucleus and speeds up the transfer of genetic improvement from nucleus into production (Bennewitz et al. (2007)). Commonly, about 8% of broiler embryos die before hatching each year; the value is much higher in turkeys with up to 15 % of fertile eggs (Beaumont et al. (1997)). Nearly the same values are reported for commercial laying hybrids (Ladislav et al. (2003)). As a complex trait, embryonic viability is influenced by a series of factors, such as nutritional deficiencies, health of breeder flock, hatching technology, egg quality and genetics (Liptoi and Hidas (2006)). The frequency of embryonic death is not equally distributed over the course of incubation – one can distinguish three distinct stages: early embryonic mortality (EEM) occurs during the first week of incubation, middle embryonic mortality (MEM) occurs after the first week of incubation and before transfer of the eggs into hatcher, and late embryonic mortality (LEM) occurs between the 18th day of incubation and hatching (Beaumont et al. (1997)). Embryonic mortality is highest during the first and the thirds phase (Kuurman et al. (2001)). The phases of embryonic mortality are associated with major changes in the metabolism of embryos influencing their survival. The main goal of this study was to determine metabolite profiles in the egg yolk and to assess possible associations with embryonic mortality.

Materials and Methods

Animals and data. In the first step hatching eggs were collected from 4 different full-pedigreed pure lines consisting of white and brown laying stock from selected commercial populations (Lohmann, company) and experimental populations (Friedrich-Loeffler-Institut, Germany). 42'688 eggs from 3319 commercial white laying hens, 38'228 eggs from 2909 commercial brown laying hens, 2903 eggs of 99 experimental white laying hens (WL) and 3080 eggs of 100 experimental brown laying hens (BL) were used. The eggs were collected daily and incubated after a storage time of 8-19 days. For analysis of reproductive traits and estimating of genetic parameters, eggs that did not show living embryos at candling as well as eggs that had not hatched at the end of the hatching period were opened and examined macroscopically to assess the true fertility and to estimate the time of embryonic death (EEM, MME and LEM) using the method of Hamburger und Hamilton (1951). In the second step, 1069 yolk samples of 380 WL and 1014 yolk samples of 350 BL hens of commercial lines with low and high breeding value for hatchability were collected to generate metabolite profiles. In the case of experimental lines, 257 yolk samples of 90 WL and 238 yolk samples of 81 BL regardless of their breeding value were collected. Single egg yolk samples from the same hen (from 2 to 4 egg yolks) were extracted and pooled. Per sample three aliquots were used separately for detecting different metabolic components via gas chromatography–mass spectrometry (GC-TOFMS). Peaks were identified and quantified using ChromaTOF Software, version 4.5 (Leco Instruments, Mönchengladbach, Germany. xcms and CAMERA open source softwares (Tauterhahn et al. (2008); Kuhl et al. (2012)), NIST 11 Mass Spectral Library (Scientific Instrumental Services, USA), FAMEs Fatty Acid Me-

thyl Esters: Mass Spectral Database (Wiley, UK, (2011)) and the Golm Metabolom Database (Kopka, (2005)) and used as metabolite profile values (Lisec et al. (2011)).

Statistical analyses. Estimation of variance components was carried out using univariate repeatability animal models with logistic link functions including poultry house, tier, site, lane and hatch number as fixed effects and random effect of animal and permanent environment using ASREML (Gilmour et al. (1998)). Heritabilities and repeatabilities were calculated using the variance on the logit link scale. This implies a correction of the residual variance by a factor of $\pi^2/3$ (Southey et al. (2003)). The metabolite data were normalized and log transformed and analyzed according to the p-rep design used for metabolite profiling (Williams et al. (2011)) with blocks corresponding to days. The model included the lines (commercial vs. experimental), egg color (WL vs. BL.), and breeding value (high vs. low, or as regression) as fixed factors. The effects related to environment of hens, i.e. house lane, side and tier were taken as random. The effects related to the laboratory i.e., block (day) and position of analysis of the sample, were considered as random and covariate, respectively. The Kenword-Roger method (1977) was used for adjusting the denominator degree of freedom for testing fixed effects.

Results and Discussion

1 summarizes the descriptive statistics for different reproductive traits. As expected, the proportion of embryonic mortality is distinctively higher during first and late stage compared to MEM. A number of factors affecting embryonic survival ability in the early stage of embryonic development such as genetics and nutrition, age of hens, time of oviposition, egg weight and quality and length of storage are described and summarized by Christensen (2001). The high embryonic mortality in this stage is partly associated with longer storage of hatchings eggs which should be considered as challenge situation.

Table 1. Hatchability traits and percent of embryonic mortality of unhatched eggs for different stages of development.

	Reproductive traits				
Lines	HAE	HFE	EEM	MEM	LEM
WL C	65.0	67.0	9.0	2.5	21.0
BL C	72.3	76.2	8.9	1.8	13.1
WL E	74.9	77.7	8.8	3.0	10.5
BL E	79.2	82.8	9.9	1.1	6.1

WL: white layers, BL: brown layers, C: commercial, E: experimental
HAE: Hatchability of all eggs set,
HFE : Hatchability of fertile eggs,
EEM: Early embryonic mortality of fertile eggs,
MEM: Middle embryonic mortality of fertile eggs,
LEM: Late embryonic mortality of fertile eggs.

In the first week of incubation the oxygen supply is limited due to the underdeveloped chorionic vascular system, so that the metabolic energy is provided by anaerobic glycolysis. At the beginning of the second stage the chorioallantois and the vascular system is fully developed assuring oxygen and carbon dioxide exchange. This embryonic stage is characterized by rapid growth of the embryo and its completion. Fatty acid from the yolk is the most important energy source for completion of the embryo in its final form (Moran (2007); de Olivera et al. (2008)). At end of these phases the maximum of chorioallantois respiration capacity is reached, defining the plateau stage of oxygen consumption. During the plateau stage after day 15 of incubation, the energetic metabolism switches from using yolk fat to carbohydrate.

The last stage is characterized by dramatic metabolic changes as well as a series of physiological events such as initiation of pulmonary ventilation, external pipping and hatch from the shell. Once oxygen is supplied by external piping energy will be provided by fatty acid β-oxidation. Yolk lipids are a major source for energy using fatty acid oxidation and are the predominant metabolite feature of the embryo. During embryogenesis complex nutrients including carbohydrate, fat and protein will be mobilized from the yolk sac and converted into simple products in the liver and transported to different embryonic tissues (Pulikanti et al. (2010)).

These processes are determined by nutrient profiles of the egg, which in turn are influenced by environmental and genetic factors and could affect the embryonic survival ability. Estimated genetic parameters for hatching traits are presented in Table 2. The estimated heritability for different reproductive traits in this study was on a low level in general, but in the usual range for fitness traits. Beaumont et al. (2007) found heritabilities for 3 different stage of embryonic mortality of 0.09, 0.07 and 0.05, respectively, obtained from the sire component in a multivariate sire model. The estimated heritability obtained from the dam component was distinctively higher (0.25, 0.20 and 0.18). This result reveals that there is a possibility for genetic improvement of hatchability traits through maternal effects.

Table 2. Estimation of heritability for hatchability and for different stage of embryonic mortality.

	Reproductive traits				
Lines	HAE	HFE	EEM	MEM	LEM
WL C	0.052	0.049	0.045	0.030	0.060
BL C	0.044	0.051	0.029	0.012	0.089
WL E	0.052	0.049	0.045	0.146	0.060
BL E	0.079	0.055	0.103	0.188	0.017

abbreviations: see table 1

A total number of 254 different metabolites in egg yolk were detected. However, a substantial number of the detected metabolites (145) could not be assigned to any known metabolites in the compound libraries used in this study and there is a need for further investigation and determination of their physiological functions.

The 109 remaining known metabolites belong to different compound families, including fatty acids (35), amino acids (25), carbohydrates (11), steroids (12), glycerides (8), vitamins, (7) and organic acids (2).

The results of the association analysis for different important metabolites with hatchability traits are shown in Table 3. Glucose provides the highest amount of energy during the early and plateau stage of development. It is hardly present in eggs. Gluconeogenesis is a major pathway for producing glucose from other carbon rich components such as amino acids and glycerol. A significant association between late embryonic survival ability and myristic acid, stearic acid, oleic acid and linoleic acid was determined by Yilmaz and Sahan (2009). In the present study, a significant relation was observed between downstream fatty acids such as 11.14-eicosadienoic acid and eicosapentaenoic acid and embryonic survival ability. Egg yolk cholesterol level is the most critical factor for the development of the embryo. The effect of cholesterol content on hatchability is not certain; however, there is a certain level of cholesterol required in the embryo and attempts to lower this level would result in reduced hatchability (Yilmaz Dikmen and Sahan (2007)).

Table 3. Results of association analysis of metabolites profiles and hatchability traits.

Metabolite	Type	Line	Trait*
Eicosadienoic acid	FA	BL C	HAE, HFR
		BL E	EEM, LEM
		WL E	
Fructose	CH	BL C	HAE, HFR
		BL E	EEM, LEM
Eicosatrienoic acid	FA	BL C	HAE, LEM
		BL E	
		WL E	
Methionine	AA	BL C	HAE, HFR
		BL E	LEM,
Cholesterol	ST	WL C	HAE, HFR
		BL E	EEM, LEM
Eicosenoic acid	FA	BL C	HAE, HFR
		WL C	EEM, LEM
		BL E	
Glycerol	CH	BL C	HFR, EEM
		BL E	LEM
Octadecatrienoic acid	FA	BL C	HAE, HFR LEM
Docosapentaenoic acid	FA	WL C	HAE, HFR
		BL E	EEM
Eicosapentaenoic acid	FA	BL E	HAE

* associations were significant at $p < 0.05$,
FA: Fatty acid,
CH: Carbohydrate,
AA: Amino acid,
ST: Steroid.
further abbreviations: see Table 1

Conclusion

The results of this study show that estimated heritability of the reproductive traits in question was low but in the usual range for fitness traits. Taking into consideration the very high reproductive ability and very short generation interval in chicken, it is feasible to select animals for improved hatchability through maternal effects. Significant associations between different metabolites and hatchability were detected. In further steps the current information will be used for detecting pathways and implementing a research plan for gene expression analysis. Additionally, the detected metabolite profiles will be applied in combination with genomic data for further association studies.

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