ABOUT CLUSTER SYSTEM OF PHOSPHOLIPIDS IN ONTOGENESIS OF BROILER CHICKENS

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Abstract

Individual growth and development is a gradual vector process. Each period of ontogenesis is characterized by certain morphological and functional changes in organs, systems and the organism as a whole. Organic and functional transformations are genetically based and realized in the course of adaptation to the external environment factors. The transformations occur at different levels of which the intracellular level is the basic one. As far as biosynthetic processes and circulation of metabolites are separated, an occurrence of some elements for their regulation seems to be possible. For instance, structural cell components probably can be most effective regulators due to ability to contribute to morphological and functional integrity in cell. These structural elements are both the product and a regulator of metabolism directly reflecting state of all intracellular events. Membrane-cell response is a key element of molecular regulation. Phospholipids, the main components of cell membrane, are extremely sensitive to external and internal factors, being at the same time relatively stable due to genotype effect and adaptation ability. Participation and the role of subclasses of phospholipids in the functions of broiler chicks are little known. In this regard we have carried out the study of phospholipids profile of ISA-15 Hubbard F15 chicks during ontogenesis, particularly in eggs before and on day 10 of incubation, and in blood serum of chicks at postnatal period on days 1, 7, 23 and 42. The experimental chicks were kept at a poultry farm in Chelyabinsk Province. To establish the functional groups of subclasses of phospholipids in the ontogeny of broilers we used the multivariate cluster analysis. It was shown that in egg before incubation the phospholipids were grouped into two separate clusters (phosphatidylcholines and cerebroside, Euclidean distance 1.08; and phosphatidylethanolamines, Euclidean distance 1.61) and one joint cluster (phosphatidylinositols with sphingomyelin and lysolecithin with cardiolipin, the Euclidean distance of 0.23). On the day 10 of incubation there were two joint clusters (lecithins with cephalins, the Euclidean distance of 1.61; phosphatidylinositols, sphingomyelins, cerebroside and lysolecithin, Euclidean distance of 2.06) and also a transitional group (cardiolipins). During postnatal ontogenesis in 1-day old chickens three groups of phospholipids were found (lecithins, the Euclidean distance of 2.07; phosphatidylethanolamine with the cardiolipin, the Euclidean distance of 0.26; lysolecithin), while in 7-day old chicks there were two combined clusters (phosphatidylcholines, the Euclidean distance of 2.03; a complex of cephalins with phosphatidylinositol, the sphingomyelin and lysophosphatidylcholine) together with an intermediate cluster (cardiolipin). In 23-day old broilers three clusters of phospholipids were found (lecithins; phosphatidylethanolamines with the phosphatidylinositol; cardiolipin together with the lysolecithin and sphingomyelin). In 42 day aged broiler chickens the presence of two functional groups of phospholipids were revealed, namely a combined one (cephalins with the cardiolipin, phosphatidylinositol, sphingomyelin and lysolecithin) and monocomponent one (phosphatidylcholine). Thus it allows characterizing phospholipids as agents possessing structural and functional organization, which presumably can mediate regulation of homeostasis in early ontogeny of broiler chickens at cellular level, and as a result, at the body level.

Keywords: phospholipids, ontogeny, metabolism, homeostasis, functional system, broiler chickens.

Ontogenesis is considered as a combination of interconnected growth and development processes [1, 2]. The body is an open system [1, 2] responding to external stimuli, in which metabolism of substances necessary to maintain vitality, growth and development involves both extraneous and assimilable nutritive, plastic and energy substrates and reused compounds formed after the degradation of previously synthesized structures. The dynamic balance of the body internal

environment is ensured via homeostasis [1, 3, 4]. The stress of the functions maintaining homeostasis limits metabolic resources which the body can allocate to development [1-3]. In other words, ontogenesis and metabolism are closely linked with homeostasis, and all of them are assumed to have a common regulation system.

Phospholipids are one of the key structures and metabolites of the body ensuring its functioning throughout the entire life [3-12]. Subclasses of phospholipids, the glycerophospholipids [13] and sphingophospholipids [14], are the elements directly combining the metabolism of lipids, proteins and carbohydrates [2, 3, 15-17]. They define all the receptor reactions [1, 6, 11, 18, 19] and participate in the processes of adaptation to changing environmental conditions at the membrane level in all the systems of the body [5, 10, 11, 13, 14] specifically through preservation of the spatial asymmetry of plasmalemma bilayer [20, 21], changes in the phospholipid composition in the membranes of myocytes in the skeletal muscles, which, e.g., result in hypersthenia in response to the increased physical activity [22]. In other words, phospholipids can be considered as a link in the system of components maintaining homeostasis [4, 23, 24].

The purpose of this work was to study the arrangement of functional groups of phospholipids participating in ontogenesis in broiler chickens.

Technique. The experiments were conducted in 2010 at Chebarkulskaya poultry farm (Chebarkulskaya Ptitsa LLC, Chelyabinsk region). The subjects of study were eggs and broilers of ISA-15 Hubbard F15 cross. The chickens were kept in cages in the grow-out house. They were fed and kept in accordance with the technology requirements and standards recommended by the All-Russian Research and Technological Institute of Poultry [25] and I.S.A. (Institut de Sélection Animale, France) [26]. Four balanced bird groups of different age (1, 7, 23 and 42 days) were formed for the experiment ($n = 10$ each). The blood samples were taken at the moment of bird decapitation at the age of 1 and 7 days and intra vitam from the jugular vein of 23-day-old and 42-day-old chickens.

The phospholipid composition of egg yolk was examined before the incubation and on day 10 of the incubation. Sample preparation included homogenization of the whole content of the yolk and tissues of the embryo. The homogenate of the yolk and embryonic tissues as well as the blood serum were analyzed for the content of phospholipid fractions using thin-layer chromatography on Silufol plates (Kavalier, Czech Republic) [27].

To determine functional groups of phospholipids, as well as to study the functional structure of their clusters during the growth and development of individual broilers, we used multivariate exploratory techniques. Clusters were analyzed [28, 29] using professional software Statistica v. 8.0 (2007) [28]. The phospholipid subclasses by functional groups were identified using hierarchical agglomerative method of minimum dispersion, the «joining (tree clustering)» [28, 29]. The euclidean distances between subclasses were calculated [28, 29]. Clustering was performed according to the weighted pair-group average method [28, 29]. Functional groups of phospholipids in ontogenesis were identified using «two-way joining» method applied to the detected subclasses taking into account some variables (periods of individual growth and development of birds) [28, 29].

Results. The anabolism and catabolism in the bodies of broilers, i.e. the processes of growth, development, decomposition and renewal of structures, are highly intense [30]. This, in turn, may impact the most sensitive organization level, the membrane level, where the so-called phospholipid clusters can reflect the vector of metabolism and the adaptive reactions. Being basically regulatory formations [13, 14], they contribute to the changes in the modality of functions of the structures and metabolites associated with them, such as membrane pro-

teins, lipo- and glycoproteids, lipoproteins [11, 17, 21]. Enhancing the efficiency of their interactions, phospholipids increase functional thresholds and as a result can extend the limits of the relative dynamic stability of the body internal environment [17, 31].

Therefore, complementarity, synergy and feedback regulation can be considered as principles of structural and functional organization of the phospholipid system in ontogenesis of broilers. Complementarity means that phospholipids supplement each other's action and can create a complete system. Synergy makes it possible to mutually increase or decrease the effects of phosphatides. Feedback principle is achieved based on the ability of blood phospholipids to regulate the amount and activity of metabolites and the condition of the membrane structures, which, in turn, defines the concentration of phosphatides in blood.

In the early periods of prenatal ontogenesis, at the stage of a zygote (an egg), phospholipids are grouped into three clusters: the first one includes phosphatidylcholines and cerebroside, the second one includes phosphatidylethanolamines, and the third twin cluster includes phosphatidylinositols with sphingomyelins, and lysolecithins with cardiolipins (Fig. 1, 2, Table 1).

Fig. 1. Clustering of phospholipids at different ontogenesis stages in ISA-15 Hubbard F15 broilers: A — prenatal period, B — postnatal period; PhCh — phosphatidylcholines, Cer — cerebroside, PhE — phosphatidylethanolamines, PhI — phosphatidylinositols, SphM — sphingomyelins, LL — lysolecithins, CL — cardiolipins (Chebarkulskaya Ptitsa LLC, Chelyabinsk Province, 2010). The minimum dispersion method applied; clustering was based on the weighted pair-group average method including calculation of euclidean distances.

Fig. 2. Color scheme of phospholipid clustering at different stages of ontogenesis in ISA-15 Hubbard F15 broilers: A — prenatal period, B — postnatal period; a — before the incubation, b — in the middle of incubation (day 10); PhCh — phosphatidylcholines, Cer — cerebroside, PhE — phosphatidylethanolamines, PhI — phosphatidylinositols, SphM — sphingomyelins, LL — lysolecithins, CL — cardiolipins (Chebarkulskaya Ptitsa LLC, Chelyabinsk Province, 2010). The two-way joining method applied. The spectral gradations indicate each separate subclass of phospholipids in the structure of the identified clusters.

We can explain the formation of these phosphatide groups as follows. Lecithins and cerebroside support functional, structural and metabolic devel-

opment and growth of an embryo, being actually the primary vital phospholipids [3-9, 11, 12]. Phosphatidylcholines are a major component of all membrane structures [3, 32, 33] and high density lipoproteins, which serve as plastic and energy substrates in embryogenesis [3-5]. Cerebrosides are the structural foundation of the nerve tissue and the future nervous system [3-5]. Similar to lecithins, they have a high affinity for cholesterol, fatty acids [3, 32, 34], defining the adaptation and stabilization of cellular membranes to the environmental changes. Finally, cerebrosides act as triggers and regulators of all embryogenesis processes at the molecular level including via interaction with regulatory proteins [9, 23, 24, 35-40].

1. Euclidean distance on the dendrogram representing phospholipid clustering in ISA-15 Hubbard F15 broilers during prenatal ontogenesis (Chebarkulskaya Ptitsa LLC, Chelyabinsk Province, 2010)

Parameter	PhCh	PhE	PhI	LL	CL	Cer	SphM
Phosphatidylcholines (PhCh)	0.00	1.61	2.78	3.06	2.90	1.08	2.71
Phosphatidylethanolamines (PhE)	1.61	0.00	1.22	1.49	1.32	1.22	1.16
Phosphatidylinositols (PhI)	2.78	1.22	0.00	0.30	0.23	2.06	0.06
Lysolecithins (LL)	3.06	1.49	0.30	0.00	0.19	2.36	0.36
Cardiolipins (CL)	2.90	1.32	0.23	0.19	0.00	2.24	0.26
Cerebrosides (Cer)	1.08	1.22	2.06	2.36	2.24	0.00	2.00
Sphingomyelins (SphM)	2.71	1.16	0.06	0.36	0.26	2.00	0.00

In turn, cerebroside activity is regulated (via feedback) by lecithins through diglycerides as intermediate links of the cycle [3]. Together with phosphatidylcholines, cerebrosides function as atheroprotectors and antitumor agents [9, 41, 42].

The main phospholipids of the nerve tissue, phosphatidylinositols and sphingomyelins [3, 5, 20], serve as predecessors during the formation of the humoral and neurohumoral regulation systems in a developing embryo [3, 19, 43-45]. Thus, phosphatidylinositols are the leading donors of diglycerides and fatty acids for the synthesis of eicosanoids including prostaglandins [3, 43]. Sphingomyelins form all the membranous and fiber neutral structures [3, 41, 42].

In bird embryogenesis, lysolecithin and cardiolipin are responsible for metabolite circulation of associated phospholipids (lecithin, phosphatidylethanolamine), as well as for etherification of cholesterol and fatty acids. The phosphatidylethanolamine acts as an independent component of membrane structures and, at the same time, as a necessary communication link between other phospholipids at the early stages of prenatal broiler ontogenesis.

On day 10 of embryogenesis (the middle period of incubation), phospholipid groups consolidated into larger clusters (see Fig. 1, 2, Table 1), which we can explain by the formation of more complicated systems in the developing and growing embryo in general. This might also be due to considerable consumption of plastic and energy substrates, and further functional induction of phospholipid groups at this stage of prenatal ontogenesis. Together with cephalins, lecithins formed the first cluster. The second cluster included phosphatidylinositols, sphingomyelins, lysolecithins and cerebrosides. In chickens these processes were apparently related to the active histogenesis and the beginning of organogenesis with the minimization of expenditures of the resources in use.

In metabolism, phosphatidylcholine and phosphatidylethanolamine affect each other and are interrelated with the circulation and functioning of all forms of cholesterol, fatty acids and their derivatives — a number of biologically active substances [2, 3]. Cardiolipin takes a transitional position between the first and second clusters.

On day 1 of postnatal chicken ontogenesis, phospholipids of blood were grouped in three clusters (see Fig. 1, 2, Table 2). Phosphatidylcholines as well as

phospholipids, the most involved in the vital processes substances, formed an independent group.

Cardiolipin was combined with phosphatidylethanolamine, which we can explain primarily by the intensity of fat metabolism and its high energy demand. The group of phosphatidylinositol, sphingomyelin and lysophosphatidylcholine was stable. Lysolecithin mainly occupied intermediate position between the groups of cephalin—cardiolipin and sphingomyelin—phosphatidylinositol (see Fig. 1, 2, Table 2).

2. Euclidean distance on the dendrogram representing phospholipid clustering in ISA-15 Hubbard F15 broilers during postnatal ontogenesis (Chebarkulskaya Ptitsa LLC, Chelyabinsk Province, 2010)

Parameter	PhCh	PhE	PhI	LL	CL	SphM
Phosphatidylcholines (PhCh)	0.00	2.07	2.56	2.50	2.03	2.60
Phosphatidylethanolamines (PhE)	2.07	0.00	0.54	0.50	0.26	0.62
Phosphatidylinositols (PhI)	2.56	0.54	0.00	0.28	0.59	0.22
Lysolecithins (LL)	2.50	0.50	0.28	0.00	0.55	0.23
Cardiolipins (CL)	2.03	0.26	0.59	0.55	0.00	0.69
Sphingomyelins (SphM)	2.60	0.62	0.22	0.23	0.69	0.00

In the 7-day-old birds, phosphatidylethanolamines were actually grouped together with phosphatidylinositols, sphingomyelins and lysolecithins. Cardiolipin occupied a separate intermediate position between the stable independent cluster of lecithins and the group of cephalins, phosphatidylinositols, sphingomyelins and lysophosphatidylcholines (see Fig. 1, 2, Table 2).

Nevertheless, we observed a trend towards cephalins aggregating with phosphatidylinositols into a group, which formed a separate functional cluster of phosphatidylethanolamines and phosphatidylinositols in the 23-day-old chickens. This can be explained by active intracellular metabolism of lipoproteins governed by the signal regulation via specific proteins and diglycerides with the participation of phosphatidylinositols. Cardiolipin joined the group of sphingomyelin and lysolecithin (see Fig. 1, 2, Table 2).

The highest consolidation of functional groups of phospholipids over the entire examined postnatal period was observed in the 42-day-old broilers. Phosphatidylcholine clustered in the first group; cardiolipin, phosphatidylinositol, sphingomyelin and lysolecithin clustered in the second group. Phosphatidylethanolamine came close to the second group (see Fig. 1, 2, Table 2). Such clustering can be explained by the ongoing active development of separate groups of skeletal muscles and the cardiovascular system, as well as by the stabilization of metabolic processes to maintain normal functioning of the liver and other internal secretion glands in the conditions of intense synthesis of proteins necessary during the hypertrophic formation of skeletal muscles.

In general, we observed a trend towards a certain retrograde reverse symmetry, i.e. the cluster system of phospholipids typical for the successive periods of broiler growth and development in postnatal ontogenesis may repeat the stages of the same system in the previous periods of prenatal ontogenesis but in the reverse order.

Thus, pre- and postnatal ontogeneses of broilers demonstrate structural and functional organization of phospholipids. Phospholipid subclasses form a functional system, which is probably inherited, is shaped by the action of endogenous and exogenous factors and is used to implement adaptation mechanisms to maintain homeostasis and preserve vital functions of the body.

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