

# Clinical Diagnostics of Adaptive Resources of the Broiler Chicks' Organism

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## Abstract

The article considers the issues of adaptive resources of broiler chicks under the industrial production conditions. A method of clinical diagnostics of adaptive resources of broiler chicks' organism is proposed on the basis of the aggregate calculation and interpretation of LipoProtein Index (LPI) and Phospholipid Index (PI) when determining the intensity of the shunt metabolic processes and functional assistance of proteins and lipids in relation to body weight gain and viability in the technological environment. The use of LPI in determining the intensity of metabolism and assessment of body weight gain in broiler chicks will allow controlling the trend and rate of metabolism in chicks during the growing period for meat that can be used for timely adjustment of feed rations, informative clinical examination and increase, thereby, the profitability of the poultry farm. Phospholipid index, together with the lipoprotein index, allows characterizing the physiological condition of chicks during rearing for meat and produce timely adjustment of parameters of the applied feeding and maintenance technologies. Besides, these indices may also be used in the breeding work for the selection of chicks with a high metabolism, and respectively, accelerated growth of body weight and high viability. Thus, in the aggregate, lipoprotein index and phospholipid index allow diagnosing the adaptive resources of the broiler chicks' organism under the technological environment conditions that is based on the physiological relationships of protein and lipid metabolites at the heart of functional systems of metabolism providing maintenance of homeostasis.

**Keywords:** Adaptive Resources, Broiler Chicks, Homeostasis, Lipoprotein Index, Metabolism, Phospholipid Index

## 1. Introduction

Adaptation of animals' organism to environmental factors, as the most labile function, is due to genetic potential, which can serve basis to manifest adaptive resources<sup>1-42</sup>. Thus, the functional activity is based on plastic and energy components of metabolism<sup>15,18</sup>. Biochemical and physiological interactions of metabolites is structurally interrelated and cyclical, providing at a given molecular membrane level of life organization primary links of adaptogenesis, which is based on the lipoproteins and lipid-membrane elements.

Thus, as far back as<sup>42</sup> noted that cyclical joints, i.e. metabolites shunts are correlated spatially and temporarily, and the range of variability of the transformations in

the synthesis and catabolism of substance defines, as a result, the span of adaptability of metabolism to changing conditions, and as a consequence, the maintenance of homeostasis. In this context, the broiler chicks (*Gallus gallus*) are of interest to model reactions of adaptogenesis under artificial conditions of controlled growth and development<sup>38</sup>. Indeed, it is programmed constitutionally and genetically that the predominant vector of broilers development is directed to formation of skeletal muscle, which is reflected in the increment of body weight<sup>39</sup>. At the same time, directed accelerated development of the chicks' body leads to the maximum activation of energy and plastic resources, which are actually spent on both the formation of body weight and adaptive response of the organism to the intense factors of the living environ-

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ment<sup>38,40,41</sup>. Indeed, the protein and fat metabolisms are the most intense metabolisms in the organism of broiler chicks<sup>1</sup>.

Proteins and lipoproteins ensure the formation of both the body and muscle mass; at that, muscle hypertrophy is compensated by increased loads on the cardiovascular system that inevitably affects the viability of broilers<sup>2,7,35</sup>.

Phospholipids, as an essential class of lipids, regulate tissue and organ building by proteins at membrane-hormonal and membrane-enzyme levels, as well as are involved in the relationship of fatty acids in the blood system, and provide interconnection of neutral fats and cholesterol with the globulins and albumins, thus defining synthetic and structural functions of lipoproteins<sup>8,17,18,25</sup>. Besides, phospholipids are signal markers for hormonal and enzyme systems in response to changes in homeostasis at the molecular-membrane, cellular and systemic levels<sup>22,23,32</sup>. Thus, the protein and lipid elements are the sensitive parts of the metabolism against the effects of endogenous and exogenous factors and are the basis of functional adaptive resources in the body of broiler chicks under the conditions of technogenic environment.

However, little is known about the range of adaptations of broiler chicks' metabolism during growth and development in artificial controlled conditions of industrial production of poultry meat.

In this regard, the aim of our work was the development and clinical testing of the method for clinical diagnostics of adaptive resources of the broiler chicks' organism based on studying the intensity of the shunt metabolic processes and functional assistance of proteins and lipids in relation to body weight gain and viability in the technological environment.

## 2. Materials and Methods

All the experiments were carried out according to the norms of humane treatment of animals. The experimental part of the work was performed at the poultry farm in the Chelyabinsk Region, Russia. The broiler chicks of Hubbard F15 cross, housed (cage housing) in cultivation section of the farm, served the objects of the study. They were split into 4 groups, 10 chicks in each group, in accordance with the principle of balanced groups, (n=10, the age of the chicks in groups was P1, P7, P23, P42 (days of postnatal ontogenesis). Feeding and stock-keeping of the experimental poultry was carried out in accordance with hygiene standards recommended by the All Russian

Institute of Poultry Husbandry (Russia)<sup>38</sup>. Research material served blood, which was obtained by decapitation of chicks at the age of P1 and P7 in accordance with the principles of humanity, set out in the directives of the European community (86/609/EEC) and the Declaration of Helsinki, as well as in vivo, by puncturing of jugular vein in P23 and P42 chicks, employing vacuum technique. The serum was analyzed in terms of the content of non-esterified fatty acid (UFA), esterified cholesterol (CE), triacylglycerides (TAG), total phospholipids (PL) and the content of phospholipids fractions by thin-layer chromatography on Silufol plates<sup>9,13</sup>. Globulins fractions (Glb) and albumins (Alb) were detected employing block type electrophoresis method, i.e. vertical electrophoresis disc in polyacrylamide gel<sup>9</sup>. Increase of body mass (in grams) was calculated for the whole cultivation plant in accordance with the chicks' age. Survivability of chicks was assessed by considering the death loss in the appropriate bird-cage. LPI and PI were calculated by appropriate formulas based on the results of the study. Measure of discrepancy and statistical significance of the obtained results were calculated using Student's t-test, employing the STATISTICA program, version 8.0<sup>11</sup>. The level of significance of value differences was assumed to be 0.05.

## 3. Results and Discussion

The target goal of the research was achieved by determining proper indices on the basis of functional and regulatory reserve activity levels of the organism during growth and development of broiler chicks in experimental groups, namely, at the molecular-membrane and systemic levels we calculated the PI, which reflects phospholipid homeostasis, and then, involving the cellular, tissue, organic and systemic levels, we determined LPI, characterizing lipoprotein balance. In the aggregate these indices allow diagnosing the adaptive resources of the broiler chicks' organism in terms of the technological housing environment (Table 1, 2, Figure 1). Thus, LPI (Table 1) was calculated on the basis of measured values of the concentrations of non-esterified fatty acid, globulins and esterified cholesterol, triglycerides, phospholipids and albumin in serum.

LPI characterized the metabolic rate, and consequently, the level of increase in body weight of chicks by means of detecting the physiological correlations and functional relationships of protein and lipid metabolites.

Lipoprotein index (LPI) was calculated by the formula (arbitrary units):

$$\text{LPI} = \frac{\text{UFA} \times \text{Glb}}{(\text{CE} + \text{TAG} + \text{PL}) \times \text{Alb}} \times 100 \quad (1)$$

where CE, TAG, PL, UFA, Glb, Alb are concentrations of the esterified cholesterol, triacylglycerides, phospholipids, non-esterified fatty acids (mmol/l); globulins and albumins (g/l) in the serum of broiler chicks, respectively; 100 – is the correction factor.

The physiological meaning of this index was defined as follows. Proteins of animals' blood plasma metabolize in close association with lipids<sup>3-5,8,10,14-16</sup>. The main substrate-energy serum complexes are lipoproteins<sup>3,8</sup>. These complexes include molecular protein fractions and fats classes, which are conjugated structurally and functionally in the course of metabolism. The lipids form with proteins metabolites, which are different in terms of molecular weight, density, spatial configuration, and accordingly the physiological load<sup>3,8,10</sup>. Their basis consists of a serum albumins complementary to non-esterified fatty acid (UFA), as well as globulin proteins, associated with lipids, such as cholesterol, triglycerides, and phospholipids. They form lipoprotein structures<sup>5,8</sup>. Normally, phospholipids and cholesterol mutually regulate the concentration of their own fractions based on the principle of biological balance<sup>3,8</sup>. Physiologically native proteins and lipids are synthesized in the same organs, mainly in the small intestine and liver<sup>3,6,10</sup>. Enzymes of lipids and proteins, as well as other organic matter, along with the enzymes pro-

viding a shunt metabolite connection between proteins and fats, are involved in metabolic process. At this level the metabolism is uniform, ensuring the same functional implementation of metabolites, common to proteins and lipids. Metabolites, in turn, insure maintaining of relative dynamic equilibrium at the molecular, cellular-tissue, organic-system, and organismic levels (Figure 1).

During the metabolism, the lipids provide energy resources for metabolism and functions of proteins as well as affinity of lipoproteins with receptor associations in tissue structures of the body<sup>3,5,8</sup>. At shunt stages, the proteins cover the possible deficit of lipids and carbohydrates, producing metabolites for the synthesis of vital fats and sugars<sup>3,5,8</sup>.

However, there is both structural and functional physiological competition of proteins and lipids, evolutionary prevailing in the organism of poultry when forming pan-tophagy<sup>3,4,8</sup>. It is caused by the dynamic needs of the body in the course of ontogeny, where at various stages of growth and development, the load on the body systems changes, and this leads consequently to changes in the level of needs in plastic and energy components, i.e. proteins and lipids.

Thus, the total metabolism, its level and intensity are determined by the biological ratio of proteins and fats, which are regulated homeostatically in the course of individual growth and development of the animal body.

LPI allows determination and evaluation of the anabolic processes intensity and the level of increase in live

**Table 1.** Blood levels of lipid, protein metabolites, lipoprotein index (LPI) and body weight gain of broiler chicks of the Hubbard F15 cross (X, ± μ, n=10)

Index	Age of the chicks, days			
	1	7	23	42
Globulins (Glb), g/L	31,0±0,08	34,99±0,09*	44,43±0,07**	49,25±0,22**
Albumins (Alb), g/L	27,91±0,05	26,93±0,03	22,72±0,04*	25,86±0,12*
Esterified cholesterol (CE), mmol/L	3,08±0,04	3,02±0,35	3,07±0,11	2,37±0,12**
Triacylglycerols (TAG), mmol/L	3,26±0,15	2,68±0,24	2,45±0,16*	1,94±0,30**
Phospholipids (PL), mmol/L	6,06±0,30	3,11±0,19***	3,07±0,03***	2,94±0,33***
Unesterified fatty acids (UFA), mmol/L	0,64±0,02	0,85±0,06**	1,74±0,29*	1,89±0,28**
Index of lipoprotein (LPI), conventional units	5,73±0,07	12,54±0,15***	39,61±0,33***	49,65±0,46***
Body weight gain, g	35,0±0,68	65,0±0,70***	671,0±1,05***	1834,0±0,64***

Note: \* – p<0,05; \*\* – p<0,01; \*\*\* – p<0,001.

weight of the poultry, since it shows the concordance of physiological relationship of proteins and lipids with the chicks' growth (Figure 1). The higher the metabolic rate the higher the body weight gain of the chicks and the better the functional relationships of protein and lipid metabolites, which are reflected in the increase of the lipoprotein index. The results of the study are presented in (Table 1).

We have revealed that the concentration of globulins and non-esterified fatty acids increased naturally with the decrease in concentration values of albumins, esterified cholesterol, triglycerides and phospholipids in the course of chicks growing.

Dynamics of globulins and free fatty acids reflects their functional role in the plastic exchange, which permanently needs main synthetic molecular structural material of own proteins and lipids to form cell and, consequently, tissue organist systems of a growing organism. At the same time, lowering the concentration of albumins and total lipids, such as esterified cholesterol and triglycerides, is explained primarily by involvement of these metabolites in the capacity of direct transport and trophic (albumins) components, as well as structural and energy (phospholipids, esterified cholesterol, triacylglycerides) components in anabolism, and also by increasing discreteness of fatty tissue in the chicks body. These processes have a physiological implementation, firstly, through the active formation of the organs ensuring all synthetic processes, such as the liver and gastrointestinal tract, and secondly, in the growth of skeletal musculature, the development of the immune system and the whole chick body in general.

The value of LPI (Table 1) shows concordance of physiological relationships of proteins and lipids to chicks' growth. The higher the metabolic rate and consequently chicks' body weight gain the better the functional relationships of protein and lipid metabolites, reflected in the increase of lipoprotein index.

The calculations of index showed that at the body weight gain of the chicks within 35.0-65.0 g (age of P1 and P7) the index value varies within the range 5.73-12.54 conventional units. In the course of increasing body mass up to 671.0-1834.0 g (age of P23 and P42) the LPI value increases to 39.61-49.65 conventional units.

We have also determined the concentrations of total phospholipids and their subclasses (Table 2). Thus, the highest content of total phospholipids was observed in the blood of day-old chicks ( $6.07 \pm 0.30$  mmol/l) (Table 2). By the age of P7 it was reduced down by 48.76% ( $p < 0.001$ ),

stabilizing further and fluctuating within the range of  $3.31 \pm 0.11$ - $3.06 \pm 0.09$  mmol/l ( $p < 0.001$ ).

That is, lipid exchange was dominant in chick's life at the early stages of postnatal ontogenesis. Starting with 7-day age, protein exchange was becoming a priority (Kolesnik and Derkho, 2014, 2015). These resulted in stabilization of the total concentration of phospholipids in the serum of broiler chicks (Table 2). At the same time, each phospholipid class was noted by peculiar age dynamics (Table 2) that is associated with various physiological load<sup>19,20,21,26,28</sup>. Thus, the content of phosphatidylcholines when approaching to the age of P7, declined by 1.97 times and in the future did not change significantly. This was probably due to the stabilization of the lecithin metabolism. The amount of phosphatidylcholines in 7-day-old chicks decreased by 1.94 times compared to that for day-old chicks. Maximal reduction corresponded to 23-day-old chicks (2.62 times). By 42<sup>nd</sup> day the concentration of phosphatidylethanolamines in the blood gradually increased (Table 2). Similar dynamics of kephalins, perhaps, is determined by the functional activity of cell membranes of the cardiovascular system and liver<sup>19,20,24,30,31</sup> and coincides with "critical" periods (P7 and P23) of ontogenesis in broiler chicks in an industrial environment conditions<sup>1,2</sup>. The change in the concentration of lysolecithin and cardioliipin in chicks' blood serum had the same pattern (Table 2). We believe that the age dynamics of the content of these phospholipids is associated with physiological activity of albumins and cholesterol during the critical periods of ontogenesis in broilers<sup>1,19,26-29</sup>, which is characterized by profound anatomical and functional change of the liver and cardiovascular system, aimed at providing high growth rates and development of the organism. The amount of phosphatidylinositols did not depend on the chicks' age, though tended to decrease (Table 2). The sphingomyelins are the most important functional agents of membrane structures of the nervous system<sup>19,20,33,34</sup>; abrupt changes in their concentrations coincided with critical periods of ontogeny (Table 2) and reflected the adaptive nature of the neuronal function formation.

Phospholipids form the structural skeleton of the cell membranes<sup>19</sup>, whose functional state determines the rate of the active metabolism of cell and its organelles with the environment that is reflected not only on the intensity of metabolism in the body, but also on the health of the animal unit. To assess the survivability of the chicks and the characteristics of the total phospholipid homeostasis, we determined the PI according to the formula:

**Table 2.** The content of phospholipids fractions in the blood (mmol/L), the values of phospholipid index (PI) and survivability of broiler chicks of Hubbard F15 cross ( $X, \pm \mu, n=10$ ).

Index	Age of the chicks, days			
	1	7	23	42
Glycerophospholipids				
Phosphatidylcholines (PhCh)	2,56±0,10	1,30±0,12***	1,17±0,13***	1,19±0,13***
Phosphatidylethanolamines (PhE)	0,97±0,08	0,50±0,03**	0,37±0,03**	0,51±0,03***
Phosphatidylinositols (PhI)	0,46±0,04	0,39±0,04	0,39±0,08	0,36±0,03
Lysolecithins (LL)	0,64±0,09	0,20±0,01**	0,44 ±0,02	0,29 ±0,05*
Cardiolipins (CL)	0,99±0,03	0,62±0,09**	0,51±0,01***	0,33±0,01***
Σof glycerophospholipids	5,61±0,33	3,01±0,24***	2,88±0,15***	2,67±0,14***
Sphingophospholipids				
Sphingomyelins (SphM)	0,45±0,03	0,20±0,02***	0,49 ±0,04*	0,40±0,03
Σof sphingophospholipids	6,07±0,30	3,11±0,19***	3,31±0,11***	3,06±0,09***
Phospholipids index (PI), conventional units	5,73±0,14	4,44±0,37	2,92±0,25***	3,13±0,26**
Survival, %	99,20± 0,01	98,70± 0,01**	96,00±0,01**	96,10±0,02**

Note: \* –  $p<0,05$ ; \*\* –  $p<0,01$ ; \*\*\* –  $p<0,001$ .

$$PI = \frac{\sum \text{PhCh} + \text{PhE} + \text{LL} + \text{CL}}{\text{SphM} + \text{PhI}} \quad (2)$$

where PhCh, PhE, PhI, LL, CL and SphM are the concentrations of phosphatidylcholines, phosphatidylethanolamine, phosphatidylcholines, lysolecithins, cardiolipins and sphingomyelins, respectively, in the chicks' serum, mmol/l. The PI values (Table 2) characterize the trend of using glycerophospholipids and sphingolipids of the blood in the formation of cell membranes (Figure 1).

Phosphatidylcholines, phosphatidylethanolamine, lysolecithins and cardiolipins are involved in building cell membranes and their organelles in different organs, while phosphatidylinositol and sphingomyelins are involved mainly in building of nervous tissue. The balance between these phospholipids in the blood determines the concordance between the development of the chick's nervous system and the intensity of its growth that affects the viability and survivability.

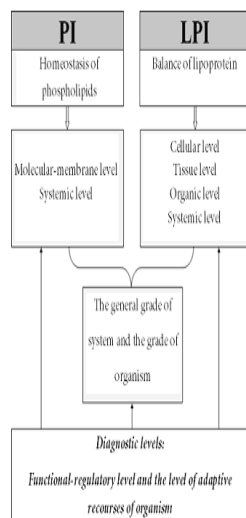
The growth of the body and a sharp increase in the number of cells result in the intense intake of glycerophospholipids for building cell membranes. Though the violation of the concordance between the rate of increase in cell number and their degree of innervations, which is measured by the concentration of sphingomyelins and

phosphatidylinositols, lead to poultry's health deterioration. At that, the total concentration of sphingomyelins and phosphatidylinositols does not significantly depend on the age of the chicks; therefore, formation of cells of the nervous system occurs slower than the growth of other organs and tissues. Comparing PI with survival rates (Table 2), we have revealed that at the PI values of 5.73-4.44 conventional units the survivability of the chicks (at the age of P1 and P7) was 99.2-98.7%. The decrease in PI down to 2.92-3.13 conventional units was accompanied by a decrease in survivability of chicks aged P23 and P42 to 96.0-96.1% (Table 2). The greatest quantity of phospholipids was found in the blood of 1-day-old broiler chicks, and it decreased with age. A specific age dynamics was found for each class of phospholipids.

## 4. Conclusion

The use of lipoprotein index to determine the intensity of metabolism and assess body weight gain in broiler chicks will allow controlling the trend and the rate of metabolism in poultry during rearing for meat that can be used for timely adjustment of feed rations and informative clinical examination to increase thereby the profitability of the poultry farm. Phospholipid index, together with

the lipoprotein index, allow characterizing the physiological condition of poultry during rearing for meat and produce timely adjustment of parameters of the applied poultry feeding and management technologies.



**Figure 1.** Diagnostics of the adaptive resources of the broiler chicks' organism.

Besides, these indices may be used in the poultry breeding to select chicks with a high level of metabolism, and, respectively, accelerated increase of body weight gain and high viability.

Thus, in combination, the lipoprotein and phospholipid indices allow diagnosing the adaptive resources of the broiler chicks' organism under the technological living conditions that is based on accounting for the physiological relationships between protein and lipid metabolites at the heart of functional systems of metabolism providing homeostasis. At the same time, we would like to mention that the problem of adaptive resources of the broiler chicks' organism in the technological living conditions is still relevant and needs further development.

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