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Short Communication

A comparative study of key enzymes in carbohydrate metabolism during embryonic development in Vanaraja and broilers chicks

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Abstract

The developmental changes from the embryonic to the post-hatching stage appear to be associated with the altered carbohydrate availability resulting from a shift from the high-fat, low-carbohydrate environment of the egg yolk to the low-fat, high-carbohydrate diet normally fed to the chicken after hatching. Hence, a comparative study was carried out in broiler and Vanaraja embryos on key enzymes of gluconeogenesis and HMP pathways, which are vital to ensure an optimal supply of glucose and nucleic acid precursors for embryo survival and early post-hatch life. The activities of Glucose-6-phosphatase(G6Pase) and Fructose 1-6 bisphosphatase (FBPase) were significantly increased towards the growth of the embryos and found to be higher in liver tissues compared to brain tissues of Vanaraja. Both the enzyme activities were found to be higher in Vanaraja compared to broilers. Glucose-6-phosphate dehydrogenase (G6PD) activity decreased significantly in both the tissues towards the growth of the embryo and it was significantly higher in brain tissue compared to liver tissue of broilers. Glucose-6-phosphate dehydrogenase (G6PD) activity was found to be lower in Vanaraja when compared to broilers. Glutathione (GSH) levels significantly decreased in both tissues towards the development of embryos and were significantly higher in Vanaraja embryos compared to broilers. In both the embryos, GSH levels were high in liver compared to brain tissue during embryonic development. Embryo weights were significantly lower in Vanaraja when compared to broilers towards the development of the embryos.

Keywords: Broiler, Carbohydrate metabolism, Enzymes, Glutathione, Vanaraja breed

Highlights:

- Vanaraja variety embryo grows slowly when compared to commercial broiler.
- Metabolic adaption from high-fat low carbohydrate to low-fat high carbohydrate as embryo grows.
- Gluconeogenic enzymes increase in the liver as the embryo grows to hatching in both genotypes.
- Glutathione levels decrease in compliance with G6PD levels as the embryo grows.
- Vanaraja chicks have better nutritional and oxidative status adaptability than broilers.

INTRODUCTION

In India, livestock and poultry farming plays a key role in socio-economic development and in the national economy of the country. Most poultry products are consumed by 25% of the people living in urban areas, who consume 75% of the eggs and almost 100% of the broiler meat produced in India. This could be because most of the commercial poultry egg and meat production is concentrated in the urban and semi-urban areas. On the other hand, rural poultry contributes to 30% of the national egg production, even though it is being neglected (Tajane and Vasulkar, 2014). The major limiting factor in the efforts to increase the consumption of egg and poultry meat in rural areas is the poor availability. Hence, this gap in the production and demand of eggs and meat in rural areas can be met by encouraging backyard poultry rearing (Nandi *et al*., 2007; Panda *et al*., 2008).

In order to develop rural poultry farming, rearing improved rural backyard poultry like Vanaraja and Gramapriya is of utmost importance. From a genetic perspective, the ICAR-Directorate of Poultry Research, Hyderabad, has developed multi-coloured germplasm (Vanaraja) that can thrive well in village conditions. It is well adapted to the local climate, resistant to avian diseases, and embryonic mortality was very low compared to other desi chicken (Kumar *et al*., 2013). This makes Vanaraja a better option for successfully rearing in a backyard system under local rural conditions. Backyard rearing of Vanaraja birds was more profitable; its rearing not only increased egg production in rural areas but also improved the economic status of rural women (Sheikh *et al*., 2018).

Broiler chickens are fast-growing chickens with deeper muscle tissue and are grown mainly for meat purposes. Recent studies have found faster embryonic

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growth, more embryonic weight, higher accumulation of protein and faster yolk consumption in broiler embryos compared to layers (Ohta *et al*., 2004; Sato *et al*., 2006). Another interesting aspect of chick embryonic development is that it assimilates a large amount of calcium into the bones from the eggshell at the stage of day 10-12 of embryonic life (Abbas *et al*., 1986). Embryo is structurally complete by 14th to 15th day of embryonic development (Moran, 2007), during 20th day pulmonary respiration begins and yolk sac is completely drawn into body (Surai, 2002).

In addition, biochemical and metabolic aspects during embryonic development also affect the embryogenesis, growth rate and hatchability. For instance, glucose oxidation increases at the end of incubation because of the energy demands and the limited O_2 availability during hatching process (Tazawa *et al*., 1983). Therefore, enough glucose must be built up and stored as glycogen before the hatching process begins (Foye *et al*., 2007). In addition to synthesize glucose, the activity of gluconeogenic enzymes increases throughout the incubation with glucogenic amino acids, glycerol, or lactate as potential precursors (Watford *et al*., 1981). On the other hand, the HMP shunt pathway ensures the indispensable supply of ribose for nucleic acid synthesis, because there are very minimal amounts of nucleic acids in a freshly laid egg (De Oliveira *et al*., 2008). The enzyme G6PD of HMP pathway produces NADPH, which is required for catalase stability and also for their generation of GSH, which are important enzymes of antioxidant system (Luzzatto *et al.*, 2001). Hence, the present study is aimed to compare the embryonic growth rates and key enzymes in carbohydrate metabolism during embryonic development in both Vanaraja and broiler poultry birds.

MATERIALS AND METHODS

Freshly laid broiler (Cobb 650 Y) strain, zero-day old fertilized eggs were procured from Sri Balaji hatcheries, Chittoor and Vanaraja eggs were procured from Department of Poultry Science, at C. V. Sc., Tirupati, weighed and incubated at 37°C with a relative humidity of 65% in an Egg Incubator. From each group,10 eggs were collected separately on the 10th, 15th and 20th day of embryonic development, embryo weights were recorded, and embryos were sacrificed on respective days to collect liver and brain tissue samples for analysis. G6Pase activity was assayed by Kings method (King, 1965). FBPase activity was measured using the Fiske and Subbarow method (Fiske and Subbarow, 1925). Assay of G6PD activity was done as per Worthington enzyme manual (Worthington and Worthington, 2023). Glutathione content was measured according to the method of Ellman (Ellman, 1959). Protein estimation was carried out by Lowry method (Lowry *et al.*, 1951). The data recorded on measured parameters were subjected to one-way ANOVA statistical analysis to detect significance between groups by the latest version of SPSS (26.0).

RESULTS

Gluconeogenic pathway enzymes

Glucose-6-phosphatase:

Brain: The G6Pase activity in brain tissue was significantly increased (P<0.05) towards the development in both broiler and Vanaraja embryos (Table 1). In brain tissues, the activity was significantly higher (P<0.05) in Vanaraja than in broiler embryos.

Liver: The mean ± SE values of G6Pase activity in liver tissue are presented in Table 1. The activity was significantly increased (P<0.05) towards the development of the embryo in both broiler and Vanaraja. In liver tissue, the activity was significantly higher (P<0.05) in Vanaraja than in broiler embryos.

Table 1. Glucose-6-phosphatase activity in brain and liver tissues of chick embryos

Groups	Glucose-6-phosphatase (µmoles of Pi liberated/hr/mg protein)		
	10th day	15th day	20th day
	Brain tissue		
Broiler (Cobb)	0.542^{Aa}±0.009	0.702^{Ab}±0.007	0.776^{Ac}±0.01
Vanaraja	0.613^{Ba}±0.016	0.744^{Bb}±0.014	0.826^{Bc} ±0.011
	Liver tissue		
Broiler (Cobb)	4.41^{Aa}±0.089	4.672^{Ab}±0.053	5.844^{Ac}±0.117
Vanaraja	4.642^{Ba}±0.054	5.15^{Bb}±0.043	6.255^{Bc}±0.085

Values are Mean ± SE (n=10). A, B different superscripts column-wise differ significantly (P<0.05). a, b, c different superscripts row-wise differ significantly (P<0.05).

Fructose-1,6-bisphosphatase:

Brain: The mean ± SE values of FBPase activity in brain tissue were presented in Fig. 1. The activity was significantly increased (P<0.05) towards the development of the embryo in both broiler and Vanaraja. In brain tissue, the activity was significantly higher (P<0.05) in Vanaraja than in broiler embryos.

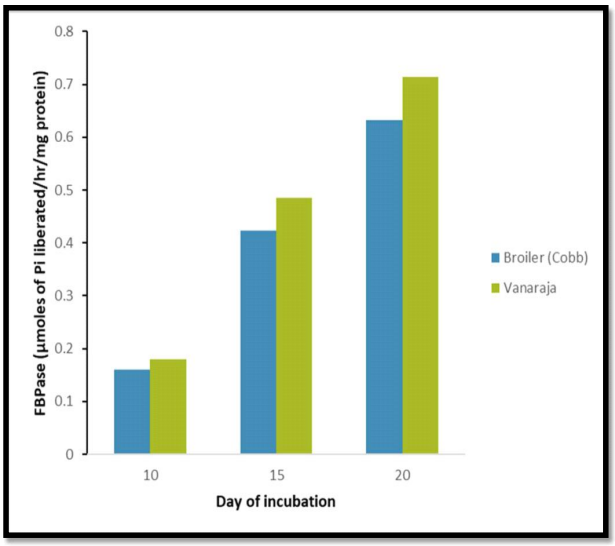

Fig. 1. Fructose-1,6-bisphosphatase activity in brain tissues of chick embryos in broiler and Vanaraja breeds

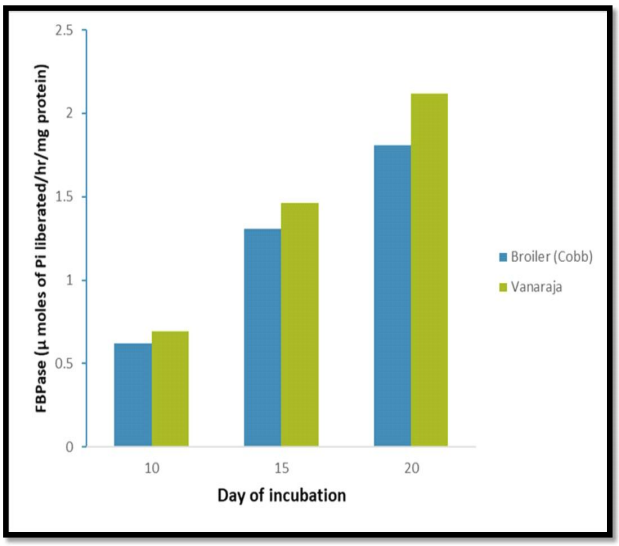

Fig. 2. Fructose-1,6-bisphosphatase activity in liver tissues of chick embryos of broiler and Vanaraja breeds

Table 2. Glucose-6-phosphate dehydrogenase activity in brain and liver tissues of chick embryos

Groups	Glucose-6-phosphate dehydrogenase (U/mg protein)		
	10th day	15th day	20th day
	Brain tissue		
Broiler (Cobb)	$0.093^{Bc}\pm0.002$	$0.085^{Bb}\pm0.002$	$0.067^{Ba}\pm0.002$
Vanaraja	$0.074^{Ac}\pm0.001$	$0.059^{Ab}\pm0.001$	$0.044^{Aa}\pm0.002$
	Liver tissue		
Broiler (Cobb)	$0.088^{Bc}\pm0.002$	$0.068^{Bb}\pm0.002$	$0.051^{Ba}\pm0.001$
Vanaraja	$0.066^{Ac}\pm0.002$	$0.052^{Ab}\pm0.001$	$0.042^{Aa}\pm0.002$

Values are Mean ± SE (n=10). A, B different superscripts column-wise differ significantly (P<0.05). a, b, c different superscripts row-wise differ significantly (P<0.05).

Table 3. Glutathione levels in brain and liver tissues of chick embryos

Groups	Glutathione (µg/mg protein)		
	10th day	15th day	20th day
	Brain tissue		
Broiler (Cobb)	$18.92^{Ac}\pm0.18$	$11.83^{Ab}\pm0.14$	$7.91^{Aa}\pm0.05$
Vanaraja	$21.18^{Bc}\pm0.27$	$15.56^{Bb}\pm0.13$	$8.42^{Ba}\pm0.11$
	Liver Tissue		
Broiler (Cobb)	$34.22^{Ac}\pm0.64$	$22.21^{Ab}\pm0.64$	$11.95^{Aa}\pm0.16$
Vanaraja	$43.17^{Bc}\pm0.91$	$28.24^{Bb}\pm0.42$	$16.33^{Ba}\pm0.47$

Values are Mean ± SE (n=10). A, B different superscripts column-wise differ significantly (P<0.05). a, b, c different superscripts row-wise differ significantly (P<0.05).

Liver: The mean ± SE values of FBPase in liver tissue are presented in Fig 2. The activity was significantly increased (P<0.05) towards the development of the embryo in both broiler and Vanaraja. It was found to be significantly higher (P<0.05) in Vanaraja than in broiler embryos.

Pentose phosphate pathway

Glucose-6-phosphate dehydrogenase:

Brain: The mean ± SE values of G6PD activity in brain tissue are presented in Table 2. The G6PD activity was significantly decreased (P<0.05) towards the development of the embryo in both broiler and Vanaraja. The activity was significantly higher (P<0.05) in broiler than in Vanaraja embryos.

Liver: The mean values of GSH in liver tissue are presented in Table 3. Results showed a significant decrease (P<0.05) in GSH levels towards the development in both broiler and Vanaraja embryos. In liver tissue, GSH levels were significantly higher (P<0.05) in Vanaraja than in broiler embryos.

Embryo weight

Mean ± SE values of embryo weights were depicted in Fig. 3. Mean embryo weight of broiler was significantly (P<0.05) higher than Vanaraja towards the development.

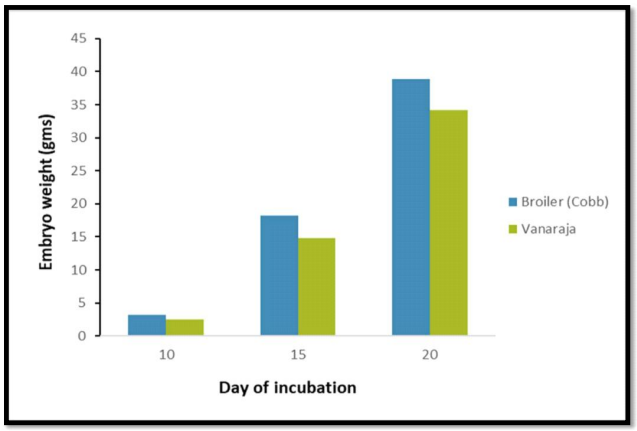

Fig. 3. Embryo weight in broiler and Vanaraja breeds

DISCUSSION

Gluconeogenic pathway enzymes: In broiler and Vanaraja embryos, both G6Pase (Table 1) and FBPase (Fig. 1 and Fig. 2) activities were increased significantly towards the course of embryonic development in both liver and brain tissues. Similar increase in their activity was also observed in other reports, where G6Pase activity in chick embryonic liver tissues (Wang, 1968; Donaldson, 1995) and in yolk sac membrane (Shibata *et al.*, 2023). Further, tissue comparison in both broiler and Vanaraja embryos for G6Pase and FBPase activities has shown higher levels in the liver than in brain tissues, which could be due to the fact that the embryonic hepatic tissue is a predominant gluconeogenic organ (Scott *et al.*, 1981; Muramatsu *et al.*, 1990; Moran, 2007). A comparable trend was also observed in quail embryos, where low activity of FBPase in the brain, heart and muscles indicated limited gluconeogenesis in these tissues (Mahapatra and Murthy, 1990). High G6Pase and FBPase activities compared to hexokinase and pyruvate kinase show the key role of these enzymes throughout the development in chick embryonic tissues (Wallace and Newsholme, 1967). Further breed comparison for G6Pase and FBPase activities in both brain and liver tissues has shown significantly higher levels in Vanaraja than in broiler embryos, which could be due to the availability of higher concentration of substrate in former breed (Bhagavan, 2002).

Pentose phosphate pathway enzymes and Glutathione: Glucose 6 phosphate dehydrogenase (G6PD) is a regulatory enzyme that catalyzes the first reaction in the HMP shunt pathway to produce NADPH, which is used to preserve the reduced form of glutathione, an important antioxidant (Schaffenburg *et al.*, 2021). In both tissues, G6PD activity decreased gradually after the 10th day of incubation towards the development of the embryos (Table 2). A similar trend was observed in other studies, where the activity decreased gradually in the later stages of embryonic development (Farnararo and Bruni, 1982). Higher G6PD activity in the brain compared to liver tissues could be due to its neuroprotective role (Wang, 1968). Similar results were reported, in previous studies where very rapid increase in G6PD activity in brain upto 8th day of development in chick embryo, followed by decline in activity throughout the remainder of the incubation period was observed (Burt and Wenger, 1961).

Glutathione levels decreased towards the growth of the embryo in both tissues and values were higher in hepatic tissues than in brain tissues (Table 3). The results are in similar lines with Wilson *et al.* (1992), who reported several-fold reductions in GSH levels in the brain compared to liver tissues in chick embryos. G6PD enzyme is required for the regeneration of GSH (Luzzatto *et al.*, 2001). As the embryo grows, a decrease in G6PD enzyme activity could be the reason for the decrease in GSH activity.

Embryo weights and GSH activity correlation

Chick embryo weight of broilers was higher than Vanaraja and it significantly increased towards hatching and growth rate was higher in broiler compared to Vanaraja (Fig. 3). Therefore, a negative correlation has been observed between embryonic growth and GSH levels in both the tissues in both breeds under study. In addition, breed-wise comparison has shown high levels of GSH in slow-growing Vanaraja when compared to fast-growing broilers. Further, other similar studies have shown an inverse relationship between GSH concentration in the liver and the growth rate of chicks and also reported higher GSH in slow-growing chicks than in fast-growing chicks (Charkey *et al.*, 1965).

Comparison of embryonic growth in confluence with metabolic alterations among Vanaraja breed and broilers has given critical insights into the variations that occur between slow-growing and fast-growing breeds of poultry in accordance with their nutritional and oxidative status requirements. In both breeds, it is observed that the gluconeogenic enzyme levels have significantly increased as the embryo grows and adapts from high fat, low carbohydrate dependent to low fat, high carbohydrate dependent by the end of embryo growth and hatching. Further, it has been observed that the liver is the chief gluconeogenic organ during embryonic growth and among the breeds, slow-growing breeds have shown better metabolic

adaptive capacity when compared to fast-growing breeds witnessed by higher levels of gluconeogenic enzymes in former at any stage of the embryo growth. In addition, the anti-oxidative and nucleic acid synthesis capability manifested by higher levels of G6PD enzyme and GSH levels in slow-growing Vanaraja shows that they have a better capacity than fast-growing broilers. Further studies with increased sample numbers and comprehensive enzymatic profiles will affirm the present study findings.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: PK, EP, AK: Study conception and design; AKG, CKTV, PK: Material preparation, data collection and analysis, and drafting of the manuscript. All authors read and approved the final manuscript.

Data availability statement: All the data supporting the research findings have been presented in this paper. The corresponding author is willing to provide the raw data upon reasonable request.

Ethics approval: The study is performed in guidelines and norms of the Institutional Animal Ethics Committee with approval no: 95/go /ReBi/S/2000/ CPCSEA/ CVSc/ TPTY/ 0112/ biochemistry/2019-20 dated 18-05-2019.

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