BIOLOGIJA. 2006. Nr. 4. P. 87–91

Correlation analysis of isozyme polymorphism, growing traits and phosphorus utilization in Cobb 500 cross broiler chicks

Vaida Tubelytė-Kirdienė[1*],

Vilma Šašytė[2],

Asta Racevičiūtė-Stupelienė[2],

Inga Ribelytė[1],

Romas Gružauskas[2],

Algimantas Paulauskas[1]

[1] *Vytautas Magnus University, Department of Biology, Vileikos 8, LT-44404 Kaunas, Lithuania*

[2] *Lithuanian Veterinary Academy, Kaunas, Lithuania*

An experiment was conducted to determine whether there are any relations among the genotype, phosphorus digestibility and body weight in broiler chicks of Cobb 500 cross. The diet of the experimental group was supplemented with 150 g/t of the enzyme phytase. Body weight was measured once a week from 14 to 49 days of age. The genetic uniformity of the control and experimental groups was determined by protein polymorphism analysis. The ANOVA linear models showed a significant linkage between the G6PD-4 locus phenotype and phosphorus utilization ($p < 0.01$). In the experimental group, broiler chicks with AA or BB phenotypes utilized by about 5–8% more of phosphorus as compared with the mean value of the control group. A significant correlation ($p < 0.05$) was found between chickens with a different MDH-2 phenotype and BW in all stages of measuring after phytase had been added to their diet. Homozygous AA and heterozygous AB individuals in the experimental group showed the highest BW and P digestibility.

Key words: broilers, biochemical polymorphism, phytase, phosphorus

INTRODUCTION

Chickens provide one of the most important and rapidly growing sources of meat protein in the world, and the majority of feeding trials are aimed at increasing productivity and meat quality. Also, there are searches for the genetic markers that can be used for selection, especially those closely related with quantitative traits. A lot of genes influencing growth and body composition, fatness, feed consumption and efficiency in chickens [1, 2] have been described. The control of excreta and litter moisture content is another major problem in the modern poultry industry to carry out environmental preservation and to enhance animal welfare as well as to reduce productivity losses [3]. About half of the phosphorus in chickens excreta comes from undigested mineral phosphates. While naturally the bird's digestion track lacks endogenous enzymes catalyzing phytate reduction, the use of phytase decreases the necessity to include mineral phosphates in the diet and reduces P excretion in broiler chickens [4, 5]. The enzymatic preparation of phytase (*Ronozyme P*) is produced from the fungus *Peniophora lycii* which is a 6-phytase, i.e. attacks the phytate ring at the 6-P position. In this respect it is similar to phytase occurring naturally in plants [6]. Feeding trials show no significant influence of *Ronozyme P* phytase supplementation of broiler diet on their body weight, but have revealed an increased utilization of phosphorus [7]. Nevertheless, experiments to show how phytase supplementation influences the growth of different genotypes are scarce.

A major factor contributing to the variation of the results in experiments is the genetic variation of individuals. The genetic uniformity of chicken groups used in feeding trials ensures more significant results. There is a variety of reports related with isozyme utilisation as genetic markers in poultry systematics [8, 9] and breeding programmes [10, 11]. In our previous study, the electrophoretic examination of biochemical markers was performed to evaluate the intra- and inter-specific genetic variability of several poultry species such as Japanese quail [12], chicken [13] and turkey [14]. No investigations have been done in search of associations of molecular markers with growth performance and other commercially important traits in phytase-supplemented chicken groups.

The aims of the present study were evaluation of isozyme polymorphism of broiler chicken cross Cobb 500 and estimation of the possible influence of phytase supplementation on body weight and phosphorus utilization in chickens of different phenotypes.

* Corresponding author. E-mail: v.tubelyte@gmf.vdu.lt

MATERIALS AND METHODS

Experimental animals. To analyse the influence of a synthetic phytase feed supplement (*Ronozyme P*) on broiler chickens' growth and feed conversion ratio, a feeding experiment was conducted using Cobb 500 cross broiler chickens 14 to 49 days of age. The chicks were divided into two groups and kept in separate cages with their own feeding trough and stationary watering containers made available for *ad libitum* consumption. The same diets were formulated for control (FYT(–)) and experimental (FYT(+)) groups according to the requirements of broiler chickens as established by the NRC [15]. The feed mixture of both groups contained a monocalcium phosphate additive (1.1% $Ca(H_2PO_4)_2$, 0.66% total P). The diet of the experimental group was supplemented with 150 g/t of phytase *Ronozyme P* (5000 FYT/g, F. Hoffmann La Roche AG, Switzerland). Phosphorus in the feeds and excrements was measured by to the methods of VDLUFA [16] and the percentage of phosphorus utilization was expressed. Body weight was determined at 14 (the begining of feeding trial), 21, 28, 35, 42 days of age and at the end (49 days of age) of the feeding experiment. At the age of 49 days the chicks were butchered and the liver tissue prepared for protein polymorphism analysis.

Isozyme analysis. Isozymes were detected by non-denaturing vertical PAGE electrophoresis according to Paulauskas & Tubelytė-Kirdienė [17]. In total, 58 individuals – 37 in the control group and 21 in the FYT(+) group were genotyped for 15 enzyme and protein loci: malate dehyrogenase (MDH-2), malic enzyme (ME-1), glucose-6-phosphate dehydrogenases (G6PD-1, 4), 6-phosphogluconate dehydrogenase (6PGD), xanthine dehydrogenases (XDH-1, 2), lactate dehydrogenase (LDH-1), superoxide dismutase (SOD-2), alcoholic dehydrogenases (ADH-1, 2), esterases (EST-1, 4), non-specific proteins (NP-1, 4). Electrophoresis was carried out at a gradient of 8.7–11.3 mA/cm for 1.30–2 h at 4 °C. Isozymes and non-specific proteins were visualized using staining system indicated by Brewer [18] with few modifications (available from the authors upon request).

Statistical analysis. Multiloci protein systems were numbered in accordance with their mobility from anode to cathode. Allele marking is a response to their expression product migration rate in gels – A (fast) and B (slow). Indistinct polymorphic loci were not included in the further analysis. The genetic variability was estimated by using the POPGENE Version 1.32 software package [19].

The SPSS 11.0.0. statistical package [20] was used to estimate the effects of phenotype on body weight (BW) and P utilization. Effects of phenotypes on the quantitative traits were considered with ANOVA linear models. Phenotype influence on the traits was expressed as a percentage from the means of the control group (Table 1).

RESULTS

The distribution of different phenotypes of the isozymes and a possible correlation with BW, BW gain and phosphorus utilization were investigated in two groups (control FYT(–) and experimental FYT(+)) of Cobb 500 broiler chicks. The control and experimental groups showed a genetic uniformity: the mean observed heterozygosity was 32%, the percentage of polymorphic loci 53.33 for both groups. The mean effective number of alleles varied from 1.886 ± 1.001 for the FYT(+) to 1.914 ± 0.966 for the FYT(–) group. No rare alleles were found, all loci were in the Hardy–Weinberg equilibrium, except 6PGD and ME in the control population ($p < 0.05$) were accumulation of AA phenotype was detected. No associations among AA phenotype, BW and P utilization were detected in those loci. A weak but statistically significant negative correlation ($r = –0.350$, $P = 0.007$) was observed between individual heterozygosity per all loci and BW gain during the feeding trial.

In both groups, a linkage was indicated between the G6PD-4, MDH-2 phenotypes and the production traits.

Table 1. **Mean body weight at the beginning and end of feeding trial, increase of body weight and phosphorus reduction in broiler chickens of Cobb 500 cross**

Trait	No.	df	P (2-tailed)	Mean	95% C.I.		S.E.
					Lower	Upper	
Control group FYT(–)							
BW 14 d of age, g	36	35	0.000	414.82	401.21	428.45	6.71
BW 49 d of age, g	36	35	0.000	2647.12	2587.68	2706.55	29.28
Increase of BW, g	36	35	0.000	2232.3	2174.76	2289.82	28.34
P reduction, %	25	24	0.000	53.54	48.84	58.23	1.49
Experimetal group FYT(+)							
BW 14 d of age, g	22	21	0.000	415.68	399.21	432.16	7.92
BW 49 d of age, g	22	21	0.000	2588.4	2506.24	2670.48	39.49
Increase of BW, g	22	21	0.000	2172.7	2090.82	2254.53	39.36
P reduction, %	16	15	0.000	54.28	50.98	57.58	1.55

Table 2. **G6pd-4 and Mdh-2 gene alleles and genotypes frequencies in broiler chickens of Cobb 500 cross**

Allele	G6pd-4		Mdh-2	
	FYT(–)	FYT(+)	FYT(–)	FYT(+)
A	0.333	0.437	0.346	0.306
B	0.375	0.312	0.327	0.278
C	0.292	0.250	0.327	0.417
Genotype				
AA	0.083	0.187	0.154	0.111
AB	0.333	0.250	0.231	0.055
AC	0.083	0.250	0.115	0.222
BB	0.167	0.125	0.154	0.333
BC	0.250	0.125	0.193	0.055
CC	0.083	0.062	0.154	0.222

The allele and genotype frequencies of these loci are shown in Table 2.

A significant association was detected among 49-d broilers' BW (F = 5.173, P = 0.034) and different G6PD-4 phenotypes. In the control group, individuals with BB phenotype of G6PD-4 locus had a BW by 7.58% higher than individuals with homozygous AA and by 10.16% higher than those with homozygous CC phenotype. In the FYT(+) group, BB homozygotes also note a 9% higher BW as compared with homozygous AA phenotype and 9.63% higher than in individuals with homozygous CC phenotype. There was a significant link-age between G6PD-4 phenotype and P utilization (F = 12.28, P = 0.002): individuals with AA or BB phenotypes in FYT(+) group utilized about 5–8% biggest amount of phosphorus in compare with mean value for control group. Figure 1 shows a relation between 49-d chickens' BW and P utilization of individuals with different G6PD-4 phenotypes.

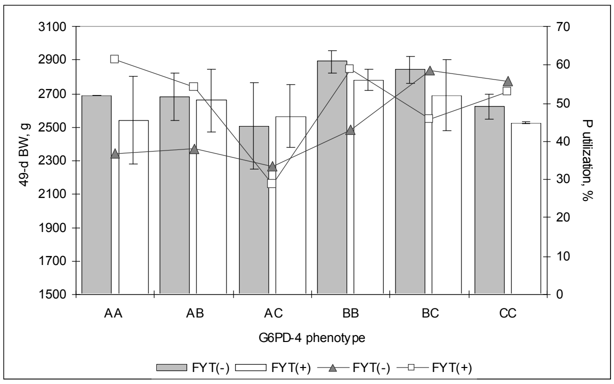

Fig. 1. Relation between 49-d chicken BW and phosphorus utilization in individuals with different G6PD-4 phenotypes

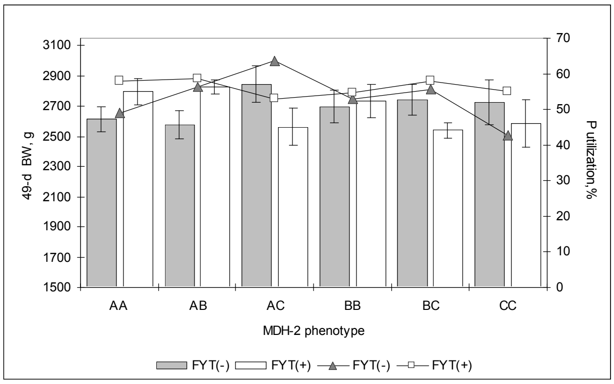

Fig. 2. Relation between 49-d chicken BW and phosphorus utilization in individuals with different MDH-2 phenotypes

Significant differences were assessed for 49-d broilers' BW (F = 4.310, p = 0.001) and MDH-2 phenotypes. Figure 2 demonstrates that the presence of A allele in the genotype has a positive association with BW

Table 3. **The influence of genetic factors on growing traits and phosphorus utilization in broiler chickens of Cobb 500 cross**

Phenotype	BW 49 d, g		Increase of BW, g		P utilization, %	
	FYT(–)	FYT(+)	FYT(–)	FYT(+)	FYT(–)	FYT(+)
G6PD-4						
AA	1.66%*	–3.97%*	1.32%	–4.81%	–16.67%**	7.64%**
AB	1.36%*	0.45%*	1.23%	0.11%	–15.34%**	0.70%**
AC	–5.24%*	–3.09%*	–4.57%	–5.20%	–20.01%**	–24.62%**
BB	9.24%*	5.03%*	12.34%	6.45%	–10.34%**	5.43%**
BC	7.36%*	1.70%*	8.37%	2.51%	4.99%**	–7.69%**
CC	–0.92%*	–4.60%*	–1.79%	–6.92%	2.19%**	–0.54%**
MDH-2						
AA	–1.28%**	5.58%**	–1.32%**	7.99%**	–4.40%	4.45%
AB	–2.69%**	6.79%**	–3.30%**	7.13%**	2.63%	5.06%
AC	7.51%**	–3.12%**	7.54%**	–3.43%**	10.08%	–0.51%
BB	1.87%**	3.31%**	2.50%**	3.16%**	–0.39%	1.13%
BC	3.53%**	–4.00%**	3.55%**	–4.64%**	2.12%	4.46%
CC	3.02%**	–2.47%**	3.97%**	–2.35%**	–10.76%	1.42%

* P < 0.05, ** P < 0.01.

gain in the FYT(+) group and a negative influence in the FYT(–) group; on the contrary, C allele influences broiler's BW negatively in the FYT(+) group and positively in the control group. During the feeding trial, in homozygous individuals with AA phenotype in the FYT(+) group BW gain is by 4.83% higher than in individuals with BB phenotype and by 10.34% higher than in individuals with homozygous CC phenotype. Homozygous AA and heterozygous AB individuals in the FYT(+) group stood out by all traits studied (Fig. 2): they had by a 5–7% higher BW again, 4–5% higher of P utilization ($p > 0.05$) as compared with the control group. The relation between MDH-2 phenotype and BW was found to be significant ($p < 0.05$) for BW measured at all stages of age – at 21, 28, 35 and 42 days.

The influence of G6PD-4 and MDH-2 phenotypes on the growth traits and P utilization in broiler chicks of Cobb 500 cross is presented in Table 3.

DISCUSSION

One of the negative sides of broiler selection for commercially useful traits is inability to utilize phytate phosphorus. There could be several solutions of this problem: to add more mineral phosphates, the most expensive nutrients, in chickens' diet; to supplement chickens' feed with the enzyme preparation phytase and/or to search for the genetic markers positively associated with chickens' growth and phosphorus utilization. Our previous investigations have shown that the deficit of phosphorus in chickens' diet decreases BW gain about 12%, and supplementation with the enzyme phytase allows avoiding this problem but not significantly increase body weight versus the mean value of the control group [7]. We evaluated the possible correlations of dietary treatment, body weight and phosphorus utilization in chickens carrying different phenotypes. Isozyme studies, with an emphasis on those involved in carbohydrate metabolism, showed a strong relation among different G6PD-4 locus phenotypes and phosphorus utilization and a moderate association with body weight in 49-d chickens. The strongest relation among broiler growth values and phenotype was found for the MDH-2 locus. This relation was significant in all periods of age. It is possible that the gene coding the cytosolic form of malate dehydrogenase is associated with chickens' growth. There is a lack of investigations to analyze this function of MDH in poultry, but experiments with steers showed that the cytosolic ratio of MDH/LDH activity in leukocytes increased significantly in the fattening process [21]. Selection of chickens showing "positive" phenotypes in MDH-2 and G6PD-4 loci for BW and P utilization not only increases BW gain in comparison with the mean values for the control group, but also exerts a positive influence on P utilization.

ACKNOWLEDGEMENTS

This work was supported by the Lithuanian State Science and Studies Foundation and Lithuanian Ministry of Agriculture.

Received 1 December 2005
Accepted 28 May 2006

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Vaida Tubelytė-Kirdienė, Vilma Šašytė,
Asta Racevičiūtė-Stupelienė, Inga Ribelytė,
Romas Gružauskas, Algimantas Paulauskas

KORELIACINIŲ RYŠIŲ ĮVERTINIMAS TARP IZOFERMENTŲ POLIMORFIZMO, AUGIMO POŽYMIŲ IR FOSFORO SUNAUDOJIMO COBB 500 LINIJŲ DERINIO BROILERIUOSE

Santrauka

Šio darbo tikslas buvo ištirti Cobb 500 linijų derinio broilerių izofermentines sistemas ir įvertinti galimus ryšius tarp fenotipo, fosforo pasisavinimo ir kūno svorio. Broileriai buvo suskirstyti į dvi grupes: kontrolinę ir eksperimentinę (jos pašarai papildyti 150g/t fitaziniu priedu). Viščiukų kūno svoris matuotas kas savaitę viso eksperimento metu (t. y. nuo 14 iki 49 dienų amžiaus). Baltymų polimorfizmo tyrimas patvirtino, kad pagal tirtas izofermentines sistemas tarp grupių nėra didelių genetinių skirtumų. Sudaryti ANOVA linijiniai modeliai leido įvertinti, ar yra patikimas ryšys tarp fenotipo, kūno svorio ir P sunaudojimo. Eksperimentinėje grupėje nustatyta, kad broileriai, kurių fenotipas G6PD-4 lokuse AA arba BB, sunaudojo 5–8% daugiau fosforo ($p < 0{,}01$) lyginant su kontrolinės grupės vidurkine reikšme. Patikimas ryšys nustatytas tarp viščiukų kūno svorio (visų matavimų) ir skirtingų fenotipų MDH-2 lokuse ($p < 0{,}05$). Eksperimentinėje grupėje homozigotiniai AA ir heterozigotiniai AB individai pasižymėjo didesniu kūno svoriu ir P sunaudojimu.

Raktažodžiai: viščiukai broileriai, biocheminis polimorfizmas, fitazė, fosforas