Plasma Components as Traits for Resistance to Coccidiosis in Chicken

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ABSTRACT: For successful genetic dissection of disease resistance it is of great importance to accurately identify the respective phenotypes. In case of coccidiosis some of the conventional phenotypes don't fully reflect the animal health status. The objective of this study was large-scale evaluation of plasma components as potential parameters for describing health status of broilers challenged with *Eimeria maxima*. The challenge was performed on 2024 Cobb500 broilers. Plasma coloration was measured on all the animals while measurement of plasma protein content and analysis of plasma protein composition were performed on subset of 184 extreme animals selected based on body weight gain. All fractions of plasma proteins associated with acute phase proteins, except β1-globulin, have been significantly elevated. We observed that the best estimation of plasma coloration variation under a coccidiosis challenge can be obtained by measuring optical density between 450 and 490 nm.

Keywords: Plasma components; Coccidiosis; Disease resistance

Introduction

For successful genetic dissection of disease resistance it is of great importance to accurately identify the phenotypes for disease resistance which has been proved to be very difficult. In case of chicken coccidiosis some of the conventional phenotypes like the oocyst count and lesion scores are technically demanding for large scale phenotyping and moreover they don't fully reflect the animal health status.

Results presented in this paper are part of a collaborative effort which included large scale challenging and phenotyping with a final aim to study the genetic architecture of resistance to coccidiosis in chicken. The project was developed to study Cobb500 broilers for coccidiosis sensibility by large scale phenotyping and genotyping with the aim of knowledge transfer to poultry breeding. The objective of this study was to evaluate plasma protein content and composition as potential parameters for describing health status of broilers challenged with *Eimeria maxima* and examine in more details plasma coloration as a trait for resistance to coccidiosis. In previous studies a strong effect of *Eimeria* infection on the loss of blood carotenoids have been detected by measuring optical density of plasma (Yvore et al. (1993)) but these plasma coloration measurements were performed on limited number of wavelengths. Up to now, there were no reports on plasma protein composition in chickens challenged with *Eimeria*. However, total plasma protein content and plasma protein composition have been known as clinically relevant parameters and they are used for obtaining information regarding a variety of physiological and immunological states in birds in veterinary practice (Harris (2009)).

Materials and Methods

Animal raising and phenotyping. The experiment was performed on 2024 Cobb500 broilers. Animals were randomly distributed in 44 (challenge) and 2 (control) boxes containing 44 birds each during the 23 days of experiment. On day 1, animals were weighed and identified; weighing was also done on days 8, 15 and 22. Animals were reared under standard conditions for broilers.

On day 16, the blood samples were collected and animals were individually inoculated per os using syringe with 50,000 *Eimeria maxima* oocysts. On day 23, the blood sampling, phenotyping and euthanasia were performed. Mortality was recorded every day and autopsy was conducted to determine the cause of death.

Plasma coloration and body weight gain were determined after the experiment on all the animals. Body weight gain was calculated as: body weight gain = 100 × (body weight at day 23 – body weight at day 16) / body weight at day 16. Plasma coloration is represented by optical density values for every 5 nm wavelength in the range from 380 to 600 nm, using Tecan Infinite® M200 PRO plate reader (Tecan Group Ltd, Männedorf, Switzerland) on 200 µl of plasma samples.

From each box of challenged animals, two birds are selected among the ones having the lowest and the highest body weight gains, forming a subset of 176 challenged birds. Moreover, 8 randomly-chosen control birds were also selected. These 184 birds have been used for measurement of plasma protein content and analysis of plasma protein composition using capillary electrophoresis. Protein capillary zone electrophoresis was performed with the MINICAP system™ (Sebia, France). All procedures were conducted in accordance with guidelines for Care and Use of Animals in Agricultural Research and Teaching (French Agricultural Agency and Scientific Research Agency).

Statistical analysis. Statistical analyses were performed using R statistical software (R Development Core Team, 2013). First, all traits were checked for outliers excluding all values being higher than four standard deviations. As large differences in mortality between boxes were observed,

Table 1: Descriptive statistics, estimates of the challenge effect and P-values for weight gain (WG), plasma protein content (PP), prealbumin (PALB), albumin (ALB), α1-globulin (A1GLB), α2-globulin (A2GLB), α3-globulin (A3GLB), β1-globulin (B1GLB), β2-globulin (B2GLB), γ-globulin (GAGLB).

Traits	Controls		Challenged						P-values
	Mean	SD[1]	High weight gain			Low weight gain			
			Mean	SD[1]	Effect	Mean	SD[1]	Effect	
WG	485.00[a]	121.19	394.4[b]	33.76	-114.75	204.75[c]	35.43	-295.75	***
PP	31.57[a]	1.90	25.34[a]	7.90	-8.50	26.65[a]	9.52	-10.00	NS
PALB	7.89[a]	3.07	5.27[a]	2.38	-5.99	5.78[a]	3.24	-5.05	NS
ALB	55.95[a]	2.88	51.10[a]	4.92	-5.90	45.17[b]	7.01	-2.50	***
A1GLB	2.92[a]	0.48	4.26[b]	0.74	1.96	5.18[c]	1.47	1.60	**
A2GLB	6.15[ab]	0.72	6.43[a]	0.66	-0.14	7.04[b]	1.11	0.27	***
A3GLB	4.35[a]	0.50	6.89[b]	1.48	4.44	8.14[c]	2.13	5.14	**
B1GLB	12.58[a]	1.37	13.84[a]	2.69	2.51	14.82[a]	2.94	-2.09	NS
B2GLB	6.81[ab]	1.09	8.32[a]	1.56	1.52	9.83[b]	3.41	1.73	***
GAGLB	3.36[a]	0.78	3.89[a]	0.97	1.60	4.04[a]	1.58	0.90	NS

Within a row means without a common superscript differ (P < 0.05).
SD: Standard deviation
NS: P-values >0.05 for differences between means
**P-values lower than 0.01
***P-values lower than 0.001

this effect was taken into account as a random box effect for further analyses. Then, challenge effect for each trait was estimated using hierarchical linear model considering box as random effect. Differences between control and challenge group were determined by ANOVA.

Results and Discussion

Plasma coloration. The challenge had a very strong effect on plasma coloration for optical density at 450 to 490 nm (P<0.0001) (Figure 1). Plasma coloration reflects the effect of *Eimeria* infection on the loss of blood carotenoids and is expressed as an optical density of plasma. Plasma carotenoids are solely of food origin and their decrease is related to changes in intestinal absorption and change in production of protein carriers and antioxidants consumption (Yvore et al. (1993)). Figure 1 shows distribution of the averaged optical density values, representing plasma coloration, measured for wavelengths between 390 and 600 nm for both experimental groups at two time points (day 16 and day 23). No significant differences were observed at day 16, even if the challenged animals seamed presenting a lower absorbance around 410 nm. At day 23, the strongest effect of the challenge on the plasma coloration was observed in range from 450 to 490 nm with the highest effects size. In addition, this wavelength interval overlaps with the peak values of plasma carotenoids absorbance spectra which are in the range between 420 and 480 nm (Breithaupt, Weller and Grashorn (2003)). Therefore, our results confirm the previous findings indicating that the plasma coloration is a very sensitive measure for evaluating the level of coccidian infection (Conway et al. (1993); Yvore et al. (1993); Ruff et al. (1974)) and strongly suggests that the best estimation of plasma coloration variation under a coccidiosis challenge can be obtained by measuring optical density between 450 and 490 nm.

Plasma protein content and composition. Total protein content has not been strongly affected in the challenged animals (Table 1) but all globulin electrophoretic fractions except the β1-globulin and γ-globulin were significantly affected by the challenge. Avian α-globulin fractions consist of acute phase inflammatory proteins such as α-lipoprotein, α1-antitypsin, α2-macroglobulin and haptoglobulin. Parasitic infections have been associated with increased levels of avian α-globulins (Harris (2009)). Furthermore, β-globulin fractions of avian plasma proteins were also constituted of acute phase proteins and their levels increase during bacterial and fungal infection, (Harris (2009)). Our results clearly suggest that all fractions, except β1-globulin, associated with acute phase proteins have been significantly elevated. Furthermore, γ-globulin fraction which is primarily composed of antibodies hasn't expressed significant difference. These findings are expected since it is early in animal's life as well as in post challenge time for detection of γ-globulins. Finally, it is known that antibodies don't play a major role in response to coccidiosis.

This is the first time that the results of capillary electrophoresis of plasma proteins are reported in chicken challenged with coccidiosis. Filipovic et al. (2007) reported results on composition of plasma protein fractions in broilers during the fattening period using agarose gel electrophoresis. However, comparison between plasma protein profiles of agarose-gel electrophoresis and capillary zone electrophoresis is not easy since agarose-gel electrophoresis lack results for prealbumin fraction and the fact that α-globulin fractions migrates in to prealbumin fraction in the case of agarose gel electrophoresis (Roman et al. (2013)).

Conclusion

The challenge strongly affected the plasma coloration and we observed that the best estimation of plasma coloration variation under a coccidiosis challenge

can be obtained by measuring optical density between 450 and 490 nm. Furthermore, plasma protein profiles and plasma coloration seem to be good indicators of animal health status. Therefore, results of plasma coloration and protein composition were shown to be a good representation of the animals' sensibility to *Eimeria maxima* challenge and are used for genome-wide association analysis which was conducted on samples from the challenge.

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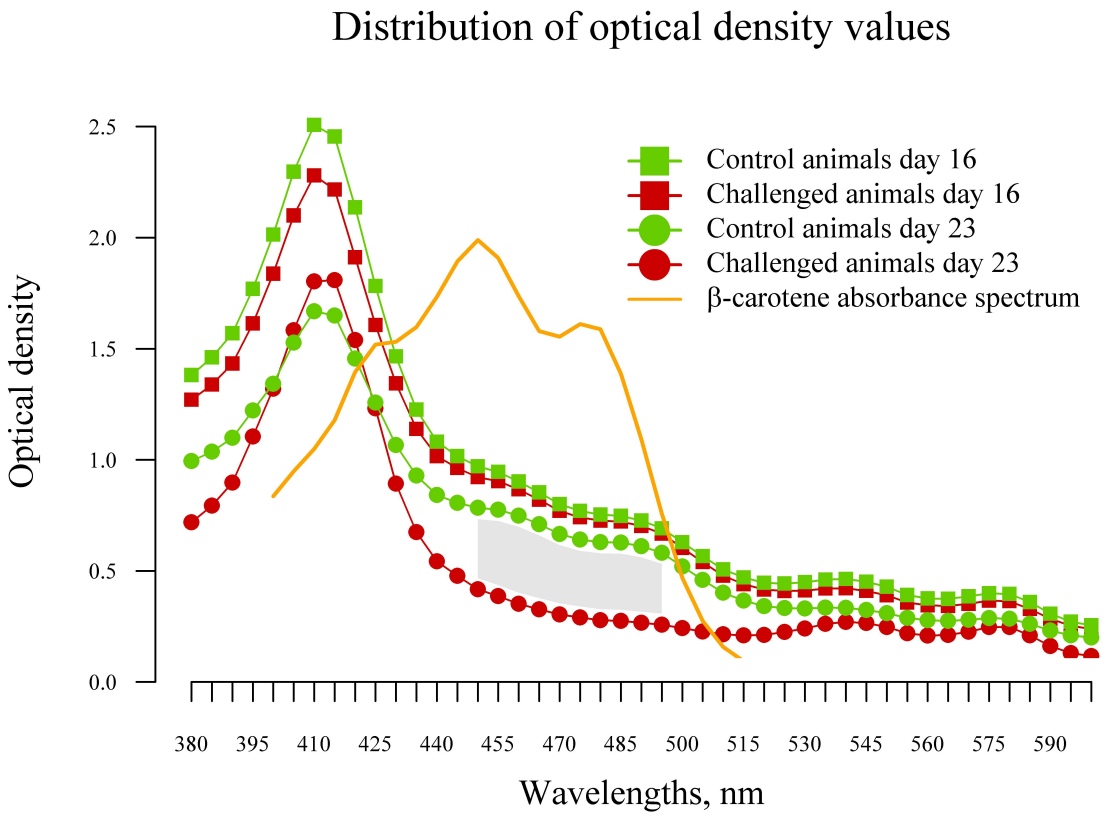

Figure 1: Distribution of the average values of optical density of plasma. Grey area indicates the range with highly significant difference between control and challenged animals on day 23 ($P \leq 0.0001$)