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Large-scale investigation of the parameters in response to *Eimeria maxima* challenge in broilers

E. Hamzic,*†‡[1] B. Bed'Hom,*†[2] H. Juin,§ R. Hawken,# M. S. Abrahamsen,# J. M. Elsen,‖ B. Servin,‖ M. H. Pinard-van der Laan,*† O. Demeure¶**

*AgroParisTech, UMR1313 Animal Genetics and Integrative Biology Unit, F-75231 Paris, France; †INRA, UMR1313 Animal Genetics and Integrative Biology Unit, F-78352 Jouy-en-Josas, France; ‡Department of Molecular Biology and Genetics, Aarhus University, 8830 Tjele, Denmark; §INRA, UE1206 Alternative Animal Husbandry and Health of Monogastric Species, F-17700 Surgères, France; #Cobb-Vantress Inc., 72761 Siloam Springs, AR 72761; ‖INRA, UMR1388 Genetics, Physiology and Breeding Systems, F-31326 Castanet-Tolosan, France; ¶ INRA, UMR1348 Physiology, Environment and Genetics for the Animal and Livestock Systems, Domaine de la Prise, F-35590 Saint Gilles, France; and **Agrocampus Ouest, UMR1348 Physiology, Environment and Genetics for the Animal and Livestock Systems, Domaine de la Prise, F-35590 Saint-Gilles, France

ABSTRACT: Coccidiosis, a parasitic disease of the intestinal tract caused by members of the genera *Eimeria* and *Isospora*, is one of the most common and costly diseases in chicken. The aims of this study were to assess the effect of the challenge and level of variability of measured parameters in chickens during the challenge with *Eimeria maxima*. Furthermore, this study aimed to investigate which parameters are the most relevant indicators of the health status. Finally, the study also aimed to estimate accuracy of prediction for traits that cannot be measured on large scale (such as intestinal lesion score and fecal oocyst count) using parameters that can easily be measured on all animals. The study was performed in 2 parts: a pilot challenge on 240 animals followed by a large-scale challenge on 2,024 animals. In both experiments, animals were challenged with 50,000 *Eimeria maxima* oocysts at 16 d of age. In the pilot challenge, all animals were measured for BW gain, plasma coloration, hematocrit, and rectal temperature and, in addition, a subset of 48 animals was measured for oocyst count and the intestinal lesion score. All animals from the second challenge were measured for BW gain, plasma coloration, and hematocrit whereas a subset of 184 animals was measured for intestinal lesion score, fecal oocyst count, blood parameters, and plasma protein content and composition. Most of the parameters measured were significantly affected by the challenge. Lesion scores for duodenum and jejunum ($P < 0.001$), oocyst count ($P < 0.05$), plasma coloration for the optical density values between 450 and 490 nm ($P < 0.001$), albumin ($P < 0.001$), α1-globulin ($P < 0.01$), α2-globulin ($P < 0.001$), α3-globulin ($P < 0.01$), and β2-globulin ($P < 0.001$) were the most strongly affected parameters and expressed the greatest levels of variation. Plasma protein profiles proved to be a new, reliable parameter for measuring response to *Eimeria maxima*. Prediction of intestinal lesion score and fecal oocyst count using the other parameters measured was not very precise ($R^2 < 0.7$). The study was successfully performed in real raising conditions on a large scale. Finally, we observed a high variability in response to the challenge, suggesting that broilers' response to *Eimeria maxima* has a strong genetic determinism, which may be improved by genetic selection.

Key words: broilers, coccidiosis, disease indicators, *Eimeria maxima*, large-scale study

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INTRODUCTION

Chicken coccidiosis is a parasitic disease of intestinal tract caused by members of the genus *Eimeria* and one of the most common and costly dis-

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[2]Corresponding author: bertrand.bedhom@jouy.inra.fr

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eases. The worldwide costs are estimated to be over US$3.5 billion (£2 billion), with a large part of costs being associated with the decreased growth in broilers and costs for preventive or therapeutic intervention (Shirley et al., 2004; Williams, 1999).

At present, coccidiosis control is based on the use of coccidiostats and vaccination. Use of coccidiostats faces 2 main obstacles: the fast development of drug resistance and increased public concern over chemical residues in food. Moreover, efficiency of multiple-species live vaccines mainly used at the moment is not fully satisfactory (Blake and Tomley, 2014). Therefore, the programs of coccidiosis control must be expanded with a complementary approach such as the use of genetic tools for improvement of host's response to the disease. Considerable variations in susceptibility to coccidiosis were observed between chicken breeds (Long, 1968) and Johnson and Edgar (1982) were the first to successfully perform a divergent selection for resistance and susceptibility to acute cecal coccidiosis. Similarly, large differences of response to *Eimeria* infection were observed between inbred and outbred lines (Bumstead and Millard, 1987; Pinard-Van Der Laan et al., 1998).

With the advent of genomics, several QTL regions associated with the resistance to *Eimeria tenella* and *Eimeria maxima* have been identified (Zhu et al., 2003; Pinard-van der Laan et al., 2009; Bacciu et al., 2014).

The objectives of this study were to evaluate the effects of the *Eimeria maxima* challenge on the measured parameters in broilers and the level of variability in the measured parameters. Additionally, our objective was to identify which parameters are the best health indicators. Finally, we estimated accuracy of prediction of intestinal lesion score and fecal oocyst count using other parameters that can easily be measured.

MATERIALS AND METHODS

Animal Raising and Measurements of Parameters

The experimental study presented in this paper was composed of 2 parts: a pilot and a large-scale challenge (Fig. 1). The pilot challenge was performed on 240 Cobb500 broilers. The building was cleaned and disinfected before arrival of day-old chicks hatched at Cobb facilities. Disinfectant used was Best Top II, which is composed of formaldehyde, glutaraldehyde, and didecyldimethylammonium chloride (Centre Technique d'Hygiene, Romans, France) and disinfection was performed according to product's instructions. Upon arrival, animals were randomly allocated to 12 litter pens (20 individuals in each) where they were kept during the 23 d of the experiment. Pen dimensions were 3 by 1 m. On d 1, animals were weighed and individually tagged. The following lighting program was used: during the first 6 d, the length of darkness was increased for 1 h per day starting with a 1-h darkness period. By d 6, the lighting program consisted of 18 h of light and 6 h of darkness and this lighting program was maintained until the end of the experiment. From d 1 to d 10, animals were fed with the starter diet (as-fed basis, 3,000 kcal/kg ME, 21.10% protein, 1.20% lysine, 0.90% methionine and cysteine, 0.25% tryptophan, 0.81% threonine, 1.03% calcium, and 0.48% available phosphorus). From d 11 to d 22, animals were fed with the grower diet (as-fed basis, 3,100 kcal/kg ME, 19.37% protein, 1.11% lysine, 0.84% methionine + cysteine, 0.22% tryptophan, 0.74% threonine, 0.96% calcium, and 0.45% available phosphorus). On d 8 of the experiment, animals were weighed. On d 16, animals were weighed and blood samples were collected from the wing vein (1 to 2 mL of blood) into BD Vacutainer K2-EDTA tubes (reference 368861; Becton Dickinson, Franklin Lakes, NJ) and stored at 4°C before use. On the same day, animals were individually inoculated per os with 50,000 *Eimeria maxima* oocysts. The inoculation was performed using a syringe without a needle and administering the inoculation dose directly into the esophagus. On d 23, blood was sampled (1 to 2 mL into K2-EDTA tubes) and the following measurements were performed on all animals: BW and measurement of rectal temperature. From each pen, 4 animals were randomly chosen, sacrificed by cervical dislocation, and measured for lesion score and fecal oocyst count. The intestinal lesion scores were estimated macroscopically by the same skilled pathologist on a scale of 0 (no lesion) to 4 (severe lesions), as described by Johnson and Reid (1970). Fecal samples were collected from the intestinal tract just after the slaughter. Oocysts were counted microscopically using a McMaster counting chamber (Dominique Dutsher, Brumath, France) and oocyst excretion was expressed as the number of oocysts per gram of feces (Conway and McKenzie, 2007).

Hematocrit, plasma coloration, and BW gain were determined after the experiment on all the animals. Body weight gain was calculated as BW gain = BW at d 23 – BW at d 16. Hematocrit level and plasma coloration were measured from the blood samples collected on d 16 and d 23. Hematocrit was determined as the volume percentage of red blood cells in a 60-µL capillary tube after centrifugation in Sigma centrifuge (model 1-15P with hematocrit rotor number 11024; SIGMA Laborzentrifugen GmbH, Osterode am Harz, Germany) at 13,684 × g for 5 min at 20°C. Plasma coloration was represented by optical density values of blood plasma for 6 wavelengths: 405, 450, 490, 550, 590, and 650 nm (Yvore et al., 1993).

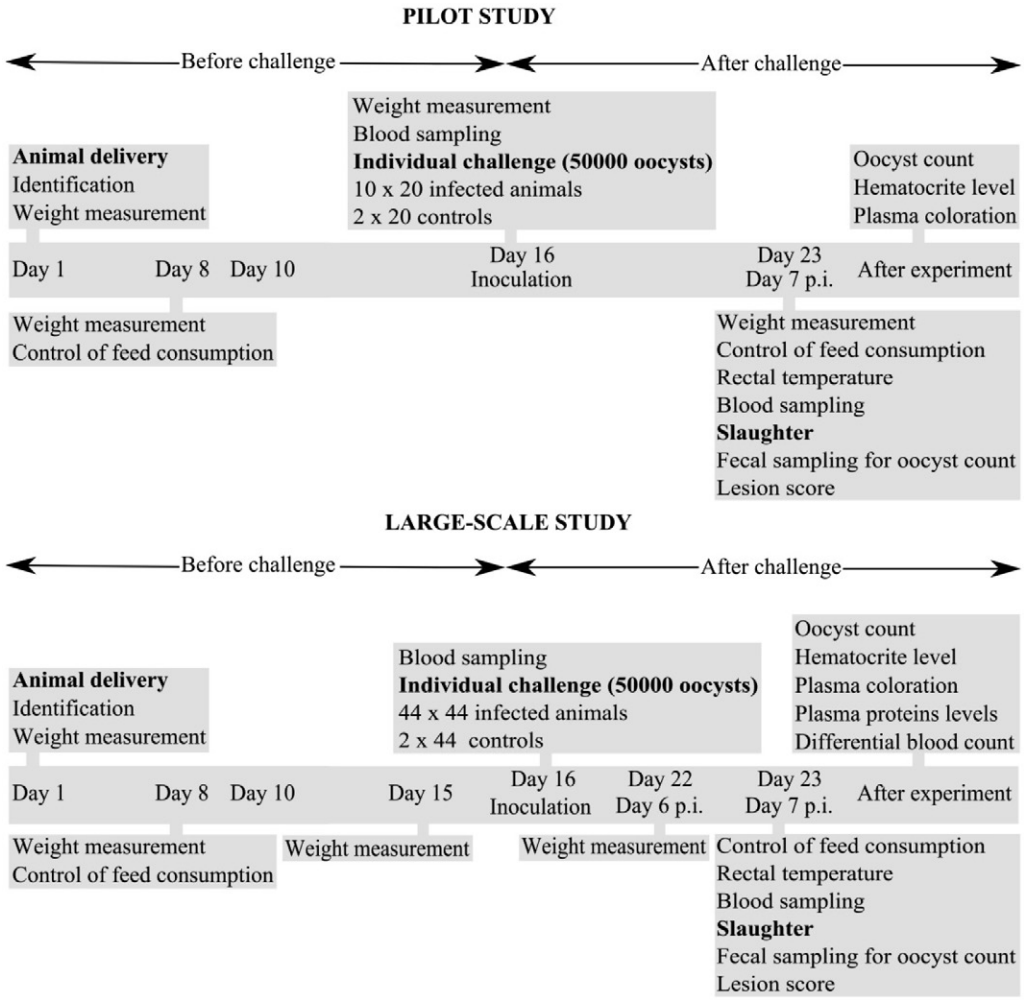

Figure 1. Experimental layout of pilot and large-scale challenge. p.i. = post-inoculation.

The experimental setup for the large-scale experiment was the same as in the pilot study (Fig. 1). The large-scale study was performed on 2,024 Cobb500 broilers randomly distributed in 44 (challenge) and 2 (control) litter pens (3 by 1 m) containing 44 birds each. Due to organization constraints, the animals were weighed at d 15 but challenged and blood sampled at d 16. For the same reasons, the last BW measurement was performed on d 22 and blood sampling, measurements, and euthanasia were done on d 23. Four selected animals from each control litter pen were selected for lesion score and oocyst count measurements. These 2 measures (on 8 animals) combined with the growth curve (on all animals; Fig. 1) were used for assessment of absence of infection in the control population.

Mortality was recorded every day and autopsy was conducted to determine the cause of death. The following parameters were measured on all animals that were still alive at d 23: BW, hematocrit, rectal temperature, and plasma coloration. All measurements except plasma coloration were performed in the same way as in the pilot study. Plasma coloration was 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 blood plasma samples. From each pen of challenged animals, 2 birds among those with the lowest and 2 birds among those with the greatest BW gains were selected based on the ranking per pen, forming a subset of 176 challenged birds. Moreover, in the same way, 8 unchallenged birds were selected from control pens. These 184 birds were used for detailed measurements that included blood cell count, plasma protein content, capillary electrophoresis of plasma proteins, oocyst count, and lesion scores. Blood cell count, plasma protein content, and capillary electrophoresis of plasma proteins were assessed on blood samples collected on d 23 of the challenge. Feces samples for oocyst count were collected at slaughter, on d 23 of the challenge. Blood cell count was performed by classical microscopic observation of blood smears (Campbell and Dein, 1984). Other blood parameters were assessed using an ABX Micro E60 (Horiba Medical, Kyoto,

Japan) hemocytometer: hemoglobin content, mean cellular volume (**MCV**), mean corpuscular hemoglobin (**MCH**), and mean corpuscular hemoglobin concentration. Protein capillary zone electrophoresis was assessed with the MINICAP system (Sebia, Evry, France) and blood analysis was performed by ORBIO Laboratory (ORBIO Laboratory, Bron, France).

All procedures were conducted under license number A176661 from the Veterinary Services, Charente Maritime, France, and in accordance with guidelines for Care and Use of Animals in Agricultural Research and Teaching (National charter on the ethics of animal experimentation; Ministry of Higher Education and Research, 2008).

Statistical Analysis

Statistical analyses were performed using R statistical software (R Development Core Team, 2014). First, data was checked for outliers excluding all values greater than 4 SD. Considering all 53 measured parameters, 267 out of 107,272 data points were regarded as outliers. Hematocrit and plasma coloration were measured twice (on d 16 and d 23). For hematocrit and plasma coloration, measurements on d 16 were considered in the model when testing for the effect of the challenge on measurements on d 23 was performed. Data transformation was applied for parameters not exhibiting normal distribution based on quantile–quantile plots (see tables for trait list). As large differences in mortality between pens were observed, the effect of pen was taken into account in the model. The challenge effects and differences between control and challenge groups for parameters measured on all 2,024 animals were estimated using linear model considering pen and status (challenged and control) as fixed effects and also including interaction between pen and status. Fit of the models was assessed using R^2.

The model described above was used for analysis of the effect of challenge on the parameters measured on the subset of 184 animals. The only difference was that status variable was composed of 3 groups: controls (unchallenged animals) and 2 challenged groups. One of the challenged groups consisted of animals selected for high BW gain and the second group consisted of animals selected for low BW gain. Comparisons of differences between the 3 groups were assessed using *t* tests.

Due to the time required for phenotyping animals, intestinal lesion score and fecal oocyst count were measured on subset of 184 animals. Therefore, we estimated accuracy of prediction for these 2 parameters based on data for parameters measured on all animals. The prediction was performed by partial least squares regression (**PLSR**) using pls package in R (Mevik and Weshrens, 2007). Partial least squares regression models were fitted for duodenum lesion score, jejunum lesion score, oocyst count, and BW gain using all parameters measured, only plasma coloration data, only blood parameters, or only plasma proteins content. The optimal number of principal components for the PLSR model were selected using following procedure: 1) Find a model with the lowest root mean squared error of prediction (**RMSEP**) and note the corresponding R^2 of prediction. 2) Check whether a model with an additional component has an increased R^2 for more than 5% of the previous model. If so, use the next model with larger number of components; otherwise, take the model with lower number of components (B. H. Mevik, University of Oslo, Norway, personal communication).

RESULTS AND DISCUSSION

Validation of the Challenge and Repeatability

The pilot study aimed at validating the challenge procedure for Cobb500 population and the parameters used for measuring the response to *Eimeria maxima*. The mortality observed in the population (6%) was in line with expectation and confirmed that the parameters of the challenge (animal age and infectious dose) were suitable for Cobb500 animals. Effects of the challenge on measured parameters were large enough as well to confirm that the experimental conditions were adapted and indicated that the variability of measured parameters in response to *Eimeria maxima* was present in Cobb500 population (Table 1). Unfortunately, in the pilot study, we observed a small number of oocysts in control animals, which was probably due to cross-contamination, which was avoided in the large-scale study. As the observed effects were very similar between the 2 challenges and the challenged animals were much more numerous in the large-scale experiment, the Results and Discussion section will be focused on results from this last experiment.

Effect of the Challenge

Body Weight Gain. The growth curves of the challenged and control animals are presented in Fig. 2 together with the expected growth curve for Cobb500 animals under standard rearing conditions. These curves clearly show that the growth of animals used in the challenge is not different from the expected growth for Cobb500 animals up to d 16 when animals were inoculated. But the difference in BW gain between the challenged and the control animals after inoculation was significant ($P < 0.001$; Table 2). In both pilot and

Table 1. Descriptive statistics for parameters measured in the pilot study, estimates of the challenge effect, and *P*-values for difference between control and challenged animals

	Control			Challenged				
Parameter	Mean	SD	CV	Mean	SD	CV	Effect[1]	*P*-value
BW gain d 22 – d 16, g	396.78	45.22	11.40	245.89	50.13	20.39	–141.95	***
Rectal temperature, °C	41.29	0.16	0.38	41.16	0.35	0.84	–0.10	*
Hematocrit, %	31.93	2.45	7.69	30.54	3.86	12.65	–1.90	†
Duodenum lesion score	0	0	0	1.20	1.26	105.4	1.20	***
Jejunum lesion score	0	0	0	2.08	0.97	46.58	2.05	***
Oocyst count (OPG)[2]	32.5	82.9	255	130,414	145,383	111.5	1.72[4]	***
Plasma OD,[3] 405 nm	2.30	0.40	17.56	1.60	0.59	37.03	–1.01[4]	***
Plasma OD, 450 nm	1.35	0.18	13.64	0.48	0.16	32.72	–1.56[4]	***
Plasma OD, 490 nm	1.04	0.15	14.12	0.28	0.08	29.44	–1.58[4]	***
Plasma OD, 590 nm	0.55	0.11	20.82	0.26	0.11	40.97	–1.41[4]	***
Plasma OD, 650 nm	0.45	0.12	27.15	0.18	0.09	47.76	–0.47[4]	***

[1]Challenge effect taking pen as a random effect (estimated in units of corresponding parameter).

[2]OPG = oocysts per gram (of feces).

[3]OD = optical density.

[4]Parameters for which data transformation was performed and challenge effect size is not in units of corresponding parameter.

*$P \leq 0.05$; ***$P \leq 0.001$; †$P \leq 0.10$.

large-scale studies, the strongest effect was observed on BW gain. The expected growth curves for Cobb500 and control animals showed no significant difference (Fig. 1). In addition, no oocysts were detected in control animals and the average lesion score was 0.25. These results indicate that control animals were free of the clinical form of infection. The importance of BW gain as a parameter for assessment of coccidiosis effect was extensively investigated. Several studies reported a weak association between BW gain and mortality (Jeffers et al., 1970; Bumstead and Millard, 1987) and Caron et al. (1997) observed a weak association between BW gain and lesion scores. On the other side, Mathis et al. (1984) observed a negative correlation between BW gain and lesion score in chickens challenged with *Eimeria tenella* and *Eimeria acervulina*. These differences in relationship between measured parameters can be due to the many factors such as species of *Eimeria* used for the challenge, sex, age, genetic background of animal, and environmental conditions. A recently published meta-analysis showed that variation in weight gain in broilers challenged with *Eimeria* spp. depends on *Eimeria* species, animal age, sex, and genetic line (Kipper et al., 2013). In addition to its major economic importance, BW gain might be directly affected by the infection and could be interesting to consider in assessment of coccidiosis resistance.

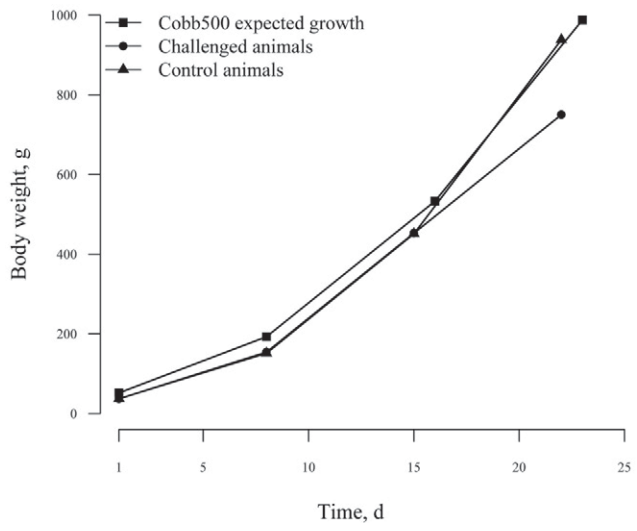

Figure 2. Comparison of BW curves between control and challenged animals and expected growth curves for Cobb500 animals during the large-scale challenge.

Mortality. During the 24 d of experiment, 593 out of the 2,024 animals died (i.e., 29.30%). Figure 3 presents the distribution of the cumulative mortality during the experiment. Only 44 animals died before the challenge, probably because of manipulation stress; all these animals were dissected and no obvious pathology was detected. The main effect of the challenge was observed at d 21, 22, and 23 with the greatest mortality at d 22 (228 dead chickens), which was expected based on the result of previous studies (Johnson and Edgar, 1982). The difference in mortality between the pilot and the large-scale study might be partially explained by an animal density per pen, which was doubled from 6.7 animals/m^2 in the pilot challenge to 14.7 animals/m^2 in the large-scale challenge. Indeed, broilers production mortality has been correlated to stocking density (Dozier et al., 2005). The mortality at d 24 was very low (16 animals), suggesting that animals that survived the most severe period of dis-

Table 2. Descriptive statistics for parameters measured on all 2,024 in the large-scale study, estimates of the challenge effect, and *P*-values for difference between control and challenged animals

	Control			Challenged				
Parameter	Mean	SD	CV	Mean	SD	CV	Effect[1]	*P*-value
BW gain (d 22 – d 15), g	487.22	52.67	10.81	302.13	57.18	18.93	–200.16	***
Rectal temperature, °C	41.64	0.40	0.96	41.42	0.42	1.01	–0.06	*
Hematocrits, %	31.42	2.90	9.23	30.00	4.16	13.87	–1.25	NS[2]
Plasma OD,[3] 450 nm	0.78	0.12	15.38	0.42	0.17	40.48	–2.02[4]	***
Plasma OD, 470 nm	0.67	0.10	14.93	0.30	0.11	36.67	–2.17[4]	***

[1]Challenge effect taking pen as a fixed effect (estimated in units of corresponding parameter).

[2]NS = not significant.

[3]OD = optical density.

[4]Parameters for which data transformation was performed and challenge effect size is not in units of corresponding parameter.

*$P \leq 0.05$; ***$P \leq 0.001$.

ease were able to recover. This mortality kinetics is in accordance with Johnson and Edgar (1982), who observed peak of mortality 120 to 168 h after inoculation and mortality decreased by 216 h.

Mortality rate is a classical parameter for assessment of coccidiosis effect and, along with BW gain and lesion score, is commonly measured in the coccidiosis challenges. Given such relatively high mortality rate, it would be worth comparing surviving and dead animals on a genetic basis.

Hematocrit. Surprisingly, even if hematocrit in the challenged animals seemed slightly decreased with a minor increase of CV, no significant effect was found on the hematocrits level ($P > 0.1$) as well as in the pilot challenge ($P > 0.05$; Table 1). We observed a significant difference for the hematocrit values when comparing individuals from subset of 184 animals (Table 3). Decreased hematocrit levels were previously observed in chicken challenged with *Eimeria tenella* and *Eimeria acervulina* (Conway et al., 1993; Fukata et al., 1997). Such result could be expected for pathogens associated with hemorrhage such as *Eimeria tenella* (Trees, 2008) but is more surprising for *Eimeria acervulina*, which is considered nonhemorrhagic. It does suggest that the effect on the hematocrit level is also strongly influenced by other factors such as infection dose of *Eimeria* species, age, and sex. However, for *Eimeria maxima* (not characterized by strong hemorrhages and bloody droppings), we did not observe a significant decrease for hematocrit level in challenged animals, in agreement with the results of Conway et al. (1993).

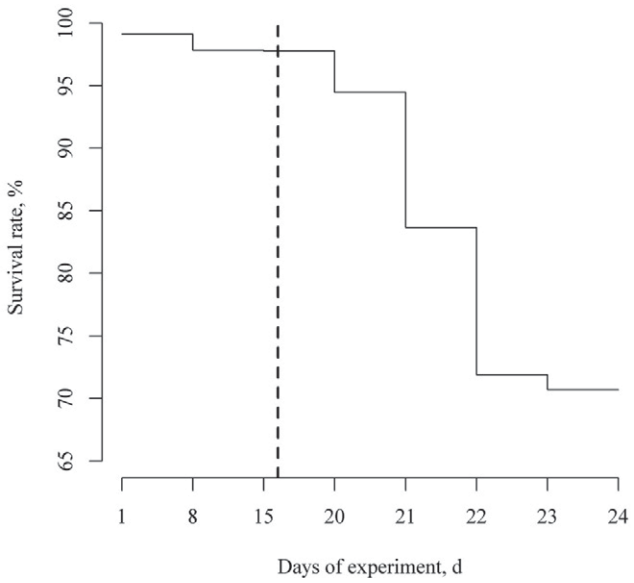

Figure 3. Mortality kinetics duringa the large-scale challenge.

Plasma Coloration. Distribution of the averaged optical density values, representing plasma coloration, measured for wavelengths between 390 and 600 nm for both experimental groups at 2 time points (d 16 and d 23) is presented in Fig. 4. This is the first time that optical density values of blood plasma were analyzed for this wide range of wavelengths. No significant differences were observed at d 16, even if the challenged animals seemed to present a lower absorbance around 410 nm. At d 23, the strongest effect of the challenge on the plasma coloration was observed in the range from 450 to 495 nm with the greatest effects size (Fig. 4). In addition, this wavelength interval overlaps with the absorbance spectra of plasma carotenoids, which is in the range between 420 and 480 nm (Breithaupt et al., 2003). More specifically, the peak absorption values for carotenoids in blood are at 450 and 470 nm and specific results for these 2 wavelengths are presented in Table 2. Blood plasma carotenoids are solely of food origin and their blood plasma decrease is related to changes in intestinal absorption, change in production of protein carriers, and their antioxidant effect (Yvore et al., 1993). Therefore, our results confirm the previous findings indicating that the plasma coloration is a very sensitive measure for evaluating the level of coccidian infection (Ruff et al., 1974; Conway et al., 1993; Yvore et al., 1993) and strongly suggests that the

Table 3. Descriptive statistics for all parameters measured on subset of 184 animals from the large-scale study, estimates of the challenge effect, and *P*-values for difference between groups

				Challenged								
	Control			High BW gain				Low BW gain				
Parameter	Mean	SD	CV	Mean	SD	CV	Effect[1]	Mean	SD	CV	Effect[1]	*P*-value
BW gain d 22 – d 15, g	485.00[a]	121.19	24.99	394.41[b]	33.76	8.56	–114.75	204.75[c]	35.43	17.30	–295.75	***
Hematocrits, %	26.71[ab]	3.50	13.10	23.42[a]	3.78	16.13	–0.50	25.23[b]	3.64	14.43	4.00	**
Plasma OD,[2] 450 nm	0.77[a]	0.14	17.96	0.54[b]	0.39	71.77	–0.36	0.49[b]	0.39	80.32	–2.02[3]	***
Plasma OD, 470 nm	0.63[a]	0.09	14.32	0.36[b]	0.23	62.83	–0.34	0.33[b]	0.23	69.99	–2.17[3]	***
Temperature, °C	41.06[a]	0.20	0.49	41.09[a]	0.42	1.03	–0.17	40.98[a]	0.54	1.32	0.13	NS[4]
Duodenum lesion score	0.25[a]	0.46	185.16	0.93[b]	0.88	94.59	0	1.53[c]	0.81	52.99	1	***
Jejunum lesion score	0.25[a]	0.46	185.16	1.72[b]	1.02	59.24	1.50	2.39[c]	0.74	31.05	2.00	***
Oocyst count (OPG)[5]	0[a]	0	0	44,693[b]	37,317	84	42,750	42,814[c]	56,531	132	2.20[3]	***
Blood parameters												
Erythrocytes count[6]	2.23[ab]	0.20	8.77	2.05[a]	0.26	12.77	0.24	2.16[b]	0.30	13.75	0.56[3]	*
Leukocytes[7]	26,498[a]	7,684	29	31,606[a]	18,243	57.72	11,593	31,040[a]	20,736	66.80	0.05[3]	NS
Heterophils, %	0.47[a]	0.12	26.45	0.53[a]	0.19	35	–0.06	0.52[a]	0.17	33.37	0.13[3]	NS
Lymphocytes %	0.53[a]	0.12	23.01	0.47[a]	0.19	39.71	0.06	0.48[a]	0.17	36.26	–0.13[3]	NS
Heterophils[7]	12,321[a]	4,076	33	15,638[a]	9,842	62	1,842	15,631[a]	15,970	102	–0.07[3]	NS
Lymphocytes[7]	14,176[a]	5,383	37	15,968[a]	13,141	82	9,751	15,410[a]	11,421	74	–0.12[3]	NS
Thrombocytes[7]	121,714[a]	17,241	14	126,238[a]	25,984	21	3,000	130,114[a]	28,281	22	0.33[3]	NS
Hemoglobin, g/dL	10.39[a]	0.88	8.44	9.40[b]	1.18	12.59	0.65	9.64[ab]	1.49	15.42	0.38[3]	*
MCV,[8] fL	144[a]	5.07	3.52	139.61[a]	5.06	3.62	–12.25	137.28[b]	5.04	3.67	–1.76[3]	**
MCH,[9] pg	46.51[a]	1.08	2.32	45.79[a]	1.68	3.68	–1.93	44.60[b]	3.17	7.12	–0.83[3]	**
MCHC,[10] g/dL	32.36[a]	1.12	3.47	32.82[a]	1.46	4.44	1.60	32.51[a]	2.36	7.25	0.76[3]	NS
Plasma protein content and composition												
Plasma protein, g/dL	31.57[a]	1.90	6.03	25.34[b]	7.90	31.20	–8.50	26.65[b]	9.52	35.74	–1.73[3]	***
Prealbumin, %	7.89[a]	3.07	38.90	5.27[b]	2.38	45.29	–5.99	5.78[b]	3.24	56	–2.18[3]	*
Albumin, %	55.95[a]	2.88	5.14	51.10[b]	4.92	9.64	–5.90	45.17[c]	7.01	15.53	–0.80[3]	***
α1-globulin, %	2.92[a]	0.48	16.33	4.26[b]	0.74	17.36	1.96	5.18[c]	1.47	28.43	1.97[3]	**
α2-globulin, %	6.15[a]	0.72	11.63	6.43[a]	0.66	10.21	–0.14	7.04[b]	1.11	15.80	0.03[3]	***
α3-globulin, %	4.35[a]	0.50	11.49	6.89[b]	1.48	21.52	4.44	8.14[c]	2.13	26.11	2.74[3]	***
β1-globulin, %	12.58[a]	1.37	10.91	13.84[a]	2.69	19.46	2.51	14.82[b]	2.94	19.85	0.17[3]	*
β2-globulin, %	6.81[a]	1.09	15.98	8.32[b]	1.56	18.68	1.52	9.83[c]	3.41	34.72	1.15[3]	***
γ-globulin, %	3.36[a]	0.78	23.18	3.89[a]	0.97	25	1.60	4.04[a]	1.58	39.16	0.99[3]	NS

[a–c]Means sharing the same superscript are not significantly different from each other ($P < 0.05$). Column with *P*-values contains the lowest *P*-values among 3 comparisons.

[1]Challenge effect taking pen as a fixed effect (estimated in units of corresponding parameter).

[2]OD = optical density.

[3]Parameters for which data transformation was performed and challenge effect size is not in units of corresponding parameter.

[4]NS = not significant.

[5]OPG = oocysts per gram (of feces).

[6]Erythrocyte count expressed in millions per millimeter^3.

[7]White blood cell and thrombocyte count expressed in absolute number per millimeter^3.

[8]MCV = mean cellular volume.

[9]MCH = mean corpuscular hemoglobin.

[10]MCHC = mean corpuscular hemoglobin concentration.

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

best estimation of plasma coloration variation under a coccidiosis challenge can be obtained by measuring optical density between 450 and 495 nm.

Rectal Temperature. Rectal temperature was also affected ($P = 0.0271$) but with a moderate variation (Table 2). Rectal temperature was not significantly affected when comparing individuals from subset of 184

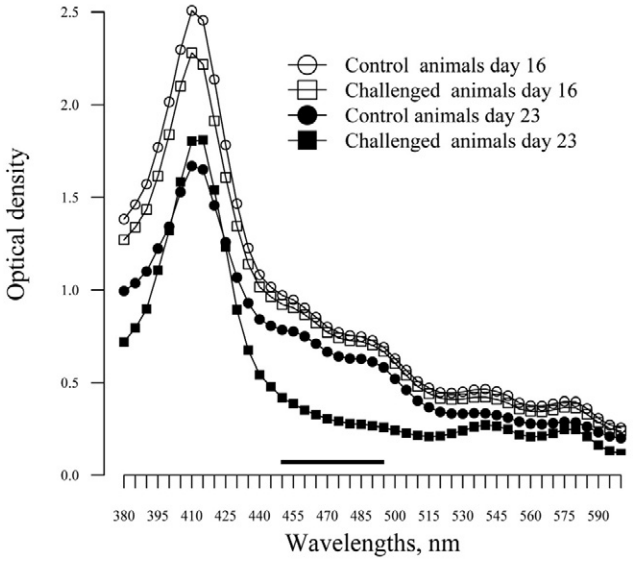

Figure 4. Distribution of the average values of optical density of blood plasma. The horizontal bar indicates the range with the strongest effect between control and challenged animals on d 23 of the large-scale challenge ($P \leq 0.001$).

animals as well as in the pilot study. Rectal temperature reflects the clinical status of animals without providing sufficient information on variability in response to coccidiosis among challenged animals. In addition, we observed low correlation between temperature and other measured parameters. However, repeated measurements at different time points during the challenge would be necessary to fully assess the potential of body temperature as a health indicator in response to coccidiosis.

In the subset of 184 animals selected for extreme BW gain values, some additional parameters were measured: oocyst counts, lesion scores, full blood count, and plasma protein content and composition. The subset of 184 animals was composed of 3 groups of animals: 8 unchallenged animals from 2 control pens, 88 challenged animals having the highest BW gain, and 88 animals with the lowest BW gain.

Lesion Scores. Lesion scores for duodenum and jejunum were significantly affected by the challenge for all challenged animals, those chosen for both low and high BW gain (Table 3). The stronger effect of the challenge on lesion score is observed for the jejunum, where *Eimeria maxima* is mainly localized (Trees, 2008).

Oocyst Count. Whereas not significant, animals selected for greater BW gain had, on average, a greater oocyst count compared to animals selected for lower BW gain (Table 3), which is in accordance with previous studies showing that oocyst count is correlated with other resistance parameters (Williams and Catchpole, 2000). No *Eimeria maxima* oocysts were detected in the control animals, suggesting that no cross-contamination occurred between challenge and control pens during the experiment.

Blood Parameters. Considering the blood parameters, only MCV, MCH, and hemoglobin level were significantly affected by the challenge (Table 3). Mean cellular volume is the standard measure in the full blood count representing the average erythrocyte volume. Decrease in MCV, MCH, and hemoglobin level is common for animals suffering from inefficient feed intake, blood loss, and/or dehydration. However, there are no reports on sensibility of MCV and MCH in chicken suffering coccidiosis. In our case, decrease in MCV, MCH, and hemoglobin level may be caused by malabsorption due to destruction of mucosal integrity. Blood cellular parameters showed low level of variation (Table 3).

Plasma Protein Content and Composition. Total protein content and all globulin electrophoretic fractions except the γ-globulin were significantly affected by the challenge (Table 3).

The decreased level of total plasma protein may be caused by several factors (Trees, 2008) and, in the case of infection caused by *Eimeria maxima*, the most likely cause is malabsorption. In veterinary medicine, reduced prealbumin serum levels are usually associated with acute phase response, trauma, or other disorders such as malnutrition (Myron Johnson et al., 2007).

Avian α-globulin fractions consist of acute phase inflammatory proteins such as α-lipoprotein, α1-antitypsin, α2-macroglobulin, and haptoglobulin. Parasitic infections were associated with increased levels of avian α-globulins (Harris, 2009). The β-globulin fraction of avian plasma is also composed of acute phase proteins and during bacterial and fungal infection, their levels increase (Harris, 2009). Our results clearly suggest that all fractions associated with acute phase proteins have been significantly elevated. Furthermore, the γ-globulin fraction, which is primarily composed of antibodies, did not express significant difference, which is expected for several reasons: it is early to observe an increase in the γ-globulin fraction just 7 d after the challenge and, additionally, antibodies do not play a major role in response to coccidiosis (Min et al., 2013).

This is the first time that the results of capillary electrophoresis of plasma proteins are reported in chicken challenged with coccidiosis. Filipović et al. (2007) reported results on composition of plasma protein fractions in broilers during the fattening period using agarose gel electrophoresis. Still, comparison with this study was not possible because that study lacked results for prealbumin fraction. Furthermore, even for other fractions, comparison between plasma protein profiles of agarose gel electrophoresis and capillary zone electrophoresis is not straightforward

due to the migration of α-globulin fractions into the prealbumin fraction in the case of agarose gel electrophoresis (Roman et al., 2013).

Altogether, results of capillary electrophoresis suggest that the plasma protein profiles are a good representation of the animal's response to the challenge.

Variation in Response to Eimeria maxima *within the Challenged Animals*

One of the goals of this study was to analyze individual variation in response to *Eimeria maxima* within challenged animals. Oocyst count showed the greatest level of variation together with lesion score for both the duodenum and the jejunum (Table 3). The results obtained for these classical parameters were in agreement with the variations observed in the pilot study and results obtained by challenges with *Eimeria tenella* (Mathis et al., 1984; Pinard-van der Laan et al., 2009). In addition, plasma coloration expressed a strong variation in response to coccidiosis, which makes it a reliable resistance parameter, especially in combination with its sensitivity and the fact that it is easy to measure. Moreover, we observed strong variation among all plasma electrophoresis fractions, suggesting that the plasma protein profile is potentially a very informative set of parameters, being already confirmed as a reliable clinical test in avian medicine (Harris, 2009).

Among significantly affected parameters, only MCV and MCH had a CV under 10%. These strong variations observed between the challenged animals are even more important because the results were obtained in a highly controlled environment, suggesting that the genetic factor had a strong contribution.

Prediction of Lesion Score and Oocyst Count

All the animals were measured for a group of traits and a subset of 184 animals was measured for additional parameters. The main reason for this was a technical limitation for acquiring parameters such as lesion score and fecal oocyst count on all animals. Therefore, one of the goals of this study was to estimate prediction accuracy for intestinal lesion score and fecal oocyst count based on parameters measured on all the animals. Along with these parameters, we included BW gain as a classical parameter for measuring response to *Eimeria* infections to estimate how much variance in BW gain is explained using the rest of the phenotype data. Partial least squares regression analysis results are listed in Table 4. The greatest amount of variance explained by PLSR analysis was obtained for duodenum (65.69%) and jejunum (66.07%) lesion scores (Table 4). Most of the information used for this prediction of lesion scores was equally brought by all 3 sources of information and most of the prediction of oocyst count was brought by plasma coloration data. On the contrary, BW gain predictions were improved when using both blood parameters and plasma protein data, even if plasma protein contributed more to the prediction.

Table 4. Proportion of variance explained and root mean squared error of prediction (RMSEP) for partial least squares regression models with ratio of the RMSEP and SD of parameter. Analysis performed on data from the large-scale study.

	R^2 [1]	RMSEP[2]	RMSEP/SD
Parameter	All parameters used in model		
BW gain d 15 – d 23	55.65	83.00	1.45
Lesion score – jejunum	66.07	0.63	0.70
Lesion score – duodenum	65.69	0.64	0.66
Oocyst count	31.93	28.16	0.80
	Only plasma coloration data		
BW gain d 15 – d 23	23.10	99.97	1.75
Lesion score – jejunum	57.38	0.66	0.73
Lesion score – duodenum	51.88	0.63	0.66
Oocyst count	31.96	30.71	0.87
	Only blood parameters data		
BW gain d 15 – d 23	34.98	88.10	1.54
Lesion score – jejunum	55.42	0.66	0.73
Lesion score – duodenum	56.00	0.66	0.68
Oocyst count	11.07	29.00	0.82
	Only plasma proteins data		
BW d 15 – d 23	42.99	82.85	1.45
Lesion score – jejunum	59.17	0.63	0.69
Lesion score – duodenum	62.48	0.60	0.63
Oocyst count	26.25	28.50	0.81

[1]Proportion of variance explained by model.

[2]RMSEP is a measure of deviation of predictions from real value.w

In addition of low amount of variance explained whatever the model used, with the greatest value not exceeding 70%, we also observed a large deviation between predicted and real values (RMSEP). Blood parameters, plasma coloration, hematocrit level, and rectal temperature were not shown to be reliable predictors of fecal oocyst count or intestinal lesion scores, which can be explained by a low correlation between lesion scores and oocyst count and other parameters measured. The greatest correlations were observed between oocyst count and erythrocyte count (0.65) and hemoglobin (0.68) whereas for all other parameters, correlation values were low (see Supplementary data).

Conclusion

The results of the pilot and large-scale studies showed a high variability among challenged animals. The challenge affected most of the measured param-

eters, but intestinal lesions scores, plasma coloration, and plasma protein profiles seem to be the most interesting as indicators of animals' health status. However, the intestinal lesion score measurement is invasive and cannot be performed on large number of animals and their prediction using blood parameters and plasma coloration data or both is not fully reliable, reducing its application for use in selection. Even if many other parameters can account for the observed variability, previous studies on coccidiosis demonstrated that the susceptibility to coccidiosis has a high genetic component (Rosenberg et al., 1954; Pinard-Van Der Laan et al., 1998). Taking advantage of the size and structure of this design, genomewide association studies will be performed to identify regions of the genome underlying this determinism. The high reproducibility of the results between the pilot and large-scale studies is encouraging, and if a large genetic component is found for these measured parameters, they could be used in the prospect of genomic selection application. We believe that new parameters such as plasma protein profiles and the large-scale measurement of blood plasma absorbance can be used to assess the effect of infection caused by other *Eimeria* species.

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