

## **RESEARCH ARTICLE**

# A comparative study on the susceptibility of different Algerian native chick phenotypes and Cobb 500 broiler strain to coccidiosis

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#### ABSTRACT

The purpose of this study was to evaluate the susceptibility to coccidiosis in three Algerian indigenous chicken phenotypes (normal feathered, crested head and naked neck) and one commercial strain Cobb 500. A total of 112 chicks were used in this evaluation. The chicks were done with a 20-fold dose ( $6 \times 10^4$  sporulated *Eimeria spp* oocysts) of a live attenuated coccidiosis vaccine. At 28 days of age, clear differences were observed between genotypes in the effects of challenge on mortality due to coccidiosis from day 1 to 7 post-inoculation. The commercial strain Cobb 500 had the highest mortality (28.5%), while the naked neck chicks showed no mortality at post-inoculation. The lesion scores and the oocyst load were also considerably higher (p < 0.05) in the Cobb 500 strain as compared to indigenous genotypes. The naked neck birds had the highest oocyst counts (p < 0.05) compared to their crested head and normally-feathered counterparts. However, there were no differences (p > 0.05) in terms of intestinal lesion score among the indigenous genotypes. Furthermore, the severity of bloody diarrhea was milder in naked neck birds than in the other genetic groups. These findings indicate that the naked neck genotype is associated with remarkable and better tolerance to coccidial infection compared to other genotypes. However, further studies should be conducted to investigate the potential utilization of naked neck birds in poultry breeding programs aimed at controlling the effects of the disease.

Keywords: coccidiosis, native chicken, major gene, resistance

# **INTRODUCTION**

Coccidiosis is the most severe and economically important disease all over the world in the avian industry (Arabkhazaeli et al., 2013). This enteric disease is caused by Eimeriaspp (Abbas et al., 2017). Exotic and domestic chickens are considered susceptible to seven species of Eimeria with Eimeria maxima being one of the prevalent causes of coccidiosis in poultry production (Boulton et al., 2018). Coccidiosis mainly affects young birds between 3 and 8 weeks of age, when the immune system is immature (Fatoba and Adeleke, 2018). The extensive damage to the intestinal epithelium caused by Eimeria has various clinical features such as decreased feed conversion rates, poor weight gain, reduced welfare, bloody feces, and high mortality (Abbas et al., 2017)resulting in enormous economic losses which have been estimated more than \$3 billion per year worldwide (Chapman, 2014). Currently, the strategy to control coccidiosis relies on chemoprophylaxis and anticoccidial feed additives. However, it has been found that long-term use of anticoccidials has led to the emergence of drug resistant strains (Xie et al., 2020). Moreover, there are some concerns about drug residues in poultry meat that threaten food security and health (Tang et al., 2018).

Increasing genetic resistance to coccidiosis represents a promising complementary control strategy (Mpenda et al., 2019). The native African chicken is a potential candidate chicken population for the selection of resistant chicken to viral infections in a changing environment attributed to climate changes (Mpenda et al., 2019). Some major genes such as the naked neck and frizzled feather are believed to confer not only adaptability to hot ambient temperatures, but also, be resistant to diseases and greatly enhance immune status (Abou-Emera et al., 2017;Dahloum et al., 2018). However, even though several studies on the performance of the native birds in Algeria have been reported (Zouaoui et al., 2023) there is a dearth of literature available on their genetic variability for resistance to diseases. Therefore, this preliminary study was set up to measure susceptibility to coccidiosis in three local Algerian chicken phenotypes to reduce the cost of the disease and increase the profitability of chicken farmers.

## MATERIAL AND METHODS

#### Animals and experimental design

The study was carried out at the Research Farm of Abdelhamid Ibn Badis, University of Mostaganem, Algeria. A total of 112 one-day-old chicks consisting of 84 Algerian local chicks of three phenotypes (28 naked neck, 28 crested head, and 28 normally feathered), and 28 Cobb 500 broiler chicks were used in the current study. One-day-old chicks of each genetic group were separated into groups of seven birds with three replications for the challenged birds, and one group of seven birds remained as uninfected control. According to Soutter et al. (2020), a minimum of five birds per treatment group is required for the evaluation of differences between lesion scores due to variability in coccidiosis response. Each group was housed in a separate cage (1m x 0.5m), and birds were kept at identical stocking density (7 birds/0.5 m2). The cages had their floor covered with clean wood shavings until 28 days of age. The cages were equipped with a hand-filled round feeder, and two nipple drinkers were supplied with drinking water. All groups were housed in similar conditions within hen houses, according to the recommendations of the Technical Institute of Livestock of Baba Ali (ITELV, Algeria). The ambient temperature was maintained at 32°C for the first two weeks of the experiment and was gradually reduced to 24°C by 21 day of age, after which no heat was provided. Light was supplied continuously throughout the experiment. The control groups were kept in an isolated enclosure to prevent accidental exposure to contamination according to Akanbi and Taiwo (2020). The birds had ad libitum access to a commercial diet from arrival until the end of the experiment. A starter diet was given from hatch to 8 days (2850 kcal/kg; 21% CP), a grower diet from 9 to 20 days (2900 kcal/kg; 19.5 % CP), and a finisher diet from 21 to 28 days (2950 kcal/kg; 18% CP). The diets used did not contain coccidiostats. Prior to the commencement of the experiment, fecal samples from all chicks in each group were examined to confirm the absence of coccidian oocysts. Eimeria oocysts in each fecal sample were detected through coprological flotation technique, employing a saturated sodium chloride (NaCl) solution as the flotation medium. The experiment lasted for a duration of 28 days.

## Coccidiosis challenge

At 21 day of age, all the experimental groups, except the control groups were challenged per os with a 20-fold dose of Paracox-8, equivalent to  $6 \times 10^4$  sporulated oocysts of Eimeria acervulina, Eimeria maxima, Eimeria mitis, Eimeria brunetti, Eimeria praecox, Eimeria necatrix, and Eimeria tenella. The control groups were given 1 ml of Hanks Balanced Salt Solution (HBSS). After the coccidiosis infestation, mortality was recorded daily for each challenge group. From day 5 to 7 post-infection, samples of chicken feces were collected daily and taken to the laboratory for calculation of the number of oocysts per gram of feces (OPG). The collected samples were cleaned of litter, thoroughly homogenized, and analyzed for Eimeria oocysts according to the modified method described by Conway and McKenzie (2007), using a McMaster counting chamber and a microscope. Briefly, a suspension was prepared by mixing 10% (w/v) of collected feces with a saturated solution of sodium chloride (aqueous NaCl). The samples were shaken, and 1 ml of the suspension was diluted with 9 ml of a saturated NaCl solution. A specific volum of the suspension

was then placed in the McMaster chamber, and light microscopy was used to count the Eimeria oocysts under 10× magnification. Triplicate counts were performed for each sample. Daily observation of the presence or absence of blood in feces was carried out for each infected group from days 3 to 7 inclusive.On day 7 post-inoculation (28 days old), three birds from each challenge group were humanely killed by cervical dislocation to evaluate the lesion score on different segments of the small intestine and ceca based on macroscopic visible lesions caused by Eimeria. The lesions included petechial hemorrhages, bloody fecal contents, thickening of the cecal wall, and mucoid discharge. Depending on the severity of the lesions, a score of 0 (no lesions), 1 (mild lesions), 2 (moderate lesions), 3 (severe lesions), or 4 (numerous severe lesions)was determined for each bird as proposed by Johnson and Reid (1970).

### Statistical analysis

Comparisons of the mean values of oocyst production and lesion score were performed by one-way analysis of variance using the GLM procedure of SPSS (v.20) followed by a post-hoc analysis using Tukey's t-test. The Chi-Square test was processed to analyze differences in survavibility rate values between the different genotypes. Statistical significance was established to be P < 0.05.

### **RESULTS AND DISCUSSION**

### Mortality

The most common diseases that cause considerable losses in poultry production in Algeria according to several Colibacillosis, Mycoplasmosis, investigations are Salmonellosis, Newcastle (local name, Ettaoun), Gumburo, infectious bronchitis, and Coccidiosis (Halbouche et al., 2009; Debbou-Iouknane et al., 2018). However, genetic research into parasitic infections of chickens is lagging behind that of viral and bacterial diseases. According to Mathis *et al.* (1984) mortality rate which is the selection trait in most coccidial selection programs is correlated with resistance to coccidiosis. In the current study, mortality due to experimental *Eimeria* coccidiosis was low (9.5%) in both normal feathered and crested head birds, while the highest mortality rate (28.5%) was observed in Cobb 500 broiler chicks (Table 1). However, no deaths occurred in the naked neck and the non infected groups. The present results are in line with what was reported by Dakpogan et al. (2012) who observed that the naked neck chicks seemed to have better survivability (100%) than silky feathered (90%) and frizzle feathered (70%) phenotypes, while normal feathered and dwarf chicks had the lowest survival rate (60%). In another study, Pinard-van der Laan et al. (2009) found a high mortality rate of 26% in Eimeria challenged White Leghorn broiler chicks and no mortality in the Egyptian Fayoumi birds.

#### **Oocyst shedding**

The three indigenous genotypes had nearly similar body weights at 3 weeks of age, ranging from 71.4 g to 74.25 g, while the Cobb 500 strain has significantly (P<0.05) the highest body weight of 237 g as compared to the three local genotypes. As shown in table 1, significant differences

(p<0.05) between genotypes for the oocyst shedding were observed. The commercial Cobb 500 birds had higher mean fecal oocyst counts (960.4  $\times$  10<sup>3</sup>) followed by the crested head (366.5  $\times$  10<sup>3</sup>) and normally feathered chicks (305.3  $\times$  $10^3$ ) while the naked neck birds showed the lowest oocvst output  $(3.25 \times 10^3)$ . However, no oocysts hedding was observed in the unchallenged groups throughout the postinoculation period. The findings of the current study are in conformity with those reported in Ethiopia by Gari et al. (2008) where the overall oocyst count in the native chickens was much lower than that in the exotic Rhode Island Red strain. Similarly, Ayssiwede et al. (2011) found that the oocyst production was significantly lower in Senegal freerange chicks in comparison with Cobb 500 and Hyline W-36 broiler chicks. Ojimelukwe et al. (2018) reported that Marshal broiler chicks inoculated orally with approximately  $23 \times 10^4$  Eimeria spp sporulated oocysts produced on average  $1023 \times 10^3$  oocysts per gram of feces from day 5 to day 7 post-inoculation. In Nigeria, Adenaike et al. (2016) observed a higher fecal oocyst output in normal-feathered chickens compared to the naked neck and frizzled counterparts, although genotype had no significant effect on this parameter. In another study, Dakpogan et al. (2012) recorded the fewest oocyst count in naked neck chicks compared to silky, frizzled, and normal-feathered chicks. Furthermore, it has been demonstrated that prolonged storage of E. tenella oocysts has a considerable impact on the peak point and pattern of oocyst count (Cha et al., 2018). In the current study, there was no oocyst production in the uninfected control groups throughout the experimental period as the chicks were not infected with Eimeria oocysts.

#### Lesion scores

The mean value of the intestinal lesion score ranged from  $0.80 \pm 0.10$  to  $2.82 \pm 0.12$ . The highest lesion score was noticed in Cobb 500 chicks. Most broiler chicks (82%) showed lesion scores of 3.0, whereas indigenous chicks had more frequent (81% on average) lesion scores of 1.0. However, there were no significant differences between the indigenous phenotypes for this parameter. The findings of the current study are in agreement with those of Ayssiwede et al. (2011) who reported the highest mean values of lesion scores in commercial strains Cobb 500 and Hy-line W-36  $(1.44 \pm 0.50 \text{ and } 1.20 \pm 0.40, \text{ respectively})$ , and the lowest score in Senegal native chicks (0.83  $\pm$  0.38). Similarly, Thakur et al. (2016) studied the genetic differences in susceptibility due to coccidial challenge in five commercial lines and Kadaknath breeds of chicken. They found a lesion score up to 4.0 in Cobb strain and Caribro-91. In naked neck and Hubbard it ranged from 1.0 to 3.0 while Kadaknath and Jabalpur dual colored breeds exhibited more resistance to coccidia with the lowest lesion score ranging from 1.0 to 2.0. In another study, Qaid et al. (2021) found a higher mean lesion score of 2.6 in the commercial Ross 308 broiler chickens challenged with 10<sup>4</sup> E. Tenella sporulated oocysts. However, Zulpo et al. (2007) found that all commercial Cobb broiler chicks infected with  $2 \times 10^4$  sporulated oocysts of E. tenella, E. maxima, and E. acervulina had a mean lesion score less than 2.0. Soutter et al. (2021) found no significant variation in lesion scores between Lohmann Brown, Hy-line Silver Brown, and Hy-line Brown layers infected with different challenge doses of E.tenella sporulated oocysts (250,4.000, 8.000, or 12.000). In this

study, although there were no significant differences (p>0.05) among the native genetic groups, the naked neck birds showed less severe lesions in comparison with their normally-feathered and crested head counterparts. Dakpogan et al. (2012) found that the naked neck and frizzle feathered birds had significantly the lowest average lesion scores (1.00  $\pm$  0.5 and 1.2  $\pm$  0.3, respectively) compared to dwarf birds counterparts  $(2.5 \pm 0.4)$  while the silky feather and normalfeathered phenotypes showed intermediate values  $(1.4 \pm 0.5)$ and  $1.5 \pm 0.4$ , respectively). The favorable effect of some major genes against susceptibility to coccidiosis has also been demonstrated by Adenaike et al. (2016) who stated the frizzled and naked neck phenotypes have clear-cut potential to resist the disease with an average lesion score of 1.0  $\pm$ 0.32 and 1.58  $\pm$  0.53, respectively, compared to normalfeathered birds  $(2.50 \pm 0.93)$ . Jatau *et al.* (2012) studied the preponderance of the Eimeria in Zaria (Northwestern Nigeria), they found that 31% of the naturally infected broilers were moderately and severely infected with coccidia, while only 8% of the infected local chickens had low grade and severe infections.

### **Blood** in feces

Except for the naked neck birds, where blood in feces was recorded from post-inoculation days 4 to 5, bloody diarrhea was observed in all infected experimental birds from postchallenge days 4 to 6 (Table 2). However, bloody diarrhea (melena) did not occur in the uninfected control groups throughout the experimental period. Divergent results were reported by Ngongeh et al. (2017) who observed that the infected Nigerian indigenous chickens passed bloody diarrhea for 6 days from day 8 to 14. According to Kaingu et al. (2017) post-infection days 5 and 6 are the most important days in the life cycle of *E. tenella*. In this study, the severity of bloody diarrhea was milder in the naked neck chicks than in the normal feathered and crested-head genotypes, while the commercial birds were associated with more severe bloody diarrhea. However, the present findings are in contrast with those of Ngongeh et al. (2017) who reported that Nigerian native chickens revealed more severe clinical signs in comparison with exotic ones. These differences might be attributed to different factors including breed, sex, and age of birds, the Eimeria strain and the dose of cocci challenge used.

**Table 1.** Survival rate (%), means (Standard errors) for oocyst output per gram fresh feces (OPG) and lesion score (LES), and distribution of LES (%) for each genetic group.

	Phenotype				
Parameter	NF	NN	CH	Cobb500	
Survivability (%)	90.5 <sup>b</sup>	100.0 <sup>a</sup>	90.5 <sup>b</sup>	71.5°	
OPG <sup>1</sup>	305.3 <sup>b</sup>	3.24 ª	366.5 °	960.4 <sup>d</sup> (9.7)	
	(4.1)	(0.12)	(11.7)		
LES <sup>2</sup>	1.00 <sup>b</sup>	0.80 ª	1.21 <sup>b</sup>	2.82 ° (0.12)	
	(0.11)	(0.10)	(0.11)		
LES (%)					
0	0	20	7	0	
1	100	80	64	0	
2	0	0	29	18	
3	0	0	0	82	
4	0	0	0	0	

<sup>a-d</sup> Values within lines with no common superscript indicate significant differences (p < 0.05) between genotypes. <sup>1</sup>10<sup>3</sup> Oocyst per gram of feces. <sup>2</sup>LES: Lesion score measured at 7 days post-inoculation. SE= Standard Error; NF: Normal-feathered; NN: Naked neck; CH: Crested head.

Group	Days post-infection				
	3 day	4 day	5 day	6 day	7 day
Normal-feathered	-	+	++	+	-
Naked neck	-	+	+	-	-
Crested head	-	+	++	+	-
Cobb 500	-	++	+++	++	-

Table 2. Blo	oody diarrhea	a degree.
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- normal, + mild, ++ moderate, +++ severe

#### CONCLUSION

The present study is the first to evaluate the genetic variability in the response of Algerian native chickens to experimental *Eimeria* induced coccidiosis. These results demonstrate that the naked neck genotype appeares to be associated with better tolerance to coccidiosis compared to other genetic groups. However, further investigations are needed to fully exploit the advantage of the naked neck gene, especially in hot climates, and to identify other genotypes that may exhibit resistance to coccidiosis among Algerian indigenous chickens with the goal of reducing disease-related damage and ultimately contributing to increased productivity and family farm income.

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