Journal of Animal & Plant Sciences, 2012. Vol. 14, Issue 3: 1978-1984
Publication date: 30/7/2012, http://www.m.elewa.org/JAPS; ISSN 2071 - 7024

Comparative sensitivity of different phenotypes of free-range chicks to *Eimeria tenella* coccidiosis in Benin

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Keywords: Naked neck, dwarf, frizzled, silky, normal feathered

1 SUMMARY

Sensitivity to coccidiosis was tested on 200 free-range 21 day--old chicks of 5 phenotypes, naked neck, dwarf, normal, frizzled and silky in a factorial design. Two chicks (male and female) per cage in ten replications were used in each phenotypic group. In total 20 chicks infected and other 20 uninfected controls in each phenotype were used. All the experimental chicks have statistically the same body weight ($P > 0.05$) and were experimentally challenged with 6×10^4 *Eimeria tenella* oocyst doses. There was similar body weight gain and significant difference ($P < 0.05$) in disease traits between phenotypes of chicks. Naked neck was the most tolerant phenotype and have the lowest lesion score (1 ± 0.5), lowest proportion of bloody feces (0% at the 6th day post infection), the highest survivability (100%), the fewest Oocysts Per Gram (OPG) (733) and the lowest reduction in packed cell volume (1.5%). The most sensitive phenotype was the dwarf with significantly higher values of lesion score (2.5 ± 0.4), mortality (40%), OPG (604400) and reduction of packed cell volume (12.5%). The other 3 phenotypes of chicks: normal feathered, silky and frizzle have similar ($P > 0.05$) sensitivity to the infection in terms of lesion score, survivability, bloody feces and oocyst excretion. The significant sensitivity variability observed in this preliminary study, suggests a great disease tolerance potentiality in free-range chicken population that can be valuably exploited in selection programs. Further studies are required to understand the real mechanism underneath the herein established *Eimeria tenella* coccidiosis divergent expression among free-range chicken phenotype.

2 INTRODUCTION

Nowadays, commercial poultry production, allows rapid propagation of diseases among the birds. High density of birds increases the risk of disease transmission, genetic homogeneity of the flock, preventing the barrier role of most resistant bird genotypes or the sanitary quality of the selected flock which disables every resistant genotype natural selection (Calenge *et al.*, 2011). Natural disease resistance is underused in poultry selection program dominated by an increasingly strong interest for high production performance driven genes. Aside from some zoonoses hardly controllable by vaccination such as salmonellosis and collibacilosis, coccidiosis control is the most costly operation in commercial poultry production system (Williams, 1999) and one of the most economically important protozoan diseases of poultry caused by *Eimeria spp* in chickens (Schwartz, 1994). It is Therefore a targeted pathology of major interest, in disease resistance designed poultry selection program (Davies *et al.*, 2009). Selection of production

disease resistant birds, particularly, coccidiosis is an attractive poultry scientific investigation field.

Early evidence of genetic differences in resistance of chickens to coccidiosis was provided by Rosenberg (1941), who found significant variations in survival of five breeds of chickens under coccidiosis challenge and Jeffers (1969), reported sex differences in resistance to *Eimeria tenella*. Bumstead and Millard (1987) exposed 3 weeks - old chicks of different breeds and inbred lines of chickens to several *Eimeria* species and measured disease resistance by changes in body weight, mortality and oocyst output. Pinard *et al.* (1998) found the Egyptian Fayoumi to be the most resistant of the five outbred lines tested based upon mortality, lesion scores and growth reduction. Recent studies conducted by Kim *et al.* (2009), pointed out a coccidiosis sensitivity variability among two genetically distinct local free-range chicken breeds. As opined by these authors, a genetic determinant exists in the Major Histocompatibility Complex (MHC) that influences the bird sensitivity to coccidiosis by controlling the local and systemic expression of immune cytokine and chemokine molecules. The increasingly high interest for drug residue-free poultry product by health-conscious consumers, the appearance of disease resistant new pathogen strains and the spectacular development of poultry genomic optimistically allow to think of better future for disease resistance driven selection program. The current study come up with the proof of some variation in sensitivity to *Eimeria tenella* coccidiosis among Benin local phenotype of free-range chicks, the normal, the silky, the frizzled feathered, the naked neck and the dwarf (Host, 1988).

3 3 MATERIALS AND METHODS

3.1 Day-old-chick: Free-range day-old chicks were produced at a Centre of the Agricultural Research Institute and reared for 21 days before being transferred into experimental cages in pens. Chicks were identified with numbered rings fixed on the radius covering skin at the right wing and housed in a wire-floured starting pen under 22-h lighting and held at initially 27°C up to 21 day-old. The chicks have free access to feed and drinking water.

3.2 *Eimeria tenella* and inoculation: *Eimeria tenella* oocysts preserved in 2% potassium dichromate solution were generously provided by the infectiology laboratory of INRA, Tour, France and kept in a refrigerator (2-5°C) until use. All the feces produced by each cage of birds, during the 24 h before the experimental infection, were examined to confirm the absence of any oocysts. Each coccidia-free chick was challenged orally with a dose of 6 x 10^4 oocysts.

3.3 Experimental groups and data collection: Twenty experimentally infected and twenty uninfected 21 days-old -free range chicks made with Lifetest procedure still in SAS (v.o. 9.2). per phenotype (normal feathered, naked neck, dwarf, silky and frizzled) were housed two (male and female) per cage with ten replications in each phenotypic group. Lesion score (Johnson and Reid, 1970), blood in feces, and total death were recorded in the first 7 days post inoculation period as well as Oocysts excretion from day 7 to day 14 post inoculation period. Blood samples were taken from the jugular vein at 7th day post inoculation and packed cell volume determined as a percentage in laboratory according to the microhaematocrit method of Benjamin (1985). At the beginning, the weights of the experimental chicks were not different ($p > 0.05$).

3.4 Statistical analysis: The descriptive and inferential analysis applied to Lesion scores, body weight gain and packed cell volume were made using the GLM procedure of SAS (v.o. 9.2). The Oocysts Per Gram was analyzed with Univariate procedure. The survival analysis with the estimation of the survival proportion (Mean ± Standard Error), was

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4 RESULTS

4.1 Survivability and lesion score: No death was found in naked neck phenotype chick group (Table 1). It survivability (100%) was significantly higher than that of the dwarf (60%) and the normal feathered chick groups (60%). Lesion scores were significantly lower ($P<0.05$) in naked neck (1 ± 0.5) and frizzle (1.2 ± 0.3) compared to the dwarf phenotype lesion score (2.5 ± 0.4). It was milder in silky (1.4 ± 0.5) and normal chick (1.5 ± 0.4) groups.

Table 1: Lesion scores, survivability and Oocysts Per Gram, (values in the same column that not share the same superscript letters are significantly different, $p<0.05$)

phenotypes phenotype	Lesion scores (Mean ± SE)	Survivability (% ± SE)	OPG (Mean ± SD)
Naked neck	1^{a} ± 0.5	100^{a} ± 0.0	733^{a} ± 465
Normal feathered	1.5^{ab} ± 0.4	60^{b} ± 0.15	154912^{b} ± 105962
Dwarf	2.5^{b} ± 0,4	60^{b} ± 0.15	604400^{c} ± 554971
Silky feathered	1.4^{ab} ± 0.5	90^{ab} ± 0.09	63280^{bc} ± 47334
Frizzle feathered	1.2^{a} ± 0.3	70^{ab} ± 0.14	104706^{bc} ± 85807

SE: Standard error, SD: Standard deviation,

4.2 Bloody feces and oocysts excretion: Bloody diarrhea was observed in all the experimental groups from the fourth to sixth day post infection (Table 2). The highest blood proportion in the feces was observed in the 6th day with frizzled chicks (41%), the normal (34.6%) and the dwarf (31%). Little blood was found with the silky (15%) chick group. No blood was ever found in the naked neck chicks feces at the 6th day post inoculation. Excreted oocysts in the naked neck chick group (733) were significantly lower than that of the other four phenotypes of chicks ($p<0.05$). It was milder with silky (63280), frizzled (104706) and normal (154912) phenotypes. The dwarf chicks group (604400) excreted significantly more oocysts than the other four phenotypic groups ($p<0.05$).

Table 2: Proportion of Bloody feces

Phenotypes	**Proportion of blood in feces % (day after infection)**				
	3	**4**	**5**	**6**	**7**
Naked neck	0	4.5	1	0	0
Normal feathered	0	4.8	27.2	34.6	0
Dwarf	0	4.7	20.8	31.6	0
Silky feathered	0	0	22.1	15.1	0
Frizzled feathered	0	0	18	41	0

4.3 Packed cell volume (PCV) and Body weight gain (BWG): No significant difference was found in packed cell volume of uninfected and infected naked neck and normal feathered phenotype chicks (Table 3); but, the uninfected control packed cell volume values of dwarf (32.0% ± 0.9), silky (29.4% ± 1) and frizzled (30.4% ± 1) phenotypes were significantly different from those of the infected dwarf (28.0% ± 1.2), silky (25.8% ± 0.9) and frizzled (26.1% ± 1.4) chicks groups ($P<0.05$). The effect of interaction between infection and chick s

phenotype on packed cell volume was less significant than the effect of the infection alone. No significant effects of infection and phenotype were noticed on body weight gain of the experimental chicks (P>0.05).

Table 3: Packed cell volume and body weight gain (values in the same row, belonging to the same caption, that not share the same superscript letters are significantly different, p<0.05)

phenotypes	Packed cell volume (%)		Body weight gain (g)	
	Control	Infected	Control	Infected
Naked neck	$26.0^{a} \pm 0.9$	$25.6^{a} \pm 1$	$2.9^{a} \pm 0.4$	$2.6^{a} \pm 0.6$
Normal feathered	$28.6^{a} \pm 1$	$27.1^{a} \pm 0.8$	$2.6^{a} \pm 0.2$	$2.4^{a} \pm 0.8$
Dwarf	$32.0^{a} \pm 0.9$	$28.0^{b} \pm 1.2$	$2.5^{a} \pm 0.2$	$2.5^{a} \pm 0.6$
Silky feathered	$29.4^{a} \pm 1$	$25.8^{b} \pm 0.9$	$2.2^{a} \pm 0.3$	$1.6^{a} \pm 0.2$
Frizzled feathered	$30.4^{a} \pm 1$	$26.1^{b} \pm 1.4$	$2.5^{a} \pm 0.3$	$2.4^{a} \pm 0.2$
Overall	$28.5^{a} \pm 0.5$	$26.4^{b} \pm 0.4$	$2.5^{a} \pm 0.4$	$2.3^{a} \pm 0.5$
Infection effect (F-value)	9.47*		1.79	
Infection*genotype effect (F-value)	3.26*		1.26	

*genotype effect is simply the F-value of the GLM procedure of SAS analysis inferential test, which inform about the significance level of the comparison. F-value superior to the value 3 often is suggestive of significant difference among measurements.

5 DISCUSSION

5.1 Survivability and lesion score: The results of the experiment are validly comparable among the different chick s phenotypes, because they were all carried out in the same laboratory, under strictly the same conditions. Several authors reported some sensitivity variability of bird to chicken coccidiosis (Kim, 2009; *et al.* Ayissiwede *et al.*, 2011). Death did occur in infected chicks groups except the naked neck which recorded the highest survivability (100%) significantly different (P<0.05) from the dwarf and normal feathered chick s groups survivability (60%). Deaths due to coccidiosis occurred during the fourth to the sixth day post infection, the subjects at risk on the third day being the same in all groups and equal to the experiment starting chick number. This is consistent with the obtained survival analysis results of William *et al.* (2001) who used the same *Eimeria* species and recorded death in the same period post infection. Ayissiwede *et al.* (2011) observed no chick mortality among both a Senegalese indigenous chick breed and two exotic phenotypes, using a mix of *Eimeria* species. Naked neck (100%) and silky (90%) chicks seem more resistant to death due to *E. tenella* coccidiosis than the normal, frizzled feathered and dwarf chicks. Lesion score was significantly lower in frizzled chicks group and especially in naked neck with the score of 1 ± 0.5 corresponding to a diarrheal chick cecal content at necropsy. The dwarf chick exhibited higher lesion score with also the highest mortality rate along with normal chick. The highest average lesion score corresponding to the middle bloody feces cecal content was observed only with the dwarf chick group. However the lesion scores observed in this study generally were less significant than that reported by Pinard *et al.* (1998). The coccidian oocyst strain (*Eimeria tenella*) and the scoring method used by these authors were the same but the *Eimeria* oocyst dose and chick breeds were different from those used in the current study.

5.2 Bloody feces and oocyst excretion: Bloody feces were found on the fourth, fifth and predominantly on the sixth day post infection with the highest values in frizzled, normal and the dwarf chicks. No bloody feces were excreted the third and the seventh day post infection, consistent with the findings of Youn *et al.* (2001) who used the same Eimeria species. This excretion of blood in feces pattern, during the

patent period, is therefore characteristic of *Eimeria tenella* species.

The excretion of oocysts was significantly affected by chicks' phenotype. There was a significant difference between the naked neck phenotype and the other four phenotype chick groups. That naked neck recorded the fewest oocysts per gram with the ratio (naked neck versus dwarf) of 1: 825. The oocyst excretion was milder with silky, frizzled and normal feathered chicks. The divergent results of oocyst yields obtained among chick phenotypes in the current study could be ascribed to the difference in initial availability of cecal epithelial cells for parasitisation and the sloughing of epithelium during the infection with formation of cecal cores, which prevent the discharge of oocysts (Tyzzer *et al*., 1932). The cell-mediated immune responses among phenotypes (Wakelin and Rose, 1990; Lillehoj and Trout, 1993; Ovington *et al*., 1995), with lymphoproliferative reaction (CD^{4+} / CD^{8+}) (Talebi and Mulcahy, 1995) and release of interferon gamma (Del Cacho *et al*., 2011), could also be involved in the divergent oocyst yields of the different local chick phenotypes used in the current study. But other studies need to be carried out to clarify or confirm these suppositions.

5.3 Phenotype measurement: The divergent results of oocyst yields obtained among chick phenotypes in the current experiment could be ascribed to the difference in initial availability of cecal epithelial cells for parasitisation and the sloughing of epithelium during the infection with formation of cecal cores, which prevent the discharge of oocysts (Tyzzer *et al*., 1932).

5.4 Packed cell volume and body weight gain: Packed cell volume or hematocrit is a proportion occupied by the red cell to the volume of the whole blood in a sample of capillary, venous or arterial blood. There was an impressive reduction of packed cell volume in dwarf (12.5%), silky (12.2%) and frizzled (7.3%) chicks groups. This reduction is little in naked neck (1.5%) and normal feathered (5.2%) chicks. Packed cell volume is an important trait of measurement of expressed resistance to coccidiosis among bird population (.Bumstead and Millard, 1987; Lillehoj and Ruff, 1987). According to Mathis *et al.* (1984), PCV is a better measure of disease resistance to *Emeria tenella* infection than to *E. acervulina* because, compared to the latter, the former causes extensive hemorrhage which substantially depresses the packed cell volume. This corroborated the packed cell volume reduction rate obtained with dwarf phenotype chick group, the most sensitive with higher reduction rate (12.5%) and naked neck phenotype, the most tolerant to *Eimeria tenella* with lower reduction rate (1.5%) with confirmative proportion of bloody feces results. But on the other hand the packed cell volume values of normal feathered and silky chicks contrasted with the proportion of blood recorded in theirs feces. In general, the various measures of response to *Eimeria* did not correlate with another and further, host resistance, generally, depends upon the *Eimeria* species involved (Bumstead and Millard, 1992). Free-range chick body weight gain was not significantly affected by both the experimental infection and phenotype. Nevertheless Youn *et al.* (2001) recorded a significant reduction of body weight gain (93.2 g) between the uninfected and the experimentally *Eimeria tenella* infected commercial broiler chick breed (Arbor Acres). Naked neck appears to be more tolerant to *E. tenella* coccidiosis than the other four phenotypes especially the dwarf in terms of lesion score, proportion of blood in feces, oocyst excretion and packed cell volume herein estimated values. Desai (1969) has reported a disease resistance character correlated to naked neck among Sudanese chicken breeds. The significant sensitivity variability observed in this preliminary study, is suggestive of a great disease tolerance potential in free-range chicken populations that can be valuably exploited in selection programs. However, further studies are required to understand the real mechanism underneath the herein established *Eimeria tenella* coccidiosis divergent expression among free-range chick's phenotypes in Benin.

6 ACKNOWLEDGMENT

The Ministry of high education is to be thanked for the financial support as well as the Agricultural Research Center of Agonkanmmey and Laboratory of Poultry Research and Zoo-Economy of Faculty of Agronomic Sciences for their logistic support.

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