

HAEMATOLOGY AND SERUM BIOCHEMISTRY OF F₁ AND F₂ PIGS PRODUCED BY ASF- RECOVERED PIGS

¹Adeoye, A.A., ²Udoh, J.E., ³Rotimi, E. A ⁴Ikeobi, C.O.N. and ⁴Adebambo, O.A.

¹ Biological Sciences Department, Ondo State University of Science and Technology, Okitipupa

² Animal Science Department, University of Uyo, Akwa Ibom

³ Department of Animal Production and Health, Federal University, Katsina State.

⁴ Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta.

Corresponding email address: adeomoh@yahoo.com

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ABSTRACT

The blood contains a myriad of metabolites and other constituents which provide a valuable medium for clinical investigation and nutritional status of human beings and animals. Data on haematological parameters and serum metabolites generated from F₁ (52) and F₂ (60) pigs farrowed by eight sows and two boars, (Four Large White sows, two Large Black sows, two Duroc sows, one Large White boar and one NigerHyb boar which are survivors of the African Swine Fever (ASF) outbreak in a farm in Abeokuta, Nigeria, in 2005) were evaluated. From these fifty-two pigs were generated in the F₁ and sixty pigs in the F₂ generation. Data obtained were subjected to General Linear Model (GLM) procedure. The serological test for determining the presence of ASF antibody showed that all the ASF recovered pigs tested positive, the Large White had more of ASF antibody compared with the other two breeds while 18.79 % of the F₁ and 6.25 % of the F₂ pigs tested positive. Genotype did not have significant effects ($P > 0.05$) on the blood parameters considered except on monocytes, lymphocytes and eosinophils among the F₁. Among the F₂, genotype effect was significant ($P < 0.05$) on lymphocytes only. Sex had significant effect on globulin, monocytes and eosinophils among F₁ pigs while among F₂ the effect was significant on creatinine and urea. ASF- infected herd showed significant variation in different types of white blood cell compared to other blood parameters.

Key words: Pigs, ASF, haematological parameters and serum metabolites

INTRODUCTION

The blood contains a myriad of metabolites and other constituents which provide a valuable medium for clinical investigation and nutritional status of human beings and animals. Hence, Coles (1986) recommended the use of blood and biochemical parameters in medical and nutritional assessment. Serum is the supernatant fluid, which forms when blood clots and the measurement of its chemical constituents is also helpful for the evaluation of physiological and pathological alteration in the animal's body (Coles, 1986). This author further noted that under healthy condition, there was equilibrium between the production and destruction of blood cells but certain pathological and physiological conditions might result in increase or decrease of blood cell abnormalities and morphology respectively. The use of differential resistance or otherwise of animals to variation in diet composition and resultant health implication have been reported (Agbede *et al.*, 1999). Different values of haematological

parameters and serum metabolites of different species of animals indicating different types of physiological and pathological conditions have been reported in the literature (Dukes, 1975; Schalm *et al.*, 1975; Friendship *et al.*, 1986; Adeshinwa *et al.*, 1999; Akinfala and Tewe, 2001; Tambuwal *et al.*, 2002; Taiwo *et al.*, 2003 and Daramola *et al.*, 2005).

The effect of sex on the red blood cell, packed cell volume and haemoglobin of guinea fowl, with the male having higher values compared to the female have been reported (Orji *et al.*, 1997). This preponderance of the male over female has been attributed to the role of testosterone. Diseases like coccidiosis, avian encephalomyelitis etc. affected blood parameters in experimental birds (Bain, 1989). Sarror and Van Veen (1987) reported that blood parameters value of Ouda and Yankasa sheep under different health conditions were affected by disease. The values of Hb, RBC, PCV and WBC from healthy flocks were similar. Expectedly, these parameters in the

infected flock had a wider spread. It was concluded that blood parameter values from clinically healthy flocks could serve as a baseline for interpreting blood data from infected local sheep (Sarror and Van Veen, 1987).

African Swine Fever (ASF) is a highly fatal contagious viral disease of pigs caused by a virus that is a member of the family *Asfarviridae* and is the only member of the genus *Asfivirus*. In domestic pigs, ASF viruses produce a range of syndromes varying from per-acute to chronic disease and apparently healthy virus carriers (OIE, 1999). The more virulent strains produce per-acute or acute haemorrhagic disease characterized by high fever, loss of appetite, haemorrhages in the skin and internal organs, and death in 2 to 10 days. Mortality rates may be as high as 100%. Less virulent strains produce mild clinical signs – slight fever, reduced appetite and depression which can be confused with other diseases in pigs. Nigeria was free of ASF up to 1996 (Ayoade and Adeyemi, 2003). Unconfirmed ASF outbreak was suspected in 1978 (FAO, 2000). It was not confirmed because of insufficient evidence to verify the diagnosis. Sufficient evidence was however available to confirm the outbreak in 1997-1998. Odemuyiwa *et al.*, 2000 isolated ASF virus from the 1997 – 1998 outbreak and thereby recorded the first confirmed outbreak of ASF in South-Western Nigeria. The second outbreak was in 2001-2002 and was confirmed by Olugasa *et al.* (2005) and Esuruoso *et al.* (2005). The third wave of the outbreak occurred between 2004 and 2005.

This study is designed to determine the effects of breed and sex of pigs on the haematological parameters of pigs produced by ASF- recovered pigs.

MATERIALS AND METHODS

EXPERIMENTAL UNITS (ANIMALS)

A total of eight sows (4-Large White, 2- Large Black and 2-Duroc) all on third parity and two boars (Large White and NigerHyb) were used as foundation stock for the production of F₁ (52 progenies) for the first phase of this study and F₂ (60 progenies) produced from F₁ for the second phase of the study. The foundation stocks were survivors of African swine fever disease outbreak

of 2005 at a Farm in Abeokuta, Nigeria. The foundation stock, F₁ and F₂ pigs were subjected to ELISA procedure to determine the presence of ASF antibody.

5ml. of blood was collected from each 5 month old grower (4 males, 4 females per group for F₁ and F₂ via the anterior vena cava using syringe and needles. 3 ml. each of the samples was collected in bottles containing ethylenediamine tetra acetic acid (EDTA) as anti- coagulant and the samples were taken to the laboratory for complete haematological analyses which included: packed cell volume (PCV), erythrocyte, leucocytes, haemoglobin and white blood cell differentials (lymphocyte, monocyte, neutrophil, basophil and eosinophil).

Serum metabolites were assessed from the remaining 2 ml. as follows:

Total Protein (TP) by Biurette method as described by Weichelbaum (1946), albumin and globulin assayed by the method of Doumas and Briggs (1972), creatinine by Folin-wu filtrate method and urea nitrogen (UN) by diacetymonoxine method. To detect the albumin content, albumen value was substituted for the total protein of control. Globulin value was determined by subtracting the albumin value from total protein value.

MATING DESIGN

Production of first filial generation

♀		♂
LW	x	LW = LWxLW
NH	x	LB = LBxNH
NH	x	D = DxNH

Production of second filial generation

♀		♂
LW	x	LBNH = LWxLBxNH
LW	x	DNH = LWxDxNH
LW	=	Large White
D	=	Duroc
NH	=	NigerHyb
LB	=	Large Black

STATISTICAL ANALYSES

Data collected on serum metabolites and haematological parameters were subjected to

General Linear Model (GLM) procedure to compare means. Duncan's New Multiple Range Test was used to separate the means that differed significantly (Gomez and Gomez, 1984). The ELISA test result was subjected to descriptive statistics.

The statistical model used is as follows:

$$Y_{jkl} = \mu + B_j + S_k + \epsilon_{jkl} \quad (\text{For } F_1 \text{ and } F_2 \text{ data})$$

Where:

Y_{jkl} = observations on the parameters

μ = Universal means

B_j = effect of genotype ($j=3$)

S_k = effect of sex ($k=2$)

ϵ_{jkl} = experimental error

RESULTS

Presence of ASF Antibody in Foundation Stock, F₁ and F₂ Pigs

The results of serological tests carried out on the survivors of ASF, their offspring up to F₂ generation to test for the presence of ASF antibody are presented in Table 1. The ten animals that survived the outbreak tested positive while 18.75 % of their offspring tested positive, 56.25 % tested negative and 25 % of the offspring were indeterminate as the procedure could not tell whether they were positive or negative. Among the F₂ individuals 6.25 % tested positive, 62.5 % tested negative while 31.25 % were indeterminate.

Table 1: Differential Diagnosis of Pig Sera

Animals	Tested	Positive	Negative	Undecided	Total
Foundation stock	10	10(100%)	-	-	10
F ₁	32	6(18.75%)	18(56.25%)	8(25%)	32
F ₂	32	2(6.25)	20(62.5%)	10(31.25%)	32
Total	74	18	38	18	74

F₁ = First filial generation

F₂ = Second filial generation

Effects of Genotype and Sex on Haematological Parameters of F₁ Pigs

The least square means and standard errors for haematological parameters of F₁ pigs as affected by genotype and sex were shown in Table 2. Genotype did not have significant effect ($p>0.05$) on virtually all the parameters except on monocytes, lymphocytes and eosinophils. The offspring of crosses between NigerHyb and Large Black (NHxLB) had the highest value of 4.67 ± 1.76 % followed by 2.50 ± 0.50 % for the cross between NigerHyb and Duroc (NHD) while the pure Large White (LW) had the lowest value of 2.13 ± 0.35 % for monocytes. Eosinophils value was highest in NHxD (4.75 ± 1.11 %) followed by

3.25 ± 1.60 % (NHxLB) and least in LW (2.40 ± 0.74 %). And lymphocytes values were 36.25 ± 2.7 %, 40.00 ± 3.83 % and 36.75 ± 4.42 % for LW, NHxLB and NHxD, respectively

Sex had significant ($p < 0.05$) effects on globulin, monocyte, and eosinophils. There was no significant effect on other blood parameters. The mean value of globulin for male (34.63 ± 2.93 g/l) was significantly ($p < 0.05$) higher than that of female (27.50 ± 1.34 g/l). The mean value of monocytes for male and female were 2.25 ± 0.45 % and 3.29 ± 0.81 %, respectively. Eosinophils value for male (4.33 ± 1.14 %) was significantly higher than that of female (2.57 ± 0.69 %)

Table 2: Effects of Genotype and Sex on Haematological Parameters of F₁ Pigs

Parameter	LWxLW N=8	NHxLB N=4	NHxD N=4	Male N=8	Female N=8	Overall N=16
TP (g/l)	67.00±2.18	65.25±3.15	64.75±2.93	67.88±2.24	64.13±1.73	66.00±1.45
AL (g/l)	36.63 ±0.91	34.25±1.50	32.25±1.65	33.25±1.25	36.63±0.67	34.94±0.81
GL (g/l)	30.38±2.81	31.00±2.74	32.50±4.48	34.63±2.93 ^a	27.50±1.34 ^b	31.06±1.81
CR (mg/dl)	1.15±0.13	0.95±0.17	0.90±0.15	0.94±0.13	1.14±0.10	1.04±0.08
UR (mg/dl)	13.53±0.87	10.88±1.66	10.38±0.97	11.81±1.14	12.34±0.91	12.08±0.71
PCV(%)	36.63±2.31	37.75±1.03	35.50±2.17	37.75±2.23	35.50±1.19	36.63±1.26
WBC x 10 ⁹ /l	12.94±1.38	16.1±0.66	16.73±2.08	15.59±1.41	13.76±1.30	14.68±0.94
RBCx10 ¹² /l)	7.03±0.43	7.23±0.19	7.35±0.25	7.48±0.35	6.84±0.25	7.16±0.22
Hb (g/dl)	12.21±0.77	12.58±0.33	12.10±0.42	13.03±0.62	11.53±0.34	12.28±0.39
Lymp %	36.25±2.74 ^a	40.00±3.89 ^a	36.75±4.42 ^b	39.00±2.89	35.63±2.56	37.31±1.90
Mono %	2.13±0.35 ^c	4.67±1.76 ^a	2.50±0.50 ^a	2.25±0.45 ^b	3.29±0.81 ^a	2.73±0.45
Neut %	60.13±3.02	53.25±3.64	55.00±5.76	55.50±3.74	58.75±2.64	57.13±2.25
Eu %	2.4±0.74 ^c	3.25±1.60 ^b	4.75±1.11 ^a	4.33±1.14 ^a	2.57±0.69 ^b	3.38±0.67

^{a,b,c}: means in the same row for genotypes and sex of pigs with different superscripts are significantly different (p<0.05)

TP- total protein

Al – albumin

Gl- globulin

CR- creatinine

PCV- packed cell volume

RBC – red blood cell

lymp – lymphocyte

Neut – neutrophil

N – Number of samples

LW-Large White

NHLB- Indigenous Large Black Dihybrid

NHD- Indigenous DurocDihybrid

UR- urea

WBC –white blood cell

Hb – haemoglobin

Mono – monocyte

Eu – eosinophil

Effects of Genotype and Sex on Haematological Parameters of F₂ Pigs

Table 3 shows the effect of genotype and sex on the blood parameters of F₂ pigs. Genotype effect was not significant (p>0.05) in almost all the parameters considered except for lymphocytes. The mean value of lymphocytes in LW (NHLB) (65.08±0.80 %) was significantly, (p<0.05) higher than that of LW(NHD) (61.25±0.75 %).

Effects of sex on creatinine and urea was significant (p<0.05), but in contrast to other parameters. Female had the highest value of 1.05 0.05 mg/dl for creatinine compared to 0.86 0.03 mg/dl for male. Urea (g/dl) values for male and female were 14.13±0.95 and 18.87±1.39 respectively.

Table 3: Effects of Genotype and Sex on Haematological Parameters of F₂ Pigs

Parameter	Genotype		Sex		
	LWxNHxLB N = 12	LWxNHxD N = 4	Male N = 8	Female N = 8	Overall N = 16
TP (g/l)	67.92 ± 1.34 ^a	67.50 ± 3.28 ^a	68.00 ± 1.49 ^a	67.63 ± 2.07 ^a	67.81 ± 1.24
AL (g/l)	34.08 ± 1.74 ^a	35.00 ± 1.78 ^a	32.13 ± 1.68 ^a	36.5 ± 1.91 ^a	34.31 ± 1.35
GL (g/l)	33.83 ± 1.75 ^a	32.50 ± 2.22 ^a	35.88 ± 2.13 ^a	31.13 ± 1.51 ^a	33.51 ± 1.39
CR (mg/dl)	0.94 ± 0.05 ^a	1.00 ± 0.07 ^a	0.86 ± 0.03 ^b	1.05 ± 0.05 ^a	0.96 ± 0.04
UR (mg/dl)	17.08 ± 1.29 ^a	14.75 ± 1.11 ^a	14.13 ± 0.95 ^b	18.87 ± 1.39 ^a	16.51 ± 1.02
PCV %	36.17 ± 0.73 ^a	36.75 ± 1.25 ^a	35.75 ± 0.75 ^a	36.88 ± 0.97 ^a	36.31 ± 0.61
WBC(x10 ⁹ /l)	12.97 ± 0.37 ^a	13.93 ± 0.78 ^a	12.85 ± 0.47 ^a	13.56 ± 0.50 ^a	13.21 ± 0.34
RBC(x10 ¹² /l)	5.03 ± 0.11 ^a	5.45 ± 0.21 ^a	5.13 ± 0.16 ^a	5.14 ± 0.14 ^a	5.13 ± 0.10
Hb (g/dl)	12.05 ± 0.24 ^a	12.18 ± 0.33 ^a	11.93 ± 0.25 ^a	12.24 ± 1.29 ^a	12.08 ± 0.19
Lymp %	65.08 ± 0.80 ^a	61.25 ± 0.75 ^b	64.0 ± 0.87 ^a	64.25 ± 1.29 ^a	64.13 ± 0.75
Mono %	1.4 ± 0.16 ^a	2.00 ± 0.41 ^a	1.63 ± 0.18 ^a	1.50 ± 0.34 ^a	1.57 ± 0.17
Neut %	31.08 ± 0.51 ^a	32.00 ± 0.71 ^a	31.25 ± 0.49 ^a	31.38 ± 0.73 ^a	31.31 ± 0.43
Eu %	2.25 ± 0.37 ^a	1.50 ± 0.29 ^a	2.25 ± 0.45 ^a	1.88 ± 0.39 ^a	2.06 ± 0.29

^{a,b} means in the same row with different superscripts are significantly different (p < 0.05)

TP- total protein
 AL- albumin
 GL- globulin
 CR- creatinine
 UR- urea
 WBC- white blood cell
 Hb- haemoglobin
 Mono- monocytes
 Eu- eosinophils

LWxNHxLB – Indigenous Large Black Trihybrid
 LWxNHxD – Indigenous Duroc Trihybrid
 CR- creatinine
 PCV- packed cell volume
 RBC – red blood cell
 lymph – lymphocytes
 Neut - neutrophils
 N – Number of samples

DISCUSSION

The serological test carried out on the survivors of ASF outbreak, their offspring and the F₂ progenies showed a decline in the percentage of pigs testing positive for ASF, from 100 % for the foundation stock to 18.79 % for the F₁ and 6.25% for the F₂. This indicates a decrease in the shedding of the virus from the recovered pigs into the environment and subsequently, a reduction in the prevailing rate of exposure of other non-infected pigs to the virus within the environment. This is to be expected because recovered pigs do continue to trap the virus within their tissues by the activities of macrophages (that eat up pathogens and infected tissues so that other parts will not be affected) and mast cells in the tissues. Together with the activities of the complement fixation antibodies (CFA), the engulfed ASF viruses are easily and effectively broken down by macrophages. This leads to continual decrease in the circulating ASF viruses in the blood and other tissues. The amount of live ASF virus particles that are shed in the urine and faeces of the older pigs originally infected by the virus dropped

significantly. This result agrees with the findings of Olugasa (2007) who reported that the level of infection is higher among the older stock (76.3% to 96.8%) than in younger stocks (13.8% to 39.7%). The result is also similar to the findings of Fasina *et al.* (2010) who reported decrease in the percentage of pigs infected with ASF from 2006 to 2008 across sixteen states of Nigeria as revealed by serological tests.

Genotype and sex had no significant effects on most of the blood parameters considered except on monocytes, lymphocytes globulin and eosinophils among the F₁. The values of monocytes, lymphocytes and eosinophils were lower in Large White progenies produced from herd with high level of ASF antibody compared with those produced from pigs with low level of ASF antibody. This showed that infection was more among the progenies of the other two genotypes (LBxNH and DxNH) compared to progenies of Large White genotype. The lower value of monocytes (2.13%) in Large White progeny indicates less ASF-virus infection as monocytes are the white blood cells that are

sensitive to ASF-virus (Enjuanes *et al.*, 1977 and Minguéz *et al.*, 1988). This may be due to high level of ASF-antibody in the body systems of their parents which called for more activities of macrophages and mast cells that trapped the virus, thus reducing the shedding of the virus into the environment by the parents. The percent eosinophils (2.41) in the Large White progeny indicated less parasitic infestation compared to the other genotypes considered. The lower value of white blood cells (12.94 ± 1.38) obtained for Large White in this study also confirmed that less infection was associated with Large White progenies than for progenies of other genotypes considered. The percentage of eosinophils (4.38%) in males was higher than 2.57% in females. This indicated that there was more parasitic infection in males compared with females across all genotypes. The lower value of monocytes (2.25%) in males indicated less ASF-virus infection associated with males compared with females as monocytes are sensitive to ASF-virus. The effect of sex on globulin was significant, with higher values in males compared with females. This agrees with the findings of Tambuwal *et al.* (2002) and Daramola *et al.* (2005) who reported higher values of globulin, RBC, PCV and Hb in males compared with females in West African Dwarf goat and in Red Sokoto goat.

In F₂ progenies where 6.25 % pigs tested positive to ASF antibody, effect of genotype was not significant on almost all the blood parameters, except for lymphocytes. This indicates that the two genotypes are of different health status. The higher value of 65.08% of lymphocytes in the Trihybrid (LW (NHLB) pigs indicated that more B-cells and T-cells were produced which showed more virus infection in the genotype compared with 61.25% in Duroc crosses [LW (NHD)]. The effect of sex on the blood parameters was significant only on creatinine and urea, with values of 0.86 mg/dl and 14.13 mg/dl for creatinine and urea respectively, for male, and 1.05 mg/dl and 18.87 mg/dl for female pigs. The higher value of urea and creatinine observed in females may be due to inflamed kidney (kidney disorder) observed in two of the females slaughtered. As kidney removes urea and creatinine from the blood. The non-significant effect of sex of pig on other blood parameters is contrary to the findings

in the literature (Orji *et al.*, 1997; Tambuwal *et al.*, 2002 and Daramola *et al.*, 2005). Orji *et al.* (1997) reported significant effects of sex on RBC, PCV and Hb of guinea fowl with males having higher values. Generally, the values observed for haematological parameters and serum metabolites were similar to earlier reports (Friendship *et al.*, 1986; Odunsi, 1991; Orji *et al.*, 1997; Akinfala and Tewe, 2001; and Rita, 2005). ASF-infected herd showed significant variations in different types of white blood cells compared to other blood parameters

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