Comparative evaluation of haematological profile of the Nigerian indigenous and Large White x Landrace crossbred pigs vaccinated with sheep RBC

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Abstract

This study was designed to evaluate haematological response between Nigerian indigenous pigs (NIP) and Large White x Landrace crossbred pigs (LWL) when vaccinated with sheep RBC. Twenty male pigs with average age of 42 ± 3 days and weighed 3.10 ± 0.29 kg (NIP) and 6.90 ± 0.43 kg (LWL), were used. They were assigned to groups A (10 NIP) and B (10 LWL); each was further divided into two with five animals in each replicate. Processing of sheep RBC followed standard procedures; feed and animal management were similar. All the pigs were vaccinated with 1 ml of 10 % sheep RBC per pig at week 4 and revaccinated 3 weeks later. Their haematological profiles were determined using standard procedures and the study lasted ten weeks. There was a rise in WBC count of both groups after initial vaccination. Mean WBC count of LWL was significantly ($p \leq 0.05$) higher at weeks 0, 1, 4 and 6, respectively. Lymphocyte count of the two groups also increased sharply after initial vaccination; values recorded for LWL showed a slight but a continuous rise while those of the NIP generally decreased and increased in value. A significant differences in mean values of neutrophil were recorded prior to initial vaccination ($p = 0.011$, $t = 8$), on weeks 1 ($p = 0.013$, $t = 8$) and 3 ($p = 0.005$, $t = 8$) with NIP maintaining higher values than LWL. There was no significance ($p \geq 0.05$) difference in the mean values of eosinophil, basophil and monocytes for both groups.

Keywords: Swine, haematology, sheep RBC, indigenous pigs, Nigeria

Introduction

Swine and poultry have made significant contributions to the provision of animal protein in many countries (Eze *et al.*, 2010; Abonyi *et al.*, 2014). According to Oluwole and Omitogun (2016), the pig plays major functions in socioeconomic life of man: it serves as source of income; it is used in fulfilling some cultural rites and contributes to food security of the rural populace. They can be reared under a variety of production systems; ranging from simple backyard pigs living on scavenger belts, to fairly operated firms, to large integrated pig industries with sophisticated biosecurity (Holnes, 1991). Whereas most exotic pigs

in Nigeria are reared under intensive system, its indigenous counterparts are generally managed extensively (Rim, 1992). The intensification of animal agriculture creates complex animal health and production problems for which there are no simple and reliable therapeutic and preventive procedures (Radostits *et al.*, 1994). These conditions adversely affect the health or welfare of the animals and impair their homeostatic mechanisms resulting in body dysfunction which may be fatal (Adenkola *et al.*, 2009). Diseases cause pain, distress, reduced productive performance and in severe cases death thereby leading to low profit making by

farmers.

The best indicator of animal's wellbeing and its potential for production is its health status. One of the important indicators of health status in animal is its haematological profile. Its determination does not only reflect physiological responsiveness of the animals to its internal and external environments (Esonu *et al.*, 2001), but is also important in disease diagnosis, prognosis, therapy and feed stress monitoring in man and animals (Jain, 1986; Mbanasor *et al.*, 2003). It is also an excellent medium for the measurement of potential biomarkers, because its collection is relatively noninvasive and encompasses an enormous range of physiological process in the body at any given time (Anderson and Anderson, 2002; Ginsburg and Haga, 2006). Haematological traits of swine have been widely studied (Radositis *et al.*, 1994; Aladi *et al.*, 2008; Oluwole and Omitogun, 2016) and have been reported to be influenced by a variety of factors including diet, age, gender, geographical location and housing (Wilson *et al.*, 1972; Egbunike and Akusu 1983; Friendship *et al.*, 1984; Abonyi, 2012). There is paucity of information on the comparative evaluation of haematological profiles of the Nigerian indigenous pigs (NIP) with their exotic crossbred; Large White x Landrace (LWL) counterparts in Nigeria. The objective of this study was to compare haematological indices of NIP with LWL crossbred pigs immunized with sheep red blood cells (RBC); a non-infectious complex antigen.

Materials and methods

Management of the experimental animals

This study was carried out at the Teaching/Research Farm of the Faculty of Veterinary Medicine, University of Nigeria Nsukka, Nigeria, located in the derived savannah zone ecological zone at latitude 6°

51' 24°N and longitude 7°23' 45°E at an elevation of 1,810 ft. (550 m) above sea level. It covers a total land area of 17.52 sq. miles (45.38 km²) (https://en.Wikipedia.org/wiki/Nsukka#cite_note-umuahia_population-0). The area experiences two distinct seasons; rainy and dry seasons. The 2006 population census report showed that Nsukka had an estimated population of 309, 633. The indigenous people are involved in agriculture, basically crop and livestock farming activities (Nwanta *et al.*, 2011). To avoid potential differences in age, gender or season, only male pigs born within the same week, during the month of January, 2015 were used. Experimental animals comprised 10 NIP and 10 LWL male pigs that averaged 42 ± 3 days of age and weighed 3.10 ± 0.29 and 6.90 ± 0.43 kg, respectively. While the LWL were purchased from the Piggery Unit of Veterinary Teaching/ Research Farm, University of Nigeria Nsukka, the NIP were progeny of a boar and two sows sourced from Katsina-Alah Local Government Area of Benue State, North Central Nigeria but had been maintained for two years at the Department of Animal Science Farm, University of Nigeria, Nsukka for teaching and research.

After selection, the pigs were stabilized for 2 weeks during which they were fed the same weaner diet, screened for haemoparasites and helminthes ova. Thereafter, they were de-wormed with Ivomec Super® (Merial, France) and given antibiotic cover using Temadox® L.A. 20% (HebeiHuarun Pharmacy Co. limited China).

Experimental design

After the two weeks of stabilization, the pigs were transferred to a fly proof grower house and allotted to two pens of equal

dimension. The two animal groups were further divided into two with five animals in each replicate. They were fed same grower diets *ad libitum* and had free access to clean drinking water throughout the study period.

Preparation and administration of sheep RBC

About 5 ml of sheep blood was collected under aseptic conditions into an equal volume of sterile Phosphate Buffered Saline (PBS). Volume of red cells required was calculated and washed three times in PBS. The washed sheep red blood cells were suspended to give a working dilution of 10% sheep RBC. Then 1 ml of the prepared sheep RBC in phosphate buffered saline was used to immunize all the experimental animals intravenously on week 4 and a booster dose administered 3 weeks later.

Haematological Techniques

Total and differential leukocyte counts of the pigs were determined before, after initial

and booster vaccinations, respectively. Thus blood was collected from all the experimental animals and the counts performed weekly for seven times. This was carried out using an improved Neubaurhaemocytometer method (Campbell, 1995).

Statistical Analysis

Data from the study was analyzed with t-test in Graph-pad Prism Statistical software version 5.02. Significance was accepted at $p \leq 0.05$.

Results

Prior to initial vaccination (week 0), there was significant difference in total WBC count between LWL and NIP. Following initial vaccination, there was sharp increase in WBC counts of both groups with LWL being significantly ($p \leq 0.05$) higher than NIP on weeks 1, 4 and 6 of study (Table 1).

Table 1: Weekly Total White blood cell count ($10^3/\mu\text{l}$) of the Nigerian indigenous and exotic cross bred pigs following vaccination with sheep RBC

Group Statistics					
Week	Group	N	Mean $\times 10^3/\mu\text{l}$	t-value	p-value
0	Local Pigs	10	12.54 $\pm$ 1.58	8	0.021
	Exotic Pigs	10	17.67 $\pm$ 0.83		
1	Local Pigs	10	23.08 $\pm$ 0.86 ^b	8	0.006
	Exotic Pigs	10	28.35 $\pm$ 1.11 ^a		
2	Local Pigs	10	25.11 $\pm$ 1.16	8	0.660
	Exotic Pigs	10	26.40 $\pm$ 2.58		
3	Local Pigs	10	23.38 $\pm$ 2.11	8	0.912
	Exotic Pigs	10	23.90 $\pm$ 3.99		
4	Local Pigs	10	22.48 $\pm$ 0.65 ^b	8	0.036
	Exotic Pigs	10	26.56 $\pm$ 1.48 ^a		
5	Local Pigs	10	21.42 $\pm$ 1.11 ^b	8	0.052
	Exotic Pigs	10	28.10 $\pm$ 2.71 ^a		
6	Local Pigs	10	19.12 $\pm$ 1.28 ^b	8	0.008
	Exotic Pigs	10	25.15 $\pm$ 1.24 ^a		

Means on the same column with different superscript are significant at 5 %

The basophil levels of the NIP remained constant at $0.60 \pm 0.25\%$ from the pre-immunization period (week 0) till week 3 of initial vaccination. On weeks 4 and 5 of

assay, the level dropped and remained at $0.40 \pm 0.25\%$ and then increased to 0.60 ± 0.25 on the final day of the study (Table 2).

The exotic group had a decline in the

basophil counts after initial vaccination from 0.60 ± 0.25 to 0.40 ± 0.25 % (Table 2). At the second week, it further dropped to 0.20 ± 0.25 % but rose to 0.40 ± 0.25 % on weeks 3 and 4. On week 5 the level was 0.60 ± 0.25 % but again fell to 0.40 ± 0.25 % on

the final day of assay (Table 2).

Notwithstanding, no significance ($p \geq 0.05$) difference in the mean values of basophils of both groups was recorded throughout the study period (Table 2).

Table 2: Weekly basophil count (%) of the Nigerian indigenous and exotic cross bred pigs following vaccination with sheep RBC

Group statistics					
Weeks	Group	N	Mean (%)	T - value	P - value
0	Local Pigs	10	0.60 ± 0.25	8	1.000
	Exotic Pigs	10	0.60 ± 0.25		
1	Local Pigs	10	0.60 ± 0.25	8	0.580
	Exotic Pigs	10	0.40 ± 0.25		
2	Local Pigs	10	0.60 ± 0.25	8	0.242
	Exotic Pigs	10	0.20 ± 0.20		
3	Local Pigs	10	0.60 ± 0.25	8	0.580
	Exotic Pigs	10	0.40 ± 0.25		
4	Local Pigs	10	0.40 ± 0.25	8	1.000
	Exotic Pigs	10	0.40 ± 0.25		
5	Local Pigs	10	0.40 ± 0.25	8	0.580
	Exotic Pigs	10	0.60 ± 0.25		
6	Local Pigs	10	0.60 ± 0.25	8	0.580
	Exotic Pigs	10	0.40 ± 0.25		

Significance taken at $p \leq 0.05$

There was no significant difference ($p \geq 0.05$) in the mean values of eosinophil for both the NIP and LWL before exposure to sheep RBC (Table 3). The LWL pigs however had a fairly higher value (1.80 ± 0.37 vs. 1.40 ± 0.25 %). After initial vaccination, the eosinophil levels for the LWL pigs remained constant at 1.60 % from week 2 to the end of assay, the value for the NIP varied and ranged from 1.40 to 1.80 % within the same period (Table 3).

Lymphocyte count of both groups showed a significant difference ($p = 0.012$, $t = 7.996$) before the initial vaccination (Table 4). At week 1 of initial vaccination, the lymphocyte counts of both breeds showed a sharp increase with LWL pigs showing continuous rise until the final day of assay (Table 4). On the other hand, the lymphocyte count of the NIP breed

generally alternated between decrease and increase in value after the increase noted on week 1 of study (Table 4)

Significant difference ($p = 0.035$, $t = 8$) in mean monocyte count of the two experimental groups was only observed on week 4 of vaccination, otherwise no other significant difference ($p \geq 0.05$) in their mean values was noted before and after their exposure to sheep RBC (Table 5).

A significant differences in the mean neutrophil values of the NIP and LWL breeds were recorded prior to initial vaccination ($p = 0.011$, $t = 8$), on weeks 1 ($p = 0.013$, $t = 8$) and 3 ($p = 0.005$, $t = 8$), respectively. Generally, the NIP maintained relatively higher neutrophil count than the LWL pigs throughout the period of study (Table 6).

Table 3 : Weekly Eosinophil count (%) of the Nigerian indigenous and exotic cross bred pigs following vaccination with sheep RBC

Group Statistics					
Weeks	Group	N	Mean (%)	t-value	p-value
0	Local Pigs	10	1.40 ± 0.25	8	0.397
	Exotic Pigs	10	1.80 ± 0.37		
1	Local Pigs	10	1.40 ± 0.25	8	1.000
	Exotic Pigs	10	1.40 ± 0.25		
2	Local Pigs	10	1.60 ± 0.25	8	1.000
	Exotic Pigs	10	1.60 ± 0.25		
3	Local Pigs	10	1.80 ± 0.20	8	0.545
	Exotic Pigs	10	1.60 ± 0.25		
4	Local Pigs	10	1.60 ± 0.25	8	0.580
	Exotic Pigs	10	1.40 ± 0.25		
5	Local Pigs	10	1.40 ± 0.25	8	0.580
	Exotic Pigs	10	1.60 ± 0.25		
6	Local Pigs	10	1.80 ± 0.20	8	0.545
	Exotic Pigs	10	1.60 ± 0.25		

Significance taken at $p \leq 0.05$

Table 4: Weekly lymphocytes count (%) of the Nigerian indigenous and exotic cross bred pigs following vaccination with sheep RBC

Group Statistics					
Weeks	Group	N	Mean (%)	t-value	p-value
0	Local Pigs	10	37.40 ± 1.63 ^b	7.996	0.012
	Exotic Pigs	10	44.80 ± 1.59 ^a		
1	Local Pigs	10	55.00 ± 2.43	8	0.012
	Exotic Pigs	10	64.80 ± 1.77		
2	Local Pigs	10	62.00 ± 4.85	8	0.195
	Exotic Pigs	10	69.40 ± 1.96		
3	Local Pigs	10	55.40 ± 3.41 ^b	8	0.004
	Exotic Pigs	10	72.80 ± 2.67 ^a		
4	Local Pigs	10	69.00 ± 2.24	7.889	0.072
	Exotic Pigs	10	75.20 ± 1.98		
5	Local Pigs	10	74.00 ± 3.83	8	0.696
	Exotic Pigs	10	75.80 ± 2.24		
6	Local Pigs	10	68.60 ± 3.66	8	0.290
	Exotic Pigs	10	73.20 ± 1.77		

Means on the same column with different superscript are significant at 5 %

Table 5: Weekly monocyte count (%) of the Nigerian indigenous and exotic cross bred pigs following vaccination with sheep RBC

Weeks	Group	Group Statistics			
		N	Mean (%)	t - value	p - value
0	Local Pigs	10	2.40 ± 0.25	8	0.242
	Exotic Pigs	10	2.80 ± 0.20		
1	Local Pigs	10	2.40 ± 0.25	8	0.217
	Exotic Pigs	10	1.80 ± 0.37		
2	Local Pigs	10	4.40 ± 0.68	8	0.817
	Exotic Pigs	10	4.20 ± 0.49		
3	Local Pigs	10	1.80 ± 0.20	8	0.195
	Exotic Pigs	10	2.20 ± 0.20		
4	Local Pigs	10	2.20 ± 0.20 ^a	8	0.035
	Exotic Pigs	10	1.40 ± 0.25 ^b		
5	Local Pigs	10	1.60 ± 0.25	8	1.000
	Exotic Pigs	10	1.60 ± 0.25		
6	Local Pigs	10	2.20 ± 0.20	8	0.667
	Exotic Pigs	10	2.40 ± 0.40		

Significance taken at $p \leq 0.05$

Table 6: Weekly neutrophil count (%) of the Nigerian indigenous and exotic cross bred pigs following vaccination with sheep RBC

Week	Group	Group Statistics			
		N	Mean (%)	t-value	p-value
0	Local Pigs	10	38.20 ± 1.85	8	0.011
	Exotic Pigs	10	30.00 ± 1.64		
1	Local Pigs	10	40.60 ± 2.20 ^a	8	0.013
	Exotic Pigs	10	31.20 ± 1.99 ^b		
2	Local Pigs	10	31.40 ± 4.76	5.578	0.232
	Exotic Pigs	10	24.40 ± 2.16		
3	Local Pigs	10	40.60 ± 3.79 ^a	8	0.005
	Exotic Pigs	10	23.00 ± 2.49 ^b		
4	Local Pigs	10	26.80 ± 2.08	8	0.090
	Exotic Pigs	10	21.60 ± 1.72		
5	Local Pigs	10	22.60 ± 3.70	8	0.607
	Exotic Pigs	10	20.40 ± 1.81		
6	Local Pigs	10	26.80 ± 3.62	8	0.326
	Exotic Pigs	10	22.40 ± 2.14		

Means on the same column with different superscript are significant at 5 %

Discussion

In the course of this study, we noted that NIP was more curious and restless than LWL; an observation that is consistent with other researchers (Fetuga *et al.*, 1977; Aladi *et al.*, 2008). It was also observed that their blood clotting time was shorter than that of LWL

pigs (data not available). Significant variation in mean count of WBC between LWL and NIP was recorded prior to initial vaccination (Table 1). Since the two animal groups used were under similar management conditions and of same sex and age, this variation could possibly be due

to differences in breed of the pigs used. This was corroborated in other studies (Sutherland *et al.*, 2005; Aladi *et al.*, 2008; Oluwole and Omitogun, 2016) where they reported that interaction between breed was significant in WBC count during pre and post weaning age in the pig possibly due to genetic control of the individual pigs (Renard *et al.*, 1998). However, our interest in evaluating haematological parameters in this study was in determination of differential white blood cell count as a means of comparing mobilization of cellular immunity against an antigen between NIP and LWL pigs. This is because the major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. In the present study, mean WBC counts of both breeds were generally higher than the normal value for all pigs (11 to $22 \times 10^3/\mu\text{L}$) as reported by Kahn and Line (2010). This leukocytosis may possibly be as a result of their contact with sheep RBC antigen that was used in their vaccination. According to Oluwole and Omitogun (2016), WBCs are important humoral immune cells in blood circulation that can recognize, kill, clear antigen and circulate immune complexes within the body system. This observed increase in WBC count is in agreement with the findings of Eze *et al.* (2010) and Oluwole and Omitogun (2016) who reported that WBC of the pigs increases above their reference values when they are challenged with an antigen. In our study, increase in WBC was detected first week of initial vaccination and is in line with the reports of Blood *et al.* (2008) that increase in circulatory WBC is one of the first signs or indicators of disease (antigen) invasion in animal and are usually detected early in

many disease processes. This early detection of WBC against disease agents through the blood immune traits has been listed as a possible criterion for selecting pigs at different ages (Oluwole and Omitogun, 2016). From our result, NIP having lower WBC count could be more exposed to high risk of disease infection, while LWL with high count may be capable of generating higher antibodies through the process of phagocytosis and could have higher degree of resistance to diseases (Soetan *et al.*, 2013) and enhance adaptability to local environmental and disease prevalent conditions (Kabir *et al.*, 2011; Okunlola *et al.*, 2012; Iwuji and Herbert, 2012; Isaac *et al.*, 2013).

This better immune performance of LWL was further authenticated by the fact they also had higher lymphocyte count. However, lymphocyte count in domestic animal could also be affected by differences in breed (Radostits *et al.*, 2003; Oluwole and Omitogun 2016). Lymphocytosis noted in the two experimental groups according to Adekola *et al.* (2008) represents biomarker of stress; may be attributed to immune-stimulatory effects of sheep RBC on lymphoid tissues of the pigs which results in antibody stimulation and increased migration of phagocytic cells (Khan and Line 2010). It is worthy to note that basophils which are rare in all common domestic animals (Khan and Line 2010) were clearly observed in the two animal groups and were within reference values. Neutrophil, eosinophil and monocyte levels of the two animal groups were also within haematologic reference ranges as reported by Radostits *et al.* (2003) and Khan and Line (2010). This could possibly be an indication that the vaccination of the experimental animals with sheep RBC did not result in basophilia, neutrophilia, eosinophilia, monocytosis or other

leukogramme abnormalities. It is also important to note that unlike the NIP, the LWL used in this trial were progeny of pigs that have been in the farm where this study was conducted for a long period. On the other hand, available breeding record at the farm where the NIP was sourced showed that in an attempt to maintain the pigs as pure line, a lot of inbreeding takes place within the herd. These factors may have also affected the haematological parameters determined in this study. Notwithstanding, within the limits of this study, data generated could provide baseline data that may be useful in further haematological studies in NIP, their conservation and utilization in swine improvement programme.

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