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HAEMATOLOGICAL PARAMETERS, ORGAN WEIGHT AND VILLI MORPHOMETRICS OF WEANER PIGS FED BISCUIT DOUGH

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Abstract

Thirty weaner (Large white x Landrace) pigs were randomly allotted to five dietary groups of six pigs each. A maize-soybean meal-based diet served as the control (T1) while diets T2, T3, T4, and T5 had 12.5%, 25%, 37.5% and 50% biscuit dough respectively as a replacement for maize. Results showed that haematological parameters had significantly different ($p < 0.05$) quadratic responses, although, pigs fed T5 had least values for white blood cell count, haemoglobin, packed cell volume and mean corpuscular volume. Significant differences ($p < 0.05$) were also observed in the weight of liver, heart, spleen and pancreas, and also in the villi length, width and crypt depth. It can be concluded that 50% biscuit dough replacement for maize weakened the body defensive mechanism, reduced the efficiency of cellular oxygen transportation, pancreatic secretion and villi width of weaner pigs, while 37.5% biscuit dough replacement for maize improved the parameters mentioned above. It is recommended that 37.5% of biscuit dough is suitable for the replacement of maize in weaner pig's diets.

Introduction

Nutrition plays an essential role in the physiology of animals (OJEDIRAN et al. 2017a). The values of blood parameters can serve as an assessment of different physiological processes in the body: this ability of blood may be due to the fact that blood is a diagnostic tool and its analysis is

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a timely way of assessing nutritional and health status of livestock on feeding trials since ingestion of dietary components has assessable effects on blood composition (OVURU and EKWEOZOR 2004, ISAAC et al. 2013). In the same vein, haematological investigations have been explored extensively to distinguish the normal state from stress induce abnormalities which could be nutritional stress (KHAN and ZAFAR 2005). A good blood composition in animals indicate excellent performance (ISAAC et al. 2013). Blood conveys nutrients and essential materials to different parts of the body. Therefore, whatever affects the blood like nutrition will have effects on the entire body adversely or moderately alter their health, growth, maintenance and reproduction (OKE et al. 2007).

Similarly, the weight of organs can indicate the response of livestock to feed intake, the growth rate or age of the animal (OJEDIRAN et al. 2016). Previous studies shows that understanding the relationship between organ weight, and body weight will help to improve organ weight interpretation about treatment effects (PIAO et al. 2013), the authors, therefore, explained that organ weight could be the most sensitive assessment for knowing the safety of feed consumed.

Profit maximisation from the use of feed formulation with least cost is therefore the target of farmers (OJEDIRAN et al. 2017b) because feed cost accounts for about 60–76% of the total cost of running a piggery. Most farmers are interested in growth while they at times do not consider other physiological indices of these animals when opting for alternative feedstuffs. The anti-nutrients in alternative feedstuffs like *Jatropha curcas* kernel meal (OJEDIRAN et al. 2014, OLADUNJOYE et al. 2014), cassava peels (SHITU et al. 2016), with the cost of processing them and seasonal availability (OJEDIRAN et al. 2017c) have prompted research into the possibilities of using biscuit dough, an industrial waste devoid of anti-nutrients as an alternative feedstuff .

Biscuit dough is an unbaked mixture of biscuit components such as wheat flour, skimmed milk powder, vegetable fat, sugar, salt and flavour material that failed to rise and is found in substantial quantities in biscuit producing industries (SHITU et al. 2016). It could be an economical feedstuff for monogastric because the bakery does not use it for production of biscuits and it is cheaper to acquire because it is a waste product in the bakery. It has been reported to have higher metabolizable energy (ME) for swine than corn grain (NRC 1998). However, little pieces of information are available on the potential of biscuit dough as an alternative component of weaner pig diet (SHITU et al. 2016) and the physiological response of pigs fed biscuit dough is not well investigated.

This study, therefore, evaluates the haematological parameters, organ weight and villi morphometry of weaner pigs fed varying levels of biscuit dough as a replacement for maize in their diet.

Materials and Method

Experimental Location

The experiment took place at the Piggery, Unit of the Ladoke Akintola University of Technology Teaching and Research Farm, Ogbomoso, Oyo State, a derived savannah zone of Nigeria, located on latitude 18°15'N of the equator and longitude 4°5'E of the Greenwich meridian (OJEDAPO et al. 2009).

Procurement and Processing of Test Ingredients

Experimental Pigs and Their Management

The test ingredient was obtained from a biscuit factory in a pasty form. It was sun-dried to 8–9% moisture content and milled before being mixed with other feed ingredients.

Thirty (30) weaner pigs of Large White and Landrace crosses were acclimatized and fed with weaner ratio of 22% CP for a week before the commencement of the experiment. The weaner pigs were randomly allotted to five dietary groups of six weaner pigs while each pig served as a replicate. The animals had access to feed and water *ad-libitum*. The experiment took 49 days. The pigs were handled and managed following the NIH Guide for the Care and Use of Laboratory Animals NIH publication No 86–23, revised 1985 and 1991) and the ethical requirements of the United Kingdom for animal experimentation (Animals scientific procedures, Act 1986)

Experimental Diet

Five experimental diets were formulated with a crude protein content of between 20–21% and metabolizable energy ranging from 2800–3000 ME $kcal^{-1}$ kg^{-1} in a Maize-soybean meal-based diet (control) as shown in Table 1. Biscuit dough was used to replace the maize in control diet at 12.5%, 25%, 37.5% and 50% in the other diets respectively.

Table 1

Gross composition of the experimental diets

Ingredients [%]	T1	T2	T3	T4	T5
Maize	56.00	49.00	42.00	35.00	28.00
Biscuit dough	0.00	7.00	14.00	21.00	28.00
Soybean meal	25.50	24.50	23.50	21.70	19.50
Fish meal	5.50	4.00	2.80	2.00	1.00
Palm kernel cake	2.00	4.50	6.70	9.30	12.50
Fixed ingredients	11.00	11.00	11.00	11.00	11.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrients					
ME [kcal kg^{-1}]	3014.04	2966.49	2919.54	2873.39	2826.83
Crude protein	21.03	20.70	20.54	20.38	20.02
Ether extract	3.78	3.72	3.68	3.68	3.68
Crude fiber	3.77	4.16	4.51	4.87	5.29

Explanations: fixed ingredients – 9.00% cassava peel meal; 0.60% – limestone, 0.60% – di-calcium phosphate; 0.10% – lysine; 0.05% – methionine; 0.20% – premix; 0.50% – salt; ME – metabolizable energy

Data Collection

At the end of the experiment, three pigs were randomly selected from each treatment and were starved overnight for 12 hours but allowed access to water *ad libitum*.

Blood samples were collected with sterile needles and syringe from jugular vein into sterilized bottles containing Ethylene Diamine Tetra-acetic Acid (EDTA) which were taken to the laboratory for analysis: haematological parameters including packed cell volume (PCV), haemoglobin (Hb), erythrocyte count (RBC), leukocyte count (WBC), differential leukocyte counts, mean corpuscular value (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), were determined using the methods described by OJEDIRAN et al. (2015).

The pigs were then sacrificed humanely and opened up for the organs which were weighed using a sensitive scale after taking the live weight of the animal.

Laboratory Procedure for Histological Processing of Villi for Its Morphometrics

Three centimetres of each pig jejunum were cut and fixed in formalin 24 h before staining for intestinal histology and were appropriately labelled. Each histological sample was dissected, fixed in10% neutral buffered formalin for further fixing before being processed in automatic tissue processor, embedded in paraffin wax and sectioned at 5 microns on a rotary microtome, and then mounted on glass slides. Staining of the slides with hematoxylin-eosin staining method was next. The villus height, villus width and crypt depth were then measured in villi per section using optical microscopy (Akpokodje et al. 2005, Carson and Christa 2009).

Statistical Analysis Method

Analysis of variance (ANOVA) was employed for all data collected in a completely randomized design using SAS statistical software package (Sas 2000) and means were separated using Duncan multiple range test of the same package.

Results

Haematological Parameters

Table 2 shows the haematological parameters of weaner pigs fed biscuit dough. There were significant ($p < 0.05$) differences in the parameters examined. A quadratic pattern of response was observed. The WBC count values ranged from 10.00–18.55 [$\cdot 10^3$ uL^{-1}]. T1 had the highest value (18.55), followed by T4 (17.75), T3 (15.45), T2 (14.55) while T5 (10.00) had the least. Values observed for Hb content ranged from 9.50–13.00 [g dL^{-1}]. T4 had the highest value (13.00), although not different from those fed T2 (12.95), while those fed T5 (9.50) had the least ($p < 0.05$). Pigs fed T2 had the highest value of 9.73 [$\cdot 10^6$ uL^{-1}] ($p < 0.05$) for RBC while those on T1 (8.18), T3 (8.13), T4 (8.13) and T5 (8.13) were not significantly different ($p > 0.05$) from each other. HCT had values ranging from 33.2–54.85 [%]. T2 had the highest value, while T5 (33.2) recorded the least ($p < 0.05$). MCV had values ranging from 57.0–60.75 [fL]. T1 had the highest value (60.75), T3 (59.05), T4 (58.60), T2 (57.0), and the least value from T5 (49.45) ($p < 0.05$).

MCH had values ranging from 13.45 to 14.50 [pg] which were significantly ($p < 0.05$) different, T4 (14.50) had the highest value, while T2 (13.45) had the least value. MCHC had values ranging from 22.90–28.65 [g dL^{-1}] with T5 (28.65) having the highest value and T3 (22.90) with the least value.

Treatment 5 had the highest value (90.75%) of lymphocyte, while T3 (62.60%) had the least value, while PLT count values ranging from 104.50 to 384.00 [· 10^3 uL^{-1}], showed that T2 had the highest value (384.00), T1 (342.50), T3 (135.00), T5 (124.50) and lastly T4 (104.50) which were significant difference ($p < 0.05$).

Table 2

Heamatological parameters of pigs fed graded levels of biscuit dough

Parameters	T1	T2	T3	T4	T5	SEM
WBC [·10^3 uL^{-1}]	18.55^a	14.55^d	15.45^c	17.75^b	10.00^e	0.80
Hb [g dL^{-1}]	11.84^b	12.95^a	11.05^c	13.00^a	9.50^d	0.35
RBC [·10^6 uL^{-1}]	8.18^b	9.73^a	8.13^b	8.13^b	8.13^b	0.17
HCT [%]	49.70^c	54.85^a	48.20^d	52.70^b	33.20^e	2.04
MCV [fL]	60.75^a	57.00^d	59.05^b	58.60^c	49.45^e	1.05
MCH [pg]	14.44^a	13.45^c	13.55^c	14.50^a	14.15^b	0.12
MCHC [g dL^{-1}]	23.70^c	23.65^c	22.90^d	24.70^b	28.65^a	0.55
PLT [·10^3 uL^{-1}]	342.50^b	384.00^a	135.00^c	104.50^d	124.50^c	32.55
LYMPH [%]	70.20^b	64.70^d	62.60^e	68.40^c	90.75^a	2.70

Explanations: ^{ab} means along the same row with different superscript(s) are significantly different ($p < 0.05$); WBC – white blood cell; Hb – haemoglobin; RBC – red blood cell; HCT – haematocrit; MCV – mean cell volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; PLT – platelet; LYMPH – lymphocyte

Organ Weight Expressed as a Percentage of Live Weight [%]

The organs weights expressed as a percentage of live weight of weaner pigs fed graded levels of biscuit dough (BD) are presented in Table 3. The weight of the liver, heart, spleen and pancreas expressed as a percentage of live weight of weaner pigs were significantly different ($p < 0.05$) while that of kidney and lungs were not significantly ($p > 0.05$) affected. The liver weight expressed as a percentage of live weight of weaner pigs fed T1 (2.67), T3 (2.59) and T4 (2.64) were significantly higher than that of T2 (2.39) and T5 (2.44). The heart weight expressed as percentage of live weight of weaner pigs fed T5 (0.71) was higher ($p < 0.05$) than other treatments, but that of T1 (0.51) and T4 (0.51) were comparable. Pigs fed T4 (0.17) had the highest spleen weight expressed as a percentage of live weight of weaner pigs compared with T2 (0.15) while other treatments were different ($p < 0.05$). The pancreas weight expressed as percentage of live weight of weaner pigs on T1 (0.26) was significantly higher than those fed T2–T5.

Table 3

Organ weight expressed as a percentage of live weight of weaner pigs fed graded levels of biscuit dough

Parameters [%]	T1	T2	T3	T4	T5	SEM
Liver	2.67^{a}	2.39^{b}	2.59^{a}	2.64^{a}	2.44^{b}	0.03
Kidney	0.40	0.38	0.39	0.41	0.38	0.01
Heart	0.51^{ab}	0.46^{b}	0.42^{b}	0.51^{ab}	0.71^{a}	0.36
Spleen	0.11^{c}	0.15^{ab}	0.13^{b}	0.17^{a}	0.14^{c}	0.01
Pancreas	0.26^{a}	0.20^{b}	0.20^{b}	0.17^{b}	0.14^{c}	0.01
Lungs	0.93	0.86	1.01	0.93	0.97	0.02

Explanations: *a*, *b* means along the same row with different superscript(s) are significantly different ($p < 0.05$)

Villi Morphometrics

The villi morphometry of weaner pigs fed varying levels of biscuit dough shown in Table 4. The villi lengths, villi widths, villi cryptal depths, were significantly ($P < 0.05$) influenced. Pigs fed T2 (0.078 inch) had the lowest value for villi length unlike those fed T3 (0.060 inch) which had the highest value for villi width while T4 (0.057 inches) has the highest value for villi cryptal depth compared to that fed T1 (0.048 inches), T3 (0.047) and T5 (0.051 inches).

Table 4

Villi morphometry of weaner pigs fed graded level of biscuit dough

Parameters	T1	T2	T3	T4	T5	SEM
Villi length [inch]	0.105^{a}	0.078^{b}	0.107^{a}	0.107^{a}	0.105^{a}	0.002
Villi width [inch]	0.021^{b}	0.023^{b}	0.060^{a}	0.025^{b}	0.017^{b}	0.006
Villi cryptal depth [inch]	0.048^{ab}	0.041^{b}	0.047^{ab}	0.057^{a}	0.051^{ab}	0.002

Explanations: *a*, *b* means along the same row with different superscript(s) are significantly different ($p < 0.05$)

Discussion

All the haematological parameters fall within the standard values for pigs (domestic boars) (THOM 2006, EZE et al. 2010). When these values fall within the normal range reported for the livestock, it is an indication that the diets are tolerated throughout the experiment, but when the values fall below the normal range, it is an indication of anaemia (TOGUN et al. 2007). Also, lower haematological values in pigs are thought to be due to

malnutrition. Immune status is a function of leucocytes, neutrophils and lymphocytes. Lymphocytes are known to play critical roles in the immune defence system of both man and animals (AMEEN et al. 2007), while, higher leukocyte count of the pig is thought to be due to chronic pneumonia and parasitism.

Moreover, when WBC (leucocytes); neutrophils and lymphocytes fall within the normal range, it indicates that the feed does not affect the immune system (AMEEN et al. 2007). Nevertheless, increased neutrophils: lymphocytes ratio is a good indicator of stress (MINKA and AYO 2007, ADEKOLA and DUROTOYE 2004), which could be nutritional stress (ETIM et al. 2014). Report shows an association between immune function and leucocyte (EHEBA et al. 2008), while other work observed that increase in PCV coupled with a marginal increase in RBC is indicative of more efficient erythropoiesis in the experimental animals (TOGUN et al. 2007). Also lower values of PCV and Hb imply a high level of blood dilution and low efficiency of cellular oxygen transportation (NWANBE and ELECHI 2009).

It is a common practice in feeding trials to use the weights of some internal organs like liver and kidney as indicators of toxicity (SHITU et al. 2016). Previous work showed that if there are toxic elements in the animal feed, abnormalities in weights of liver and kidney could be observed. The abnormalities will arise because of the increased metabolic rate of the organs in an attempt to reduce these toxic elements or anti-nutritional factors to nontoxic metabolites (AHAMEFULE et al. 2006). However, the reported on biscuit waste showed that biscuit waste has no anti-nutritional factor; therefore, could make a suitable replacement for maize (ADEYEMO et al. 2013). This position could be buttressed by the quadratic pattern observed by these organs except the linear decrease in the weight of pancreas as the BD increases suggesting hypo-secretion of pancreatic juice, which suggest that addition of BD to the absolute limit may reduce the production of acid by the lumen as shown in this study.

Higher villi area (length and width) could indicate a greater surface area for absorption of nutrients (OJEDIRAN et al. 2017b) which is also in line with previous observation and concluded that efficient utilization of feed could be a function of the response of the villous to the feed form (NKUKWANA 2014).

Conclusion

It can be concluded that 50% biscuit dough replacement for maize weakened the body defensive mechanism, reduced the efficiency of cellular oxygen transportation, pancreatic secretion and villi width of weaner pigs,

while 37.5% biscuit dough replacement for maize improved the parameters mentioned above. It is, therefore, recommended that 37.5% of biscuit dough is suitable for the replacement of maize in weaner pig's diets.

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