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Physiological response of growing pigs to qualitative and quantitative feed restriction in a humid tropical enviroment

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Abstract

Feed restriction is a common management practice with market pigs to improve carcass quality and feed efficiency while decreasing production cost and carcass fat. But some scientists have argued that feed restriction poses stress on pig. Arising from this, a 3 x 3 factorial experiment was carried out to determine the physiological responses of growing pigs to qualitative and quantitative feed restriction. Fifty four mixed breed pigs with initial average weight of 6.72±0.38 kg were divided into 9 treatment groups. Treatments 1-3 consisted of pigs fed *ad-libitum* with rations containing 20, 18 or 16% crude protein, respectively, pigs on treatments 4-6 were fed 90% of *ad-libitum* with rations containing 20, 18 or 16% crude protein, respectively, while treatments 7-9 were fed 80% of *ad-libitum* with rations containing 20, 18 or 16% crude protein, respectively. Pulse rate and rectal temperature were monitored on weekly basis while blood samples were collected on the 12th week for haematological and serum analysis. Quality of feed offered had no significant effect ($p>0.05$) on the haematological and serum biochemistry parameters considered. Neutrophil, lymphocyte, neutrophil:lymphocyte, monocyte, total protein and cortisol values were significantly ($p<0.05$) influenced by quantity of feed offered. Rectal temperature and pulse rate were not significantly ($p>0.05$) influenced by the quality of feed, but they were significantly ($p<0.05$) affected by the quantity of feed offered. It can be concluded that quantitative not qualitative restriction had effect on some physiological (rectal temperature and pulse rate) and serum biochemistry parameters of growing pigs.

Keywords: Biochemistry, haematology, qualitative, quantitative, restriction, rectal

Introduction

Stress has been described as chemical and physical factors that may result to physiological reactions that contribute to bodily or mental discomfort, disease and –or death (Martinez-Porchas *et al*., 2009). It inhibits the immune system of domestic animals (De Groot *et al*., 2000). Stress can be grouped into two phases: the healthy and bad stress (Selye, 1985). The healthy stress can be defined as the physiological changes that optimize organism's biological functions while the bad stress involves physiological changes that may compromise organism's integrity. Organism response to stress is characterized by the stimulation of the hypothalamus, which results in the activation of the neuroendocrine system and a subsequent flow of metabolic and physiological changes (Lowe and Davison, 2005; Martinez-Porchas *et al*., 2009). These changes enhance the tolerance of an organism to environmental variation or an adverse condition while maintaining a homeostasis status.

The physiological state of domestic animals is influenced by several factors, among which is nutrition (Renaudeau *et al*., 2012). Nutritional status of an animal is dependent on dietary intake and effectiveness of metabolic processes. These can be determined by either or combinations of chemical, anthropometric, biochemical or feeding methods (Bamishaiye *et al.,* 2009). Addass *et al*. (2012) reported that nutrition influences blood values of domestic animals. Adeyemi *et al*. (2015) asserted that the haematological indices of animals are affected by nutritional status of the animals and they are valuable components in routine screening for health and physiological state of the domestic animals. Blood profile and some vital signs (rectal temperature, heart beat and respiratory rate) studies have been found useful for disease prognosis and for therapeutic and stress monitoring (Mohamed and Abdelatif, 2010). It helps to distinguish normal state from state of stress which can be nutritional (Aderemi, 2004). Several nutritional strategies have been proposed to alleviate the adverse effects of environmental stress (Renaudeau *et al*., 2012) among which is feed restriction. Feed restriction is a feeding method that involves the manipulation of animals' ration in order to decrease growth and metabolic rate to some extent, hence, minimising the incidence of some metabolic diseases and as well as improving feed efficiency. Most feed restriction studies investigated the effects of limited feeding (qualitative and quantitative) on growth performance, nutrient digestibility, carcass yield and economic efficiency (Lebret, 2008; Njoku *et al*., 2013) without regard to the physiological state of the animal being subjected to this vital management strategy. To this effect, this study was carried out to elucidate the physiological responses of growing pigs to qualitative and quantitative feed restriction, acknowledging the fact that the physiological states of domestic animals determine the level of productivity.

Materials and Methods

Experimental site

The experiment was carried out at the Piggery Unit of the Teaching and Research Farms Directorate (TREFAD) and Meat Processing Laboratory of Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The farm lies within latitude 7°10´N, longitude 3°2´E and altitude 76 mm. It is located in the derived savannah zone of South-Western Nigeria. It has a humid climate with mean annual rainfall of about 1037 mm and temperature of about 34.7°C. The relative humidity ranges from 63 to 96% in the rainy season (late March to October) and from 55 to 82% in the dry season (November to early March) with an annual average humidity of 82%. The seasonal distribution of annual rainfall is approximately 44.96 mm in the late dry season (January-March); 212.4 mm in the early wet season (April-June); 259.3 mm in the late wet season (July-September) and 48.1 mm in the early dry season (October-December) as documented by Federal University of Agriculture, Abeokuta Meteorological Station.

Experimental animals and management

A total of 54 mixed breed pigs weighing 6.72±0.38 kg were bought from a reputable farm within Abeokuta metropolis. The pigs were grouped on weight equalisation basis into 9 treatments with 3 replicates of 2 pigs per replicate. The floor of the housing unit was washed with detergent and disinfectant. On arrival the pigs were given water and feed containing anti-stress and multi-vitamins. The pigs were injected with Ivomec® (Ivermectine) against endo- and ecto-parasites and allowed to acclimatize for 7 d before the commencement of the experiment. The pen had a floor area of 3 m by 2 m, equipped with concrete feeding and watering trough. The pens were half-walled of about 1.4-1.6 m high and the rest were open-sided for proper ventilation. Routine management practices were carried out on a daily basis, fresh water was supplied *ad libitum* throughout the 150-d experimental period.

Experimental design

The experimental design was a 3 × 3 factorial arrangement with factor A consisting of 3 levels of qualitative feed restriction (20%, 18% and 16% crude protein) and factor B involving quantitative feed restriction (*ad libitum*, 90% and 80% of *ad libitum* feeding). *Ad libitum* feed intake of pigs of the same class had been previously determined in a preliminary study. Groups 1, 2 and 3 consisted of pigs fed ration containing 20, 18 and 16% crude protein at *ad libitum* feeding level, respectively, groups 4, 5 and 6 consisted of pigs fed ration containing 20, 18 and 16% crude protein ration at 90% of *ad-libitum* feeding level, respectively, and groups 7, 8 and 9 consisted of pigs fed ration containing 20, 18 and 16% crude protein ration at 80% of *ad-libitum* feeding level, respectively.

Experimental diets

Three experimental diets were formulated to meet the body requirements of growing pigs as shown in Table 1. The ration contained 20.30, 18.00 or 16.16% crude protein and metabolisable energy of 2677.50, 2870.09 or 2932.37 kcal/kg DE (Auto Feed Formulator, NRC) for diets 1, 2 and 3, respectively. Feed was offered to the experimental pigs at 08:00 hour daily throughout the experimental period.

Table 1: Percentage composition of experimental diets

Ingredients	Diet 1	Diet 2	Diet 3
Maize	29.00	40.00	45.50
Groundnut cake	18.00	13.00	8.00
Wheat offal	30.00	20.00	19.05
Palm kernel cake	19.65	23.65	24.10
Bone meal	2.60	2.60	2.60
Salt	0.35	0.35	0.35
Lysine	0.05	0.05	0.05
Methionine	0.05	0.05	0.05
*Premix	0.30	0.30	0.30
Total	100.00	100.00	100.00
Determined Analysis (%)			
Crude protein	20.30	18.00	16.16
Fat	6.81	7.34	7.26
Crude fibre	5.09	4.47	4.25
Calcium	0.77	0.79	0.84
M.E (Kcal/kg feed)	2677.50	2870.09	2932.37

*To supply the following per kg diet; VitA 12600 IU; Vit. D3 2800 IU; Vit E 49 IU; Vit K3 2.8 mg; Vit B1 1.4 mg; Vit B2 5.6 mg; Vit. B6 1.4mg; Vit B12 0.014mcg; Niacin 21 mg; Pantothenic Acid 14 mg; Folic Acid 1.4 mg; Biotin 0.028 mcg; Choline Chloride 70 mg; Manganese 70mg; Zinc 140 mg; Iron 140 mg; Copper 140 mg; Iodine 1.4 mg; Selenium 0.28 mg; Cobalt 0.7 mg; Antioxidant 168 mg.

Data collection

Physiological indicators like pulse rate and rectal temperature were measured on weekly basis throughout the experimental period. The pulse rate determines the frequency of the heart beat per minute. A stethoscope and a stop watch were used in determining the pulse rate of the growing pigs. Rectal temperature was determined by inserting a digital rectal thermometer through the anus to the rectum of the pig and the temperature was read once the thermometer beeped. These parameters were carried out on weekly basis at 09:00 hour before feeding.

Haematological and biochemical parameters

Two pigs per treatment were selected randomly and bled in order to determine the haematological, serum chemical and physiological stress parameters. The pigs were restrained and bled through the anterior vena cava. About 2.5 ml blood sample was collected into a labelled bottle containing ethylene diamine tetra acetate (EDTA) and another 5 ml was collected into a sterile new 10-ml hypodermic syringe. The blood sample in the EDTA bottle was used for the determination of haematological parameters while the one in hypodermic syringe was refrigerated for 6 h, after which the serum was separated into clean sterile test tubes bottles to be used for the biochemical analyses. The haematological parameters determined were white blood cell count, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration. An auto haemo-analyser machine was used in determination of these parameters. The total serum protein was determined according to the method of Nufield (2010) while the serum albumin, globulin and cholesterol were determined spectrophotometrically using commercial Bio-La-Tests (Pliva-LaChema Brno Ltd; Czech Republic), and serum creatinine was determined using enzymatic creatinine assays as described by Daly *et al*. (1996). Serum glucose was estimated using a commercial glucose colorimetric assay kit (Cayman® Chemical Company, Ann Arbor, USA) and the protocol used was according to the manufacturer's instruction.

Statistical analysis

The experimental layout was a 3x3 factorial arrangement. Data generated were subject to two-way Analysis of Variance using SAS (2000). Differences between means for the variables were detected for significance using New Duncan Multiple Range Test.

Results and Discussion

Effect of qualitative restriction on haematological and serum biochemistry of growing pigs

Table 2 shows the effects of qualitative feed restriction on haematological and serum biochemistry of the growing pigs. Levels of dietary protein in the ration of growing pigs had no significant ($p>0.05$) effect on all haematological and serum biochemical parameters considered. Packed cell volume (26.00, 21.83, 21.33%), haemoglobin (8.53, 7.33, 7.20%), red blood cell (4.30, 3.60, 3.55 x 10^6), neutrophil (32.83, 32.17, 29.50%) and neutrophil:lymphocyte (0.50, 0.49, 0.43) values decreased with decreasing levels of crude protein while basophil (0.17, 0.50, 1.17%) and mean corpuscular haemoglobin concentration (32.90, 33.83, 33.93 g/l) increased with decreasing level of protein in the pigs' diets. The serum biochemistry revealed that total protein (5.92, 5.62, 5.55 g/dl), albumin (3.33, 3.32, 2.98 g/dl), globulin (2.58, 2.30, 2.23 g/dl), cholesterol (80.83, 71.50, 68.53 mg/dl), glucose (72.00, 64.17, 63.00 mg/dl) and cortisol (11.73, 11.13, 10.28 ng/ml) values decreased with decreasing levels of dietary crude protein of growing pigs. White blood cell, lymphocyte, eosinophil, basophil, monocyte and mean corpuscular volume showed no definite pattern.

Table 2: Effect of qualitative restriction on haematological and serum biochemistry parameters of growing pigs

	Dietary crude protein level			
Parameters	20%	18%	16%	SEM
Haematological parameters				
Packed cell volume (%)	26.00	21.83	21.33	2.67
Haemoglobin (%)	8.53	7.33	7.20	0.81
Red blood cell (10^6/µl)	4.30	3.60	3.55	0.40
White blood cell (10^9/l)	9.05	7.52	7.90	0.91
Neutrophil (%)	32.83	32.17	29.50	1.54
Lymphocyte (%)	66.33	66.17	68.33	1.36
Neutrophil:Lymphocyte	0.50	0.49	0.43	0.27
Eosinophil (%)	0.57	0.50	0.50	0.29
Basophil (%)	0.17	0.50	1.17	0.32
Monocyte	0.50	0.67	0.50	0.19
Mean Corpuscular volume (fl)	59.82	60.65	59.83	1.56
Mean corpuscular Haemoglobin(pg)	19.67	20.50	20.22	0.37
Mean Corpuscular haemoglobin concentration (g/l)	32.90	33.83	33.93	0.59
Serum biochemistry				
Total protein (g/dl)	5.92	5.62	5.22	0.32
Albumin (g/dl)	3.33	3.32	2.98	0.20
Globulin (g/dl)	2.58	2.30	2.23	0.18
Cholesterol (mg/dl)	80.83	71.50	68.53	6.65
Glucose (mg/dl)	72.00	64.17	63.00	5.23
Cortisol (ng/ml)	11.73	11.13	10.28	0.64

Effect of quantitative restriction on haematological and serum biochemistry parameters of growing pigs

The effects of quantitative feed restriction on haematological and biochemical indices of growing pigs are shown in Table 3. All the parameters studied except neutrophil, lymphocyte, neutrophil:lymphocyte, monocyte, total protein, globulin and cortisol were not significantly ($p>0.05$) affected by the amount of feed offered. Mean neutrophil level of pigs fed *ad libitum* (34.17%) was not different from that of pigs fed 90% of *ad libitum* (32.67%) but varied significantly ($p<0.05$) from 27.67% documented for those on 80% of *ad libitum* feeding level. Lymphocyte values ranged from 64.67% in pigs fed *ad libitum* to 70.00% in pigs fed 80% of *ad libitum*. Pigs on *ad libitum* and 90% *ad libitum* feeding had similar monocyte values (2.30, 2.34%, respectively) that were lower ($p<0.05$) than that for pigs on 80% *ad libitum* feeding (3.00%). The serum biochemistry revealed that total protein, globulin and cortisol increased significantly with increasing levels of feed offered. The pigs on *ad libitum* feeding had the highest total protein value of 6.32 g/dl while those on 80% feeding level recorded the least value of 4.93 g/dl. The values obtained for globulin ranged from 1.98 g/dl in pigs on 80% *ad libitum* feed offered to 2.75 g/dl in pigs fed *ad libitum*. The highest cortisol value documented for pigs on *ad libitum* feeding (12.53 ng/ml) compared to 10.90 and 9.71 ng/ml noted for pigs on 90 and 80% of *ad libitum* feeding, respectively.

Table 3: Effect of quantitative restriction on haematological and serum biochemistry parameters of growing pigs

Parameters	Restriction level (% *ad libitum*)			
	100%	90%	80%	SEM
Haematological parameters				
Packed cell volume (%)	27.00	22.83	19.33	2.67
Haemoglobin (%)	8.98	7.57	6.52	0.81
Red blood cell (%)	4.48	3.68	3.32	0.40
White blood cell (10^9/l)	9.35	8.3	6.83	0.91
Neutrophil (%)	34.17^a	32.67^a	27.67^b	1.54
Lymphocyte (%)	64.67^b	66.17^{ab}	70.00^a	1.36
Neutrophil:Lymphocyte	0.53^a	0.49^{ab}	0.40^b	0.24
Eosinophils (%)	0.33	0.17	0.67	0.29
Basophil (%)	0.50	0.67	0.67	0.32
Monocyte (%)	2.30^b	2.34^b	3.00^a	0.19
Mean Corpuscular Volume (fl)	59.45	62.13	58.72	1.56
Mean Corpuscular Haemoglobin (pg)	20.00	20.58	19.80	0.37
Mean Corpuscular Haemoglobin Concentration (g/l)	33.77	33.13	33.77	0.56
Serum Biochemistry				
Total protein (g/dl)	6.32^a	5.50^{ab}	4.93^b	0.32
Albumin (g/dl)	3.57	3.12	2.95	0.20
Globulin (g/dl)	2.75^a	2.38^{ab}	1.98^b	0.18
Cholesterol (mg/dl)	83.50	73.33	64.33	6.65
Glucose (mg/dl)	74.33	66.17	58.67	5.23
Cortisol (ng/ml)	12.53^a	10.90^{ab}	9.71^b	0.64

[ab]Means within the same row with different superscripts are significantly ($p<0.05$) different

Table 4: Interaction between qualitative and quantitative restriction on haematological serum biochemistry parameters of growing pigs

Qualitative (% Crude protein)	20%			18%			16%			
Quantitative (% *ad libitum*)	100%	90%	80%	100%	90%	80%	100%	90%	80%	
Parameters/Treatments	1	2	3	4	5	6	7	8	9	SEM
Haematological parameters										
Packed cell volume (%)	31.50	25.50	21.00	23.00	21.50	19.50	26.50	21.50	17.50	4.62
Haemoglobin (%)	10.15	7.95	8.85	8.5	7.00	7.20	6.95	6.65	5.95	1.41
Red blood cell (%)	5.10	3.85	4.45	4.1	3.40	3.50	3.15	3.40	2.80	0.69
White blood cell (10^9/l)	10.80	8.20	9.05	8.85	8.50	7.50	7.50	7.00	6.00	1.57
Neutrophil (%)	36.50^{a}	33.50^{ab}	32.5^{ab}	33.5^{ab}	28.50^{ab}	36.00^{a}	28.50^{ab}	26.50^{b}	28.00^{ab}	2.66
Lymphocyte (%)	63.00	65.50	65.50	66.00	70.00	63.00	70.00	69.50	70.50	2.36
Eosinophil (%)	0.00	0.50	0.50	0.00	0.50	0.00	0.50	0.50	1.00	0.50
Basophils (%)	0.00^{b}	0.50^{ab}	1.00^{ab}	0.00^{b}	0.50^{ab}	0.00^{b}	0.00^{b}	2.00^{a}	0.00^{b}	0.55
Monocyte (%)	0.50^{ab}	0.00^{b}	0.50^{ab}	0.00^{b}	0.00^{ab}	0.50^{b}	1.00^{ab}	1.50^{a}	0.50^{ab}	0.33
Mean corpuscular volume (fl)	63.25	58.15	58.45	62.15	63.25	61.00	56.15	57.50	62.50	2.70
Mean Corpuscular Haemoglobin(pg)	19.70^{ab}	20.50^{ab}	19.80^{ab}	20.7^{ab}	20.60^{ab}	20.45^{ab}	18.60^{b}	19.55^{ab}	21.25^{a}	2.65
Mean corposcular haemoglobin concentration(g/l)	32.25	35.10	33.95	33.30	32.60	33.50	33.15	34.10	34.05	1.01
Serum biochemistry										
Total Protein (g/dl)	6.75^{a}	5.60^{ab}	6.60^{ab}	5.85^{ab}	5.05^{ab}	5.60^{ab}	5.15^{ab}	5.0^{ab}	4.65^{b}	0.56
Albumin (g/dl)	3.60	3.20	3.90	3.2	2.90	3.25	3.2	2.85	2.80	0.35
Globulin (g/dl)	3.15^{a}	2.40^{ab}	2.70^{ab}	2.65^{ab}	2.15^{ab}	2.35^{ab}	1.95^{b}	2.15^{ab}	1.85^{b}	0.32
Cholesterol (mg/dl)	95.00	72.50	83.00	79.50	69.50	71.00	68.00	64.50	60.5^{b}	11.51
Glucose (mg/dl)	83.00	67.00	73.00	71.00	63.00	64.50	62.00	59.00	55.00	9.05
Cortisol (ng/ml)	13.4^{a}	11.10^{ab}	13.10^{ab}	11.6^{ab}	10.00^{ab}	11.10^{ab}	10.20^{ab}	9.75^{ab}	9.20^{b}	1.12

[ab]Means within the same row with different superscripts are significantly ($p<0.05$) different

Interaction between qualitative and quantitative restriction on haematological indices and serum biochemistry of growing pigs

The interaction between qualitative and quantitative feed restriction had significant ($p<0.05$) effect on some of haematological parameters (neutrophil, basophils, monocyte and mean corpuscular haemoglobin) and serum biochemical indices (total protein, globulin and cortisol). Significant difference observed in neutrophil had highest value of 36.50% recorded for pigs fed *ad libitum* with 20% crude protein ration and the least value of 26.50% was noted for pigs on 16% dietary crude protein ration at 90% of *ad libitum* feed offered. Also significant differences were seen in basophil, monocyte and mean corpuscular haemoglobin. For the serum biochemistry, significant differences were seen in total protein, globulin and cortisol. Pigs fed *ad libitum* with 20% dietary crude protein ration had the highest total protein value (6.75 g/dl) while their counterparts on 80% of *ad libitum* feeding containing 16% crude protein had the least value (4.65 g/dl). The same trends were noted for the globulin and cortisol values.

Effect of qualitative restriction on rectal temperature and pulse rate of growing pigs

Table 5 shows the effect of qualitative feed restriction on rectal temperature and pulse rate of growing pigs. Rectal temperature and pulse rate were not significantly ($p>0.05$) influenced by the levels of dietary crude protein in diets of growing pigs. Rectal temperature and pulse rate decreased with decreasing crude protein content in the diets. Rectal temperature and pulse rate ranged from 39.48^{o}C and 149.84 heart beats min^{-1} in pigs fed 16% crude protein diet to 39.61^{o}C and 155.62 heart beats min^{-1}, respectively, for pigs fed 20% crude protein diet.

Table 5: Effect of qualitative restriction on physiological parameters of growing pigs

Parameters	Crude protein level			SEM
	20%	18%	16%	
Rectal temperature (°C)	39.61	39.54	39.48	0.41
Pulse rate (heat beat/minute)	155.62	152.48	149.84	2.17

Effect of quantitative restriction on rectal temperature and pulse rate of growing pigs

Rectal temperature and pulse rate were significantly ($p<0.05$) affected by quantitative feed restriction (Table 6). Rectal temperature decreased significantly (39.70, 39.54, 39.39°C) with decreasing levels of feed offered. Pulse rate values ranged from 147.95 heart beats min^{-1} in pigs on 80% of *ad libitum* feeding to 155.68 heart beats min^{1} in pigs fed *ad libitum.*

Table 6: Effect of quantitative restriction on physiological parameters of growing pigs

	Restriction level (% *ad libitum*)			
Parameters	100%	90%	80%	SEM
Rectal temperature (°C)	39.70[a]	39.54[b]	39.39[c]	0.31
Pulse rate (heat beat/minute)	155.68[a]	155.30[a]	147.95[b]	1.97

[abc]Means within the same row with different superscripts are significantly ($p<0.05$) different

Interaction between qualitative and quantitative restriction on rectal temperature and pulse rate of growing pigs

The interaction between qualitative and quantitative restriction significantly ($p<0.05$) influenced the rectal temperature and pulse rate of growing pigs (Table 7). The rectal temperature values ranged from 39.26°C (pigs fed 80% of *ad libitum* with ration containing 18% dietary crude protein) to 39.75°C (pigs fed *ad libitum* with 18% crude protein ration). Pulse rate values ranged from 140.72 heart beats min^{-1} (pigs subjected to 80% *ad libitum* feed restriction with 18% crude protein ration) to 161.90 heart beats min^{-1} (pigs fed 90% of *ad libitum* with 20% crude protein diet).

Table 7: Interaction between qualitative and quantitative restriction on physiological parameters of growing pigs

Qualitative (% crude protein)	20%			18%			16%			
Quantitative (% *ad libitum*)	100%	90%	80%	100%	90%	80%	100%	90%	80%	
Parameters/Treatments	1	2	3	4	5	6	7	8	9	SEM
Rectal Temperature (°C)	39.73[a]	39.70[a]	39.39[bc]	39.75[a]	39.63[a]	39.26[c]	39.62[a]	39.29[c]	39.53[ab]	0.72
Pulse rate (heart beat/minute)	153.90[a]	161.90[a]	151.05[ab]	155.62[a]	153.19[a]	140.72[b]	157.52[a]	150.81[ab]	150.09[ab]	3.76

[abc]Means within the same row with different superscripts are significantly ($p<0.05$) different

Nutritional level is among the major factors that affect the physiology of domestic animals (Renaudeau *et al*., 2012). It plays a vital role in health, productivity and profitability of farm animals. Haematological indices are important indicators of health and disease conditions in domestic animals. It reflects the physiological responsiveness of the animals to its internal and external environment (Esonu *et al*., 2001). The result of this present study showed that the packed cell volume, haemoglobin and red blood cell values numerically decreased with decreasing levels of dietary crude protein with the range values of 21.33 to 26.00%, 7.20 to 8.53% and $3.55x10^6$ to 4.30 $x10^6$/µl, respectively, which were lower than the reference range values of 32.0-50.0%, 10.0-16.0% and $3.55\text{-}4.30x10^6$/µl, respectively, as recommended by RAR (2009) and Coronado (2014). The decreasing levels of packed cell volume, haemoglobin and red blood cell with reduction in dietary crude protein corroborated the tendency of decreasing blood components with appropriate nutrient reduction in the diet of domestic animals, leading to a decrease in growth rate (Kermanshahi *et al*., 2011). The homeostasis system of the pigs was not affected by the reduction in dietary crude protein of growing pigs which indicated that growing pigs could tolerate the changes in crude protein levels as indicated by the blood parameters. Hence, the physiological process involved in erythropoietin production was not compromised by dietary crude protein reduction since the haematological indices values were comparable. This observation contradicted the report of Machebe *et al*. (2010) who observed that 14-16% dietary crude protein were inadequate to meet the physiological and developmental needs of breeding gilts as a specific quantity of protein is needed to maintain protein homeostasis (Beitz, 1993). The variation in these reports may be linked to environmental differences, breed, sex, sampling techniques and testing methodology (RAR, 2009; Etim *et al*., 2014). The non-significant effects observed in the white blood cell differential count (lymphocyte, eosinophil, basophil and monocyte) and other indices connected with iron metabolism (mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration) are in line with the reports of Machebe *et al*. (2010) and Korniewicz *et al*. (2012). These values fall within the standard reference values of pigs as outlined by Research Animal Resources (RAR, 2009), Etim *et al*. (2013) and Coronado (2014). Decrease in mean corpuscular haemoglobin concentration with increase in dietary crude protein level is in line with the study of Ahmed *et al*. (1994) that observed a decline in mean corpuscular haemoglobin concentration with increasing dietary protein level. Etim *et al*. (2014) reported that when the leucocytes, neutrophils and lymphocytes fall within the reference range, it indicates that the feeding strategy does not compromise the immune system. The serum biochemistry values noted in this study showed that dietary crude protein level had no significant effect on total protein, albumin, globulin, cholesterol, glucose and cortisol values. These findings are in agreement with Bindas (2009) who noted that early-weaned piglets subject to different protein level diets showed no significant effect on the biochemical parameters (total protein, albumin, glucose, cholesterol etc). But was in contrast with Korniewicz *et al*. (2012) who indicated that the reduction of protein level in sow ration had a significant influence on the indices of protein transformation (total protein, globulins and urea).

According to Daramola *et al*. (2005), haematological values could serve as baseline information for comparisons of nutrient deficiency, physiology and health status of domestic animals. Deviation in haematological indices is often used to determine stresses due to nutrition and other factors (Afolabi *et al*., 2011). Feeding regimen as practiced in this present study had no significant effect on packed cell volume, haemoglobin and red blood cell of growing pigs. The current observations are consistent with the findings of Bratte (2011) and Adeyemi *et al*. (2015) of similarities in the haematological indices of full-fed and feed-restricted broiler chickens. The packed cell volume, haemoglobin and red blood cell values were below the standard reference values as reported by RAR (2009) and Coronado (2014). The decrease in these parameters from standard may be an indication of anaemic condition (Etim *et al*., 2014) which could not be associated to the restriction effects since control groups had similar values. Although, De *et al*. (2014) opined that the cause of low values for haematological parameters in pigs was due to malnutrition. The similarities in values of white blood cells which fell within the normal range values as documented by RAR (2009) indicated that the growing pigs were healthy. Reilly (1993) opined that normal range of values for white blood cells indicated that the animals were healthy because a decrease in number of white blood cells below the normal range is an indication of allergic conditions, anaphylactic shock and certain parasitism. The significant decrease in neutrophils with reduction in feed offered indicated that the immune defence mechanism of the pigs was not compromised by quantitative feed restriction. Neutrophil values in this study fell within the reference standard values for pigs as outlined by Coronado (2014). Etim *et al*. (2014) enthused that when leucocytes, neutrophils and lymphocytes fell within the normal range, it indicated that the feeding pattern did not affect the immune system. The significant increase in lymphocyte values with increasing restriction levels might not be unconnected to harsh environmental condition (thermal stress) of the tropics in which the pigs were raised. Togun *et al*. (2007) reported that a significantly higher lymphocyte count was an indication of an inclement in the ability of the experimental animals to produce and release antibiotics when physiologically challenged. Monocytes frequencies have been reported to positively correlate with the level of feed restriction imposed (Hocking *et al*., 1996). This assertion is in acquiescence with the results of this present study where the monocytes values of the restricted pigs varied significantly with the level of restriction. The monocytes values fell within the normal reference values as documented by RAR (2009), Etim *et al* (2013) and Coronado (2014). The significant reduction of neutrophil:lymphocyte ratio with increasing level of feed restriction indicated that the level of restriction practiced in this study posed no physiological stress on the pigs. Bratte (2011) posited that increase in neutrophil and lymphocyte ratio is a good indicator of stress in domestic animals. Also, Hocking *et al*. (1993) averred that psychological and physical stressors like fasting, frustration, water restriction and high stocking density increase the heterophil:lymphocyte ratio of domestic fowls. The significant decrease in total protein and globulin values with increasing level of feed restriction designated that the body conditions of the pigs were affected to some extent due to lower protein synthesis (Piotrowska *et al*., 2011). Swenson (1993) asserted that the synthesis of serum proteins drastically reduced when the supply of amino acid for digestive process was inadequate. It has been documented that urea, total protein and creatinine levels in the blood depended on both quantity and quality of dietary protein (Iyayi and Tewe, 1998). The decrease in serum total protein and globulin values with increasing levels of restriction was in line with the observation of Mohamed (2012) who noted that restricted-fed rams had significantly lower serum total protein compared to their counterparts on *ad libitum* feeding regimen. Blood glucose appears to be one of the major factors affecting nutrients cyclicity in domestic animals and a minimum level of 40-60 mg/ml is required to maintain the physiological functions of the body (Ahmad *et al*., 2004). The non significant values of blood glucose in this study might be an indication that the level of restriction studied did not require further glucose catabolism. Ebeid *et al*. (2012) observed that quantitative restriction did not significantly influence the plasma glucose level of growing rabbits. Although the values obtained in this study are within the standard reference values as outlined by Mercks (2004), the values decreased numerically with decreasing level of feed offered.

Growing pigs are sensitive to high temperature since genetic improvement; breeding intensity and low thermolytic ability affect their reactions to heat stress factors (Fagundes *et al*., 2008). In hot environment, high planes of feeding decrease the heat tolerance of pigs due to excess thermal loads resulting from metabolic activity. Cortisol concentration has been used for decades as a physiological marker of stress in domestic animals (Yates *et al*., 2008). Its synthesis is stimulated by physical stress, emotional or psychological stress and hypoglycaemia (Okeudo and Moss, 2005). The decreasing concentration of cortisol with increasing level of feed restriction in this study may be attributed to reduction in metabolic heat production which may have a positive impact on the welfare of the pigs. Heat increment of metabolism was connected with the feeding level and the quality of feed (Mohamed, 2012). Serum cortisol increased at different stress conditions and duration (Fagundes *et al*., 2008). Yoshioka *et al*. (2004) observed that both normal pigs and heterozygous pigs with Halothane gene showed an increase in cortisol levels after subjecting to stress condition with increase of rectal temperature and respiratory rate as clinical signs.

The levels of crude protein in the diets of the study had no effects on rectal temperature and pulse rate of growing pigs and the values obtained increased with increasing levels of dietary crude protein. The explanation for the increase in rectal temperature and pulse rate with increasing dietary crude protein content might be linked to high heat increment of crude protein. Pond *et al*. (2012) posited that protein presents the higher heat increment among nutrients. Also, Le Bellego *et al.* (2001) observed that diets with reduced crude protein level and fat addition resulted to lower heat production in pigs. Since body temperature in homeotherms is largely dependent upon oxidative phosphorylation, increment in rectal temperature in terms of dietary crude protein level is likely to be a reflective increase in the rate of oxygen required for the combustion of extra unit of dietary crude protein. Renaudeau *et al*. (2001) stated that the elevation of peripheral temperature can be attributed to an increase of blood flow in the skin's vessels for dissipation of body heat.

Energy for maintenance of vital physiological processes is derived from the organic matter in the feed. Higher feeding levels increase the metabolic heat production and thereby increase the rectal temperature and pulse rate while low levels of feeding decrease the metabolic heat production and thereby reduce rectal temperature and pulse rate (Schemidt, 1995). This is in line with the present study where higher feeding regimen resulted in significant increase in rectal temperature and pulse rate of the growing pigs. It was observed that farm animals on high plane of feeding tended to pump more blood from the heart to digest and transport nutrients to the various parts of the body resulting to an increase in the pulse rate, rectal temperature and respiratory rate (Mohamed *et al*., 2010). It is a known fact that many farm animals respond to an energy deficit by reducing their body temperature (Lane *et al*., 1996). Matsumoto *et al*. (1990) reported a positive correlation between feed intake and pulse rate in domestic animals.

Conclusion

The results from this study showed that some physiological (rectal temperature and pulse rate) and serum biochemistry parameters of growing pigs were adversely affected by quantitative but not by qualitative restriction of feed intake. Haematological indices, serum biochemistry, rectal temperature and pulse rate are indicators that qualitative restriction may result in a discernible improvement in the welfare of growing pigs resulting in high production efficiency and economic gains.

Ethical Standard

The experimental protocols involving the use of animals were in compliance with the animal welfare requirements for care and management of experimental animals and approved by the Animal Welfare Committee of the Federal University of Agriculture, Abeokuta, Nigeria.

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