Nutrient digestibility and physiological implication of feed withdrawal periods and ascorbic acid supplementation in growing pigs under tropical environment

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ABSTRACT

A study was conducted to evaluate nutrient digestibility and physiological impact of feed withdrawal periods and ascorbic acid supplementation in pigs reared in tropics. Unsexed, 8 weeks old mixed breed pigs (54) with mean body weight of 5.5±0.2 kg were arranged in a 3×3 factorial layout. The pigs were grouped on weight equalization into 9 treatments of 3 replicates with 2 pigs per replicate. Vital signs data were obtained on weekly basis. Three pigs from each treatment group were selected at tenth week and arranged in clean disinfected metabolic cages for a 7 day adaptation and a 5 day quantification of feed intake and faecal output. Faeces collected were dried in a forced air cabinet and analysed for proximate composition. About 5 ml blood sample was collected, 2.5 ml each was placed in EDTA and sterile bottles for haematological and biochemical indices evaluation. Data obtained were subjected to a 2-way analysis of variance. The feed withdrawal period had no impact on nutrient digestibility. Ascorbic acid supplementation significantly influenced excreted faeces, faecal dry matter output, dry matter and ash digestibility. Neutrophils increased with increasing period of feed withdrawal while lymphocytes decreased significantly with increasing period of feed withdrawal. Feed withdrawal period and ascorbic acid supplementation had no influence on serum biochemical parameters. It can be concluded that feed withdrawal periods influenced neutrophil, lymphocyte and neutrophil-lymphocyte ratio whereas ascorbic acid enhanced nutrient digestibility of pigs but had no impact on vital signs and blood indices.

Key words: Ascorbic acid, Blood indices, Feed withdrawl, Growing pigs, Nutrient intake, Pigs, Pulse rate

Altering feeding management techniques such as change in feeding time or frequency of feeding are proficient tools utilised by pig farmers to avoid excessive heat load and promote the survivability of their pigs. Feed denial during the hot hours of the day could be a management technique that can promote the welfare of pigs by reducing the rate of metabolic heat production since digestion takes place during the cool period of the day. Digestion process generates heat and attains maximum level several hours after ingestion (Njoku *et al.* 2017). Altering feed supplies to pigs consequently during the hot hours of the day can eliminate some latent superfluous heat from digestion process. This modification can lead to improvement in production index by increasing the rate of feed consumption or compensates the low feed intake during the stress period.

Ascorbic acid also creates anti-stress hormones that assist to combat the effect of environmental stress in pigs. Under normal environmental condition, biosynthesis of ascorbic acid in the renal medulla and contex equates to the

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physiological needs of the pigs, hence, dietary supplementation of ascorbic acid is not necessary (Khan and Sandar 2005). However, when physiological requirements of ascorbic acid exceeds the level being synthesized in the body, pigs may demand for extra dietary ascorbic acid in order to cope and perform optimally. Biobaku *et al.* (2017) recommended dietary utilization of ascorbic acid as an anti-stress agent to curb the negative impact of the environment since ascorbic acid plays a vital role in the biosynthesis of corticosterone which is a hormone that optimises the supply of energy during stressful condition.

Little information exist on the effects of feed withdrawal period during the hot hours of the day and ascorbic acid supplementation on nutrient digestibility and physiological parameters of growing pigs reared in a prevailing tropical environmental, in attempt to enhance the welfare and production indices. Hence, this study was conceived and assessed.

MATERIALS AND METHODS

The experiment was carried out at the Piggery Unit of the Teaching and Research Farms of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The farm is geographically located within tropical rainforest zone on latitude 7°13' N and longitude 3°26' E and altitude 76 m above sea level. The climate is humid with a mean annual rainfall of 1037 mm. The annual mean temperature and humidity are usually 34°C and 82%, respectively.

Unsexed (54) 8-weeks old pigs with mean weight of 5.5 kg were bought within Abeokuta metropolis. The pigs were assigned to 9 treatment groups with 6 pigs per treatment based on weight equalization method. Each treatment was replicated thrice with 2 pigs serving as replicate. The floor dimension of each pen housing the replicate group was 2 m × 2 m and furnished with concrete feeding trough, water drinkers and wallowing bath. Prior to the arrival of the experimental pigs, the pens were washed and disinfected. On arrival, the experimental pigs were given water and feed containing anti-stress and multivitamins. The pigs were also injected with ivermectine to take care of endo and ectoparasites. The pens were half-walled of about 1.4-1.6 m high, and the rest were open sided for proper air circulation. Routine management practices were adhered to strictly, and fresh water was provided ad lib. throughout the 90 days experimental duration.

 $A 3 \times 3$ factorial layout was adopted for this study, factor A consisted of three (3) levels of daily feed withdrawal periods during the hot hours of the day (0, 2 and 4 h) and factor B involved three (3) levels of ascorbic acid supplementation (0, 1500 and 2500 mg per kilogram feed offered). Pigs in Gr 1 were fed ad lib. without ascorbic acid supplementation; pigs in Gr 2 and 3 were supplied feed at 07:00 h and withdrawn at 12:00 h but later returned at 14:00 and 16:00 h of the same day, respectively. The daily ration offered to the pigs in Gr 1 to 3 had no ascorbic acid supplementation. Pigs in Gr 4 were fed ad lib. with ascorbic acid supplementation at the rate of 1500 mg/kg of feed. The feeders of pigs in Gr 5 and 6 were removed at 12:00 h and later returned at 14:00 h and 16:00 h respectively. The feed offered to the pigs in Gr 5 and 6 were supplemented with 1500 mg/kg ascorbic acid. Pigs in Gr 7 were fed ad lib. while those on Gr 8 and 9 were withdrawn at 12:00 h and later returned at 14:00 and 16:00 h respectively. The ration of pigs in Gr 7, 8 and 9 were supplemented with ascorbic acid at the rate of 2500 mg per kilogram diet. The experimental diet was formulated to meet up with the physiological need of growing pigs (Table 1). The ration contained 18:0% crude proteins and 2986.70 kcal/kg metabolizable energy.

The study protocol was approved and conducted in line with the Animal Ethics Committee guidelines of Federal University of Agriculture, Abeokuta, Nigeria (FUNAAB 2013). Three pigs (one from each replicate group) from each treatment group were selected at the end of tenth week of the study and placed in a clean, disinfected metabolic cage for a 7-day acclimatization period and a 5-day faecal collection. Total collection method as described by Cullison (1982) was adopted for faecal sample collection. The collected sample was stored in a freezer until required for analysis. After collection period, faeces were pooled

Table 1. Gross composition of the experimental diet

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0
39
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*Contained the following per kg diet: Vit A, 10000000 IU; vit D3, 2500000 IU; vit E, 40000 IU; vit K3, 4000 mg; vit B1, 750 mg; vit B2, 2000 mg; vit B6, 2000 mg; vit B12, 10 mcg; niacin, 13000 mg; pantothenic acid, 5000 mg; folic acid, 1000 mg; biotin, 10 mcg; choline chloride, 50000 mg; manganese, 50000 mg; zinc, 100000 mg; iron, 80000 mg; copper, 50000 mg; iodine, 1000 mg; selenium, 200 mg; cobalt, 500 mg; antioxidant, 120000 mg.

together based on replicate group, thawed, homogenized and dried in a forced air cabinet. The oven dried faeces and feed sample were milled to 2 mm and analyzed for proximate composition as described by AOAC (2005) protocols. Digestibility of nutrients in the experimental diets was determined using the formula:

$$Dry \ matter \ digestibility = \frac{Feed \ intake \left(\frac{g}{Dm}\right) - Faecal \ output \left(\frac{g}{Dm}\right)}{Feed \ intake \left(\frac{g}{Dm}\right)}$$

$$Nutrient \ digestibility (\%) = \frac{Nutrient \ in \ feed \ (g) - Nutrient \ in \ faeces \ (g)}{Nutrient \ in \ feed \ (g)} \times 100$$

The pulse rate and rectal temperature of the experimental pigs were monitored on weekly basis throughout the experimental period. The frequency of heart beat per minute of the growing pigs was monitored with the aid of stethoscope and a stop watch. Rectal temperature of the pigs was assessed by inserting a digital thermometer through the anus of the pig to the rectum; the temperature was read once the thermometer beeps. These physiological indicators were carried out before 09:00 h on weekly basis.

At the end of the twelfth week, three pigs per treatment (one pig per replicate) were selected and bled through anterior vena cava. About 5 ml of blood was drawn and 2.5 ml of it was poured into ethylene diamine tetra acetate (EDTA) bottles and the remaining into plain bottles. The blood sample in EDTA bottles were used to determine haematological parameters of the growing pigs while the one in plain bottles were refrigerated for 6 h, thereafter, the

serum was separated into sterile test tubes to be used for the determination of biochemical constituents of the blood. The red blood cell, white blood cell, haemoglobin and packed cell volume were determined as described by Jain (1986). The procedure of Campbell (2012) was used in calculating the mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. Total serum protein was determined using the Biuret method as described by Kohn and Allen (1995). Albumin was determined using Bromocresol Green (BCG) method. Serum creatinine was determined using enzymatic creatinine assays as outlined by Daly *et al.* (1996). Serum cholesterol was determined spectrophotometrically using commercial Bio-La-Tests. Serum glucose was estimated using a commercial glucose colorimetric assay kit.

Generated data were analyzed using two-way Analysis of Variance as contained in SAS (2000). New Duncan Multiple Range test was utilised to separate significant means (P<0.05) among treatments.

RESULTS AND DISCUSSION

Nutrient digestibility parameters considered in this study were not significantly (P>0.05) affected by feed withdrawal periods. Feed intake, dry matter intake, excreted faeces, excreted faeces/dry matter intake and faecal dry matter output decreased numerically with increasing level of feed withdrawal period. The similarity in the feed intake and dry matter intake values of pigs on full feeding and those on feed withdrawal periods implied that compensatory intake of feed occurred when the feeders were returned after the withdrawal periods. The pigs denied full access to feed during the hot periods of the day tend to consume more feed in order to meet up with their expected daily requirement before nightfall. This observation is line with the studies of Hassanabadi and Moghaddam (2006) and Sahraei and Shariatmadari (2007) that enthused that feed denial increased feed intake of broiler chickens. The slight decrease in feed intake with increasing feed withdrawal periods can be related to the longer period of feed accessibility by the pigs on ad lib. feeding regimen over those of 2 or 4 h feed withdrawal periods. The similarity in

the values of feed intake and dry matter intake is in consonance with the observation of Njoku et al. (2017) who observed statistically similar values in growth performance of rabbits subjected to 4 h feed withdrawal period. The slight decrease in excreted faeces, faecal dry matter output and excreted faeces per dry matter intake with increasing feed withdrawal periods could be linked with the numerical increase in dry matter intake. It has been established that there is a negative relationship between the level of feed intake and digestive efficiency (Njoku et al. 2013), as a small decrease in digestibility led to much larger increase in faecal output as observed in the present study. Higher feed intake resulted in increased losses of dry matter and gross energy in the large intestine of growing pigs (Haydon et al. 1984). Pigs on ad lib. feeding regimen had relatively better dry matter digestibility and crude fibre digestibility compared to the values noted for pigs on other treatment groups. The marginal decrease in dry matter and crude fibre digestibility with increasing levels of feed withdrawal period could be attributed to the longer digesta retention time in ad lib. fed pigs that must have attained satiation level before the cool of the day when the feeders were restored to those in different withdrawal periods. The higher gut fill in pigs on feed withdrawal periods before nightfall could have led to the poor marginal dry matter and crude fibre digestibility as recorded in this present study. Pigs on two hours feed withdrawal period had the highest numerical crude protein and ash digestibility values than those on ad lib. and four hours feed withdrawal period. Abdel-Wareth et al. (2015) surmised that restricted feeding regimen enhanced digestion due to longer transit time of nutrients in the guts of farm animals. The present observation is in variance with the study of Njoku et al. (2013) that reported significant differences in the dry matter and crude fibre digestibility of finishing pigs offered different levels of feed. The differences in these studies could be associated with the duration of feed restriction adopted, method of restriction and the age of the animals in these studies.

Ascorbic acid supplementation had no significant impact on feed and dry matter intakes of pigs in this study. This observation concurs with the previous report of Blaha and

Table 2. Effect of feed withdrawal periods and ascorbic acid supplementation on nutrient digestibility of growing pigs

Parameters	Fee	d withdrawal pe	riod	Ascort	oic acid supplement	ntation
	0 hour	2 h	4 h	0 mg/kg	1500 mg/kg	2500 mg/kg
Feed intake (g/day)	647.22±47.8	644.44±34.1	552.77±65.2	583.33±74.2	669.44±43.03	591.67±26.9
Dry matter intake (g/day)	592.21±40.6	589.67±34.1	505.79±59.6	533.75.67.9	612.54±39.4	541.38±24.6
Excreted faeces (g/day)	308.33±40.6	305.56±37.8	244.44±37.2	369.44±22.9a	255.56±45.2b	233.33±29.8b
Faecal dry matter output (g/day)	292.22±38.5	290.41±37.3	232.05±35.8	352.37±21.4a	243.81±42.8b	218.49±28.3b
Excreted faeces/dry matter intake	0.56 ± 0.06	0.53 ± 0.06	0.52 ± 0.06	0.68 ± 0.04^{a}	0.43 ± 0.07^{b}	0.42 ± 0.03^{b}
Dry matter digestibility (%)	55.85±3.04	53.02±5.54	54.90±4.31	48.53 ± 2.32^{b}	53.31±5.26ab	61.93±3.81a
Crude protein digestibility (%)	60.41±5.05	61.97±8.21	45.57±5.95	62.23±8.31	48.72±5.13	57.01±6.47
Ether extract (%)	56.92±4.72	55.61±7.02	61.24±5.35	56.40±7.26	50.75±4.15	66.62±4.04
Crude fibre digestibility (%)	56.46±5.56	42.78±6.80	45.65±6.28	40.32±7.56	49.11±6.43	55.47±4.07
Ash (%)	34.55±5.69	34.78±5.99	33.89±8.00	22.69 ± 2.82^{b}	32.66±7.28ab	47.87±5.58 ^a

abMeans in the same row with different superscripts are significantly (P<0.05) different.

Krerosna (1997) which asserted that feed intake of broiler chickens was not affected by ascorbic acid supplementation. However, it differs from the study of Adeyemi et al. (2015) that noted significant difference in the feed intake of broiler reared in a high temperature environment placed on ration supplemented with ascorbic acid. The numerical increase in feed and dry matter intakes though not significant in this present study points to the fact that ascorbic acid supplementation improved nutrient intake of domestic animals as pointed out by earlier researchers (Adeyemi et al. 2015). Excreted faeces, faecal dry matter output, excreted faeces/dry matter intake, dry matter digestibility and ash were significantly influenced by the level of ascorbic acid supplementation. The highest excreted faeces was documented for pigs fed ration without ascorbic acid while the least value was noted in pigs on diet containing 2500 mg ascorbic acid supplementation per kg feed. Faecal dry matter output of pigs fed diets containing 1500 and 2500 mg ascorbic acid per kg feed were statistically lower than the value noted for pigs fed ration without ascorbic acid supplementation. Dry matter digestibility significantly increased with increasing levels of ascorbic acid supplementation. Ash also increased significantly with increasing inclusion level of ascorbic acid. Many studies are of the opinion that environmental stress decreases serum vitamins like A, C and E in farm animals (Sahin et al. 2002, Sahin et al. 2001). The significant increase in dry matter, ash digestibility and the numerical increase in crude fibre digestibility with increasing levels of ascorbic acid supplementation could be associated to improved metabolic activity in pigs on ascorbic acid supplementation as a result of increment in the secretion of digestive enzymes. Tatli-Seven (2008) postulated that ascorbic acid supplementation enhanced iron assimilation by decreasing Fe³⁺ to Fe²⁺, that are better assimilated in the intestine, thereby improving the rate of resistance to infections. The favourable digestibility of dry matter and ash components of the feed with increasing levels of ascorbic acid supplementation could be a pointer to an improved welfare through temperature regulation in the pigs on ascorbic acid supplementation over their counterparts on diets without ascorbic acid supplementation. According to Panda et al. (2008), ascorbic acid supplementation is vital for collage and biosynthesis of adrenaline corticosterones secretion, regulation of body temperature and body immunity boosting. Likewise, Khan et al. (2012) chorused that dietary supplementation of ascorbic acid alleviates the negative impact of stress on metabolic process, enhances production and boosts immunity. In the study of Bain (1996), ascorbic acid has been associated with the conversion of body proteins and fat into production energy and survivability through enhanced secretion of corticosterone.

Interaction between feed withdrawal periods and ascorbic acid supplementation on nutrient digestibility of growing pigs is shown in Table 3. Feed intake, dry matter intake, excreted faeces, faecal dry matter output, excreted faeces/dry matter intake, dry matter digestibility, crude

Table 3. Interaction between feed withdrawal periods and ascorbic acid supplementation on nutrient digestibility of growing pigs

Z 4 4/2242		0 h			2 h			4 h	
rarameters/AAS	0 mg/kg	1500 mg/kg	2500 mg/kg	0 mg/kg	1500 mg/kg	2500 mg/kg	0 mg/kg	1500 mg/kg	2500 mg/kg
Feed intake (g/day)	675.0 ± 24.1^{ab}	758.3 ± 62.6^{a}	508.3 ± 81.8^{b}	600.0 ± 19.3^{ab}	550.0±28.9ab	783.3±19.3 ^a	500.0 ± 51.2^{b}	700.0 ± 77.0^{ab}	458.3±68.4 ^b
Dry matter intake (g/day)	617.6 ± 22.0^{ab}	693.9 ± 57.2^{a}	465.1±74.8 ^b	549.0 ± 17.6^{ab}	503.3 ± 26.4^{ab}	716.8 ± 17.6^{a}	457.5±5.9 ^b	640.5 ± 70.4^{ab}	419.4 ± 15.1^{b}
Excreted faeces (g/day)	341.7±4.8 ^{abc}	416.7 ± 57.7^{a}	166.7 ± 0.0^{d}	216.7±19.3 ^{cd}	325.0 ± 4.8^{abc}	375.0 ± 10.1^{a}	141.7 ± 14.8^{d}	366.7 ± 28.9^{ab}	225.0±52.9abcd
Faecal dry matter output (g/day) 320.1±10.2abc	320.1 ± 10.2^{abc}	397.6 ± 53.1^{a}	158.9 ± 0.8^{d}	203.7±21.3 ^{cd}	310.7±2.5 ^{bcd}	356.8 ± 94.9^{a}	131.6 ± 1.5^{d}	348.9 ± 27.7^{ab}	215.7 ± 51.0^{bc}
Excreted faeces/dry matter intake 0.56±0.03ab	: 0.56±0.03 ^{ab}	0.63 ± 0.13^{a}	0.39 ± 0.06^{ab}	0.40 ± 0.05^{ab}	0.65 ± 0.04^{a}	0.53 ± 0.15^{ab}	0.31 ± 0.01^{b}	$0.58{\pm}0.02^{ab}$	0.67 ± 0.12^{a}
Dry matter digestibility (%)	47.8±3.51 ^{cd}	56.9±2.75abcd	62.8±5.84abc	66.8 ± 2.56^{ab}	43.4 ± 0.62^{d}	$48.9\pm14.56^{\text{bcd}}$	71.2 ± 0.33^{a}	45.3±1.71 ^{cd}	48.21±4.19 ^{bcd}
Crude protein digestibility (%)	42.2 ± 6.50^{bc}	68.0 ± 3.60^{a}	71.0 ± 1.01^{a}	67.9 ± 14.35^{a}	$35.1\pm2.80^{\circ}$	82.9 ± 9.85^{a}	60.9 ± 8.86^{ab}	$43.0\pm0.09^{\rm bc}$	$32.8\pm12.0^{\circ}$
Ether extract digestibility (%)	51.4±1.48	52.2±13.0	67.2±4.15	70.8±1.82	46.9±4.87	49.1 ± 19.75	77.7±2.12	53.2±1.50	52.9±11.60
Crude fibre digestibility (%)	43. 0±4.37bc	72.8 ± 6.67^{a}	53.7 ± 8.86^{abc}	56.8 ± 6.19^{abc}	$34.4\pm4.40^{\circ}$	$37.1\pm18.68^{\circ}$	66.7 ± 0.30^{ab}	$40.2\pm1.98^{\rm bc}$	30.2 ± 10.7^{c}
Ash (%)	$28.8 \pm 4.97^{\rm bc}$	$21.1\pm 8.26^{\rm bc}$	53.8 ± 1.40^{a}	51.7 ± 2.26^{a}	18.6 ± 1.08^{bc}	34.0 ± 12.26^{b}	63.1 ± 6.14^{a}	$28.4\pm0.83^{\rm bc}$	$10.15\pm2.28^{\circ}$

abdMeans on the same row with different superscripts are significantly (P<0.05) different.

protein digestibility, crude fibre digestibility and percentage ash were significantly influenced by the interactive effects of feed withdrawal periods and ascorbic acid supplementation. Feed intake and dry matter intake of pigs on zero withdrawal period fed ration that contained ascorbic acid at the rate of 1,500 mg per kg feed offered and their counterparts on 2 h feed withdrawal period fed diet containing 2,500 mg ascorbic acid per kg feed offered were statistically similar but higher than those fed ad lib. diet containing 2,500 mg ascorbic acid per kg feed and their counterparts on 4 h feed withdrawal period fed ration with 0 and 2,500 mg ascorbic acid supplementation. Excreted faeces values ranged from 141.67 g (pigs on 4 h feed withdrawal period fed diet that did not contain ascorbic acid) to 416.67 g noted for pigs fed ad lib. with ration that contained 1,500 mg per kg feed offered. The highest faecal dry matter output was noted for pigs on ad lib. feeding regimen and ascorbic acid supplementation of 1,500 mg per kg feed while the least value was recorded for pigs on 4 h feed withdrawal period without ascorbic acid supplementation. Excreted faeces/dry matter intake values ranged from 0.31 noted for pigs on 4 h feed withdrawal period fed ration without ascorbic acid supplementation to 0.67 documented for their counterparts on 4 h feed withdrawal period fed ration containing 2,500 mg ascorbic acid per kg feed. Pigs on 4 h feed withdrawal period fed diet that had zero ascorbic acid supplementation recorded the highest dry matter digestibility while the lowest digestibility value was noted for pigs on 2 h feed withdrawal period fed diet that had 1,500 mg ascorbic acid per kg feed. Significantly highest crude protein digestibility value was noted for pigs on 2 h feed withdrawal period and fed ration that contained ascorbic acid at the rate of 2500 mg per kg feed while the least value was recorded for pigs on 4 h feed withdrawal period and ascorbic acid supplementation of 2500 mg per kg feed offered. Crude fibre digestibility ranged from 30.19% documented for pigs on 4 h feed withdrawal period with ascorbic acid supplementation at 2500~mg per kg feed to 72.79% noted for pigs on zero feed withdrawal period and ascorbic acid supplementation of 1500 mg per kg feed. Pigs on 4 h feed withdrawal period without ascorbic acid supplementation had the highest percentage ash value while their counterparts on 4 h feed withdrawal period and ascorbic acid supplementation of 2500 mg per kg feed recorded the least value.

Effects of feed withdrawal periods and ascorbic acid supplementation on some physiological indicators of growing pigs. Feed withdrawal periods had no significant (P>0.05) effect on all physiological welfare indicators

considered. The findings of Keim et al. (2002) indicated that rectal temperature is the best physiological indices that one can employ to objectively monitor the welfare of farm animal especially those managed in tropical environment. Rectal temperature rises when the physiological mechanisms of farm animals fail to repudiate the excessive heat load. The resultant effects of continuous exposure of pigs to heat stress include a decline in feed intake, disturbances in water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolisms (Marai et al. 2007). Normal physiological function of pig is efficient if the body temperature is maintained within narrow limits or is kept constant. Edward (2015) posited that the rectal temperature reference values ranged from 30°C-40°C. The rectal temperature values noted in this present study is within this reference value for healthy growing pigs. The rectal temperature of this present study decreased numerically with increasing level of feed withdrawal period. The decreasing trend in rectal temperature values with increasing feed denial periods is an indication that feed withdrawal during the hot hours of the day provides beneficial physiological effect against stress caused by high environmental temperature. Thuy and Andre (2005) reported that lower feed intake in pigs during the hot hours of the day assists in the reduction of heat production and necessary heat loss. The pulse rate values obtained in this present study followed no definite pattern, but the values were higher than the standard values of 60 to 100 beats per minute recommended for healthy pigs by Edward (2015). The change in the pulse rate is an adaptive response of pigs to maintain its homoeothermic nature. Pigs on ad lib. feeding regimen require more blood flow from the heart to the tissue for ease of digestion and transportation of nutrients within the body system as more feed is consumed (Mohamed and Abdelatif 2010). From this present study, the pulse rate values decreased by 2.49% and 1.56% in pigs on 2 h and 4 h daily withdrawal periods respectively compared to the value observed in ad lib. fed pigs. Positive correlation between levels of feed intake and pulse rate in farm animals have been established by Matsumoto et al. (1990).

Likewise, ascorbic acid supplementation had no significant impact on the physiological welfare indicators of growing pigs with values obtained showing no definite pattern. Ascorbic acid supplementation had no significant impact on the rectal temperature of growing pigs though values increased numerically with increasing levels of ascorbic acid supplementation. The increasing value of rectal temperature with the rise in ascorbic acid

Table 4. Effect of feed withdrawal periods and ascorbic acid supplementation on rectal temperature and pulse rate of growing pigs

Parameter	Fee	ed withdrawal per	riod	Ascort	pic acid suppleme	ntation
	0 h	2 h	4 h	0 mg/kg	1500 mg/kg	2500 mg/kg
Rectal temperature (°C) Pulse rate (beats per minute)	39.47±0.10 128.30±3.21	39.16±0.08 125.11±3.03	38.84±0.12 126.30±2.25	39.06±0.10 125.33±3.09	39.17±0.11 128.89±2.76	39.25±0.11 125.48±2.72

supplementation is in variance with the observation of Kassah and Mohammed (2014) who reported that significant decrease in rectal temperature of sheep on ascorbic acid supplemented diets compared with nonsupplemented group. The authors attributed the variation in the rectal temperature values to the ameliorating effects of ascorbic acid in animals exposed to environmental stressors. The values noted in this present study were within the recommended values for physiologically healthy pigs as indicated by Edward (2015). Pulse rate values documented in this study were higher than the values of 60 to 100 beats per minute recommended for healthy pigs (Edward 2015). The higher pulse rate values can be linked with the impact of high environmental temperature under which these set of pigs were managed not as a result of treatment effect since the control group was affected too. Renaudeau et al. (2011) noted that livestock managed under tropical and subtropical conditions are heat challenged most part of their life time and that they respond to the heat challenge through physiological means which include alteration of metabolic rate and heat loss, changes in cardiovascular system, behaviours and general morphology of the animals.

Table 5 shows the interaction between feed withdrawal periods and ascorbic acid supplementation on some

physiological welfare indicators of growing pigs. Rectal temperature and pulse rate were not significantly influenced by the interaction between feed withdrawal periods and ascorbic acid supplementation. Pigs fed *ad lib*. without ascorbic acid supplementation recorded the highest rectal temperature while the least value was observed in pigs on 4 h daily feed withdrawal period and ascorbic acid supplementation at 2500 mg/kg feed offered. Pigs on daily ration that contained 1500 mg of ascorbic acid per kilogram feed offered had numerically highest pulse rate while least pulse rate value was documented for pigs on 4 h feed withdrawal period without ascorbic acid supplementation.

Effects of feed withdrawal period and ascorbic acid supplementation on haematological parameters of growing pigs are shown in Table 6. According to the results of present study, the packed cell volume was statistically similar, with the values numerically decreasing with increasing feed withdrawal periods. The decreasing values observed in this study could be linked with the rate of nutrient intake which was lower in pigs on feed withdrawal periods. Since packed cell volume is associated with the rate of nutrients and oxygen transportation within the body cells of an animal (Isaac *et al.* 2013), reduction in feed intake could have led to marginal packed cell volume values observed in pigs on feed withdrawal periods compared to those on full feeding

Table 5. Interaction between feed withdrawal periods and ascorbic acid supplementation on rectal temperature and pulse rate of growing pigs

Feed withdrawal		0 h			2 h			4 h	
Parameters/AAS	0 mg/kg	1500 mg/kg	2500 mg/kg	0 mg/kg	1500 mg/kg	2500 mg/kg	0 mg/kg	1500 mg/kg	2500 mg/kg
Rectal temperature (07.07=0.1.	39.11±0.20	39.62±0.17	38.95±0.15	39.14±0.15	39.40±0.11	38.53±0.14	39.27±0.20	38.73±0.22
Pulse rate (beats/minutes	128.22±5.9 s)	130.44±5.8	126.22±5.3	117.33±5.8	127.78±5.1	130.22±4.6	130.44±3.9	128.44±3.4	120.00±4.1

AAS, Ascorbic acid supplementation.

Table 6. Effect of feed withdrawal periods and ascorbic acid supplementation on haematological parameters of growing pigs

Parameter	Fee	d withdrawal per	riods	Ascorbi	ic acid supplem	entation
	0 h	2 h	4 h	0 mg/kg	1500 mg/kg	2500 mg/kg
Packed cell volume (%)	38.33±3.40	34.33±2.82	33.17±2.70	37.67±3.00	37.00±1.86	31.67±3.50
Haemoglobin (g/dl)	12.28±0.82	11.35±0.75	10.95±0.79	12.03±0.60	12.28±0.50	10.27±0.98
Red blood cell (10 ¹² /l)	6.32±0.56	5.83 ± 0.41	5.43 ± 0.42	6.30 ± 0.46	6.22±0.30	5.07±0.49
White blood cell (10 ³ /l)	11.07±0.46	11.75±0.64	11.55±0.66	11.13±0.43	11.05±0.79	12.18±0.37
Neutrophil (%)	27.50 ± 1.52^{b}	30.67±1.63ab	34.50±1.65a	30.67±1.15	30.83±2.87	31.17±1.74
Lymphocyte (%)	71.67±1.15 ^a	68.00±1.29ab	64.50 ± 1.48^{b}	67.83±0.79	68.17±2.64	68.17±1.64
Eosmophil (%)	0.03 ± 0.21	0.33 ± 0.21	0.17 ± 0.17	0.33 ± 0.21	0.33 ± 0.21	0.17 ± 0.17
Basophil (%)	0.50 ± 0.34	0.17 ± 0.17	0.50 ± 0.34	0.50 ± 0.34	0.50 ± 0.34	0.17 ± 0.17
Monocyte (%)	0.33 ± 0.21	0.67 ± 0.33	0.67 ± 0.33	0.67 ± 0.42	0.67 ± 0.21	0.33 ± 0.21
Mean corpuscular volume (µm ³)	66.70±0.35	59.73±1.04	60.93±0.65	59.70±0.69	68.58±0.40	61.08±0.98
Mean corpuscular haemoglobin (×10 ¹ /g)	19.65±0.59	19.65±0.23	20.42±0.58	19.25±0.55	20.18±0.59	20.28±0.17
Mean corpuscular haemoglobin (%)	32.38±1.06	32.93±0.72	33.17±0.63	32.28±1.06	32.92±0.61	33.28±0.71
Neutrophil/ Lymphocyte ratio	0.39 ± 0.03^{b}	0.45 ± 0.04^{ab}	0.53 ± 0.03^{a}	0.45 ± 0.02	0.46 ± 0.06	0.46 ± 0.03

^{ab}Means on the same row with different superscripts are significantly (P<0.05) different.

regimen. The trend observed in the packed cell volume, haemoglobin and red blood cell in this study though not significant is an indication of moderate anaemic condition induced by low metabolic intake caused by feed withdrawal periods. Restricted feeding has been reported to result to trend towards lower haemoglobin, packed cell volume and erythrocytes values (Etim et al. 2014). According to Coronado (2014), the reference value of white blood cell of pigs is 11 to 22 (× 1000), the white blood cell values gotten in this present study fall within the reference values which indicate that feed withdrawal period imposes no immunological abnormalities in the pigs. Eheba et al. (2008) asserted that reduction in white blood cell below the reference value reflected a decline in the production of defensive contrivance to fight infection. Neutrophil and neutrophil-lymphocyte ratio increased significantly with increasing feed withdrawal periods while a decreasing trend was observed for lymphocyte. The significant impact of feed withdrawal periods on neutrophil, lymphocytes and neutrophil: lymphocytes ratios reflect the impact of feed withdrawal periods on status of the immune system of pigs. According to Ameen et al. (2007), neutrophil and lymphocytes play a vital role in maintaining the integrity of the immune system of domestic animal. The neutrophil values obtained in this study remained within the reference range indicating that feed withdrawal period had no deleterious effects on the tissues of the pigs. The significant rising neutrophil with increasing feed withdrawal period could point to the fact that longer feed withdrawal period could compromise the welfare of pigs resulting from nutritional frustration. DeLeeuw and Ekkel (2004) reported that feed frustration in pigs led to behavioural changes, indicative of stress condition. Afolabi et al. (2010) enthused that deviation in haematological parameters is used to access nutritional stress or other factors that predispose animal to stress. The significantly higher lymphocyte values in this study above the standard reference values (Etim et al. 2013, RAR 2009) can be connected to environmental stress caused by high temperature in the tropics, as the value noted for the control group was higher than the reference value. The decreasing lymphocyte values with increasing feed withdrawal period indicate better physiological stability of pigs on feed withdrawal periods over those on ad lib. feed offered. Togun et al. (2007) stated that significantly higher lymphocyte count was an indication of increased ability of the pigs to produce and release antibodies when physiologically challenged. Neutrophil: lymphocyte ratio can be defined as the ratio of absolute neutrophil count to the absolute lymphocyte count (Alexander 2016). It has been considered as a marker of the immune response to aberrant agent. Increasing values of neutrophil: lymphocytes ratio is a potential indicator of stress especially that of nutritional basis (Etim et al. 2014). The values obtained in this study revealed a significant increase in neutrophil: lymphocyte ratio with increasing feed withdrawal periods. The highest neutrophil to lymphocyte ratio observed in pigs on feed withdrawal periods compared to the control group

Table 7. Interaction between feed withdrawal periods and ascorbic acid supplementation haematological parameters of growing pigs

Feed withdrawal periods		ad lib.			2 h			4 h	
Parameters/AAS	0 mg/kg	1500 mg/kg	2500 mg/kg	0 mg/kg	1500 mg/kg	2500 mg/kg	0 mg/kg	1500 mg/kg	2500 mg/kg
Packed cell volume (%)	43.00±8.00	42.00±2.00	30.00 ± 1.00	34.00 ± 1.00	35.50 ± 2.50	33.50±1.50	36.00 ± 5.00	33.50±10.50	30.00 ± 8.00
Haemoglobin (g/dl)	13.25 ± 1.25	13.70 ± 0.80	9.90 ± 0.10	11.45 ± 0.55	11.60 ± 0.40	11.00 ± 2.80	11.40 ± 1.40	11.55 ± 0.15	9.90 ± 2.40
Red blood cell $(10^{12}/I)$	7.05 ± 1.25	7.00 ± 0.30	4.90 ± 0.10	5.85 ± 0.35	6.20 ± 0.10	5.45 ± 1.45	6.00 ± 0.80	5.45 ± 0.15	4.85 ± 1.15
White blood cell $(10^3/1)$	11.40 ± 0.60	10.00 ± 0.40	11.80 ± 0.90	11.15 ± 1.45	12.40 ± 1.80	11.70 ± 0.30	10.85 ± 0.35	10.75 ± 1.75	13.05 ± 0.15
Neutrophil (%)	$30.50\pm2.50^{\rm bc}$	$25.50\pm3.00^{\circ}$	$26.50\pm1.50^{\rm bc}$	32.00 ± 2.00^{abc}	39.00 ± 2.00^{a}	32.50 ± 1.50^{ab}	29.50 ± 2.50^{bc}	28.00 ± 2.00^{bc}	34.50 ± 2.50^{ab}
Lymphocyte (%)	68.50 ± 1.50^{abc}	74.00 ± 1.00^{a}	72.50 ± 0.50^{ab}	$66.00\pm1.00^{\circ}$	60.50 ± 2.50^{d}	67.00 ± 1.00^{bc}	69.00 ± 1.00^{abc}	70.00 ± 1.00^{abc}	65.00 ± 3.00^{cd}
Eosmophil (%)	0.00 ± 0.00	0.50 ± 0.50	0.50 ± 0.50	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.50	0.00 ± 0.00
Basophil (%)	1.00 ± 1.00	0.50 ± 0.50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.50	0.50 ± 0.50	1.00 ± 1.00	0.00 ± 0.00
Monocyte (%)	0.00 ± 0.00	0.50 ± 0.50	0.50 ± 0.50	1.00 ± 1.00	1.00 ± 0.00	0.00 ± 0.00	1.00 ± 1.00	0.50 ± 0.50	0.50 ± 0.50
$MCV (\mu m^3)$	60.90 ± 0.60	60.00 ± 0.30	61.40 ± 0.80	58.25±1.75	60.30 ± 0.00	60.65 ± 3.15	59.95±0.35	61.45 ± 0.50	61.40 ± 1.90
$MCH (\times 10^{1}/g)$	19.15 ± 2.05^{ab}	19.60 ± 0.30^{ab}	20.20 ± 0.60^{ab}	1960 ± 0.20^{ab}	19.10 ± 0.00^{ab}	20.25 ± 0.25^{ab}	19.00 ± 0.20^{b}	21.85 ± 0.95^{a}	20.40 ± 0.10^{ab}
MCHC (%)	31.45 ± 3.65	32.65 ± 0.35	33.05 ± 1.45	33.65 ± 0.65	31.66 ± 0.00	33.55 ± 2.15	31.75 ± 0.55	34.50 ± 1.10	33.25 ± 0.85
NEU/ LYM	$0.45\pm0.05^{\rm bc}$	0.35 ± 0.05^{c}	0.37 ± 0.02^{c}	$0.49\pm0.04^{\rm bc}$	0.65 ± 0.06^{a}	$0.49\pm0.03^{\rm bc}$	$0.43\pm0.44^{\rm bc}$	$0.40\pm0.03^{\rm bc}$	0.53 ± 0.06^{ab}

abod Means within rows followed by different superscripts are significantly different (P<0.05). AAS, Ascorbic acid supplementation.

could be a reliable indicator of slight physiological stress as a result of treatment effect as affirmed by Post *et al.* (2003). The values obtained were within the standard reference values by Coronado (2014). Other haematological parameters considered were not significantly affected by feed withdrawal periods. Packed cell volume, haemoglobin and red blood cell values decreased numerically with increasing feed withdrawal periods while mean corpuscular haemoglobin concentration increased numerically with increasing feed withdrawal periods.

Ascorbic acid has been used in the management of issues pertaining to stress in livestock husbandry. Haematological values are of great diagnostic importance in practical livestock husbandry since they reflect the reaction of domestic animal to its environment and pathological organisms. Haematological indices can act as vital supports to prognosis and may expose adverse conditions even when the pigs did not show apparent symptoms of illness. A deviation from normal haematological values is an indication of abnormal physiological state of the body (Eze et al. 2010). In accordance with the data obtained in this study, ascorbic acid supplementation had no significant alteration on haematological values of growing pigs, which were within the standard reference values documented by Coronado (2014) and RAR (2009) for pigs except in lymphocytes value that was higher. Packed cell volume, red blood cell, mean corpuscular haemoglobin concentration and neutrophil increased numerically with increasing dietary ascorbic acid inclusion. Urban-Chmiel et al. (2009) and Powers and Jackson (2008) reported that ascorbic acid being a chain-breaking antioxidant, plays a pivotal role in the prevention and constraint of free radical chain establishment and proliferation, consequently, maintaining the structural integrity of the blood cells from oxidative damage. The higher lymphocyte values noted among pigs in this present study in comparison with the reference values could be linked to stress due to heat load from the environment where the pigs were raised not as a result of treatment effect since control pigs had higher lymphocyte value compared with the reference standard. Celik et al. (2009) asserted that leucocytosis could be due to amplified leucocyte recruitment which is directly proportional to the degree of the causative stress condition. Increased lymphocytes values could be the means by which the defence mechanism of the body is activated (Yousef et al.

2003). Borges *et al.* (2007) surmised that heat load causes changes in the amount and structure of blood cells of growing pigs reared in high environmental temperature.

The effect of feed withdrawal periods and ascorbic acid supplementation on serum biochemical parameters of growing pigs is presented on Table 7. Feed withdrawal period had no impact (P>0.05) on biochemical parameters considered in this present study. Albumin, albumin:globulin ratio and serum glucose of the pigs increased numerically with increasing level of feed withdrawal period. Total protein values of pigs on different feed withdrawal periods ranged from 7.73 g/l (pigs on 4 h feed withdrawal period) to 8.60 g/l (pigs on 2 h feed withdrawal period). Pigs on 2 h feed withdrawal period recorded the least creatinine value of 1.12 mg/dl while the highest value of 1.12 mg/dl was documented for pigs on 4 h feed withdrawal period. Feed withdrawal periods did not induce positive changes in the levels of biochemical indices of growing pigs. The similarity in the serum parameters simply indicate that the pigs on feed deprivation periods easily adapt to the situation and compensatory response must have occurred since feed denial was only for few hours of the day. Catabolism of tissues did not occur in the pigs on feed withdrawal periods in order to meet up with the metabolic requirement of the pigs. Many information in literature indicated that compensatory responses are based on severity and duration of restriction, genetic factors and age of animals at which feed deprivation was imposed (Sivanagendra et al. 2018). Zulkifi et al. (2006) reported that plasma corticosterone, glucose and neutrophil: lymphocyte ratios are commonly used physiological indices of stress caused by feed refutation or restriction. The assessment of biochemical parameters assist in the evaluation of the physiological state of the pigs, and the changes in metabolic rate as a result of stress conditions (Fadare et al. 2012). The insignificant in the biochemical parameters in this present study is in line with the report of Boostani et al. (2010) that reported similarity in the values of total protein, albumin and globulin of broiler chickens on restricted feeding compared with full fed birds. Also, Njoku et al. (2016) reported that four hours feed withdrawal period had no influence on the biochemical components of growing rabbits.

No significant differences (P>0.05) were also observed in all serum biochemical parameters of growing pigs subjected to ascorbic acid supplementation. Total protein,

Table 8. Effect of feed withdrawal periods and ascorbic acid supplementation on serum biochemical parameters of growing pigs

Parameter	Fe	ed withdrawal per	riod	Ascor	bic acid suppleme	ntation
	0 h	2 h	4 h	0 mg/kg	1500 mg/kg	2500 mg/kg
Total protein (g/l)	7.52±0.34	8.60±0.53	7.73±0.41	7.65±0.37	8.52±0.61	7.68±0.30
Albumin (g/dl)	4.13±0.19	4.78±0.31	4.83 ± 0.25	4.27±0.20	4.73±0.36	4.30±0.20
Globulin (g/dl)	3.38 ± 0.17	3.82 ± 0.23	3.35 ± 0.16	0.95 ± 0.17	0.95 ± 0.26	0.85 ± 0.12
Creatinine (mg/dl)	0.90 ± 0.20	0.73 ± 0.17	1.12 ± 0.15	3.38 ± 0.23	3.78±0.16	3.38±0.16
Albumin/Globulin ratio	1.23±0.33	1.27±0.02	1.32 ± 0.02	1.27 ± 0.22	1.27 ± 0.03	1.28 ± 0.03
Cholesterol (mg/dl)	114.17±5.23	123.17±4.23	116.17±5.70	116.00±5.60	121.83±6.17	115.67±3.52
Glucose	89.17±3.16	91.67±4.29	99.83±4.61	90.00±3.49	97.67±6.06	93.00±2.68

 Table 9. Interaction between feed withdrawal periods and ascorbic acid supplementation on serum biochemical parameters of growing pigs

Feed withdrawal period		0 h			2 h			4 h	
Parameters/AAS	0 mg/kg	1500 mg/kg	2500 mg/kg	0 mg/kg	1500 mg/kg	2500 mg/kg	0 mg/kg	1500 mg/kg	2500 mg/kg
Total protein (g/l)	8.20 ± 0.80^{b}	7.00 ± 0.40^{b}	7.35 ± 0.45^{b}	8.00 ± 0.30^{b}	10.20 ± 0.00^{a}	7.60 ± 0.50^{b}	6.75 ± 0.35^{b}	8.35±0.55b	8.10 ± 0.80^{b}
Albumin (g/dl)	4.55 ± 0.45^{b}	3.80 ± 0.10^{b}	4.05 ± 0.15^{b}	4.05 ± 0.15^{b}	5.70 ± 0.00^{a}	4.20 ± 0.30^{b}	3.83 ± 0.20^{b}	4.70 ± 0.30^{b}	4.65 ± 0.55^{b}
Globulin (g/dl)	3.65 ± 0.35^{b}	3.20 ± 0.30^{b}	3.30 ± 0.30^{b}	3.55 ± 0.15^{b}	4.50 ± 0.00^{a}	3.40 ± 0.20^{b}	2.95 ± 0.15^{b}	3.65 ± 0.25^{b}	3.45 ± 0.25^{b}
Creatinine (mg/dl)	1.05 ± 0.65	0.60 ± 0.10	1.05 ± 0.10	0.50 ± 0.10	1.20 ± 0.00	0.50 ± 0.30	1.30 ± 0.00	1.05 ± 0.45	1.00 ± 0.30
Albumin/Globulin	1.25 ± 0.05	1.20 ± 0.10	1.25 ± 0.15	1.25 ± 0.05	1.30 ± 0.00	1.25 ± 0.05	1.30 ± 0.00	1.30 ± 0.00	1.35 ± 0.05
Cholesterol (mg/dl)	124.00 ± 12.00^{ab}	106.50 ± 6.50^{ab}	112.00 ± 8.00^{ab}	121.50 ± 4.50^{ab}	132.00 ± 10.00^{a}	116.00 ± 4.00^{ab}	102.50 ± 5.50^{b}	127.00 ± 8.00^{ab}	119.00 ± 9.00^{ab}
Glucose	93.00 ± 5.00^{ab}	84.00 ± 5.00^{b}	90.50 ± 7.50^{ab}	96.00 ± 4.00^{ab}	109.00 ± 13.00^{a}	94.50 ± 2.50^{ab}	81.00 ± 4.00^{b}	100.00 ± 6.00^{ab}	94.00 ± 6.00^{ab}

^{ab}Means on the same row with different superscripts are significantly (P<0.05) different. AAS, Ascorbic acid supplementation.

albumin, creatinine, cholesterol and glucose values were numerically highest in pigs on 1500 mg ascorbic acid per kg feed while the least value of total protein, albumin and glucose were noted in full fed pigs. The non-significant values obtained in the biochemical components of pigs on supplemental ascorbic acid compared with the control group indicates that 2 to 4 h daily feed withdrawal periods improve the well-being of pigs as it poses no stress in the pigs that may require the alleviating effects of antioxidant agent like ascorbic acid. Sivanagendra et al. (2018) reported that stress induces changes in serum biochemical profile of farm animals. Pardue et al. (1985) affirmed the need for supplemental ascorbic acid as quantity of ascorbic acid produced by farm animals under specific environmental and physiological conditions could be inadequate to meet the biological need of the animal. Earlier workers have provided evidence supporting the utilization of ascorbic acid decreasing different kinds of stress in farm animals (Powers and Ayo et al. 2006, Jackson 2008).

Interaction between feed withdrawal periods and ascorbic acid supplementation on blood indices of growing pigs is shown in Table 8. Neutrophil, lymphocyte, mean corpuscular haemoglobin and neutrophil:lymphocyte ratio values were significantly influenced by the interactive effect between feed withdrawal period and ascorbic acid supplementation. Growing pigs on 2 h feed withdrawal period with 1500 mg ascorbic acid supplementation per kg feed recorded the highest neutrophil and neutrophil: lymphocyte ratio values while the least values of were noted for the pigs fed ad lib. with diet that contained 1500 mg ascorbic acid supplementation per kg feed offered. The highest lymphocyte value was observed in pigs on ad lib. feeding regimen and ascorbic acid supplementation at the rate of 1500 mg per kg feed offered. The least lymphocyte value was documented for pigs on 2 h feed withdrawal period, given ascorbic acid at the 1500 mg per kg feed offered. Mean corpuscular haemoglobin values of growing pig ranged from 19.00 pg (pigs on 4 h feed withdrawal period with zero ascorbic acid supplementation) to 21.85 pg documented for pigs on four hours feed withdrawal period offered ration containing 1500 mg ascorbic acid per kg feed. Other haematological parameters considered were not influenced significantly by the interaction between feed withdrawal periods and ascorbic acid supplementation. Interaction between feed withdrawal periods and ascorbic acid supplementation positively influenced total protein, albumin, globulin, cholesterol and glucose of growing pigs. The highest total protein, albumin and globulin values were observed in pigs whose daily feed was withdrawn for 2 h and ascorbic acid supplementation at the rate of 1500 mg per kg feed. The lowest total protein and globulin values were recorded for pigs on 4 h feed withdrawal period and 0 mg ascorbic acid supplementation per kg feed offered. The lowest albumin value was documented for pigs on ad lib. feeding regime and ascorbic acid supplementation at 1500 mg/kg feed. The better positive interactive effects observed in the pigs on 2 h feed withdrawal period and supplemented

with ascorbic acid at the rate of 1500 mg/kg feed offered over their counterparts on control and those on 4 h feed withdrawal period with ascorbic acid supplementation of 2500 mg/kg feed in terms of serum total protein, albumin and globulin values could be linked with the slight higher crude protein digestibility in this set of pigs. This present result reinforced the necessity of a transient feed restriction and ascorbic acid supplementation in enhancing the gut microbiota of growing pigs as reported by Le Floch et al. (2014). Sahin and Kucuk (2001) reported that dietary supplementation of ascorbic acid increased the nutrient digestibility of Japanese quails managed under hot environmental condition. Sivanagendra et al. (2018) reported positive correlation between the rate of dietary nutrient intake and concentration of blood metabolites. The present result is in line with the study of Sivanagendra et al. (2018) who concluded that serum total protein and albumin were significantly influenced by limited feed offered in sheep and goats. The highest cholesterol and glucose values were noted for pigs on 2 h feed withdrawal periods and 1500 mg ascorbic acid supplementation per kg feed offered while the lowest mean values were recorded for pigs whose daily ration was withdrawn for 4 h without ascorbic acid supplementation. This study indicates that 2 h feed withdrawal period acted synergistically with ascorbic acid supplementation at the rate of 1500 mg/kg feed, resulting to the highest significant differences in serum cholesterol and glucose levels. Bain (1996) posited that ascorbic acid is connected with the conversion of tissue protein and fat into energy for production and subsistence through inclement in corticosterone secretion. Whang et al. (2003) reported that pigs subjected to a period of dietary restrictions utilized feed more efficiently during realimentation period than their unrestricted counterparts. It has been reported that restricted feeding influenced serum lipid concentration especially cholesterol in lambs (Sivanagendra et al. 2018). The authors attributed the resultant changes to catabolism of the tissues.

It can be concluded from this study that 2 to 4 h daily feed withdrawal periods foisted no positive impact on nutrient digestibility and serum biochemical parameters but influenced neutrophil, lymphocyte and neutrophil: lymphocyte ratio of growing pigs. Whilst ascorbic acid supplementation improved nutrient digestibility (excreted faeces, faecal dry matter output, dry matter and ash digestibility) and some biochemical indices (total protein, albumin, globulin, cholesterol and glucose), but had no impact on haematological parameters of growing pigs. Hence, feed withdrawal periods and ascorbic acid supplementation can be used as feeding strategies to enhance the economic measures and welfare of growing pigs.

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