Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
Journal of Agricultural Faculty of Gaziosmanpasa University
http://ziraatdergi.gop.edu.tr/

Araştırma Makalesi/*Research Article*

JAFAG
ISSN: 1300-2910
E-ISSN: 2147-8848
(2021) 38 (1), 20-27
doi:**10.13002/jafag4704**

Effect of Weight Asymmetry on Serological Parameters, Tibia Bone Characteristics and Gut Morphology of Pigs

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Alındığı tarih (Received): 18.06.2020 Kabul tarihi (Accepted): 18.02.2021
Online Baskı tarihi (Printed Online): 25.02.2021 Yazılı baskı tarihi (Printed): 30.04.2021

Abstract: Sorting and rearing of pigs on equal weight basis is one of management options used in improving the welfare of pigs. A sixteen-week study was carried out to investigate the serological responses, tibia bone characteristics and gut morphology of pigs to weight asymmetry. Fifty-four Large White growing pigs (15.33±0.2 kg) were assigned to three treatments (Homozygous heavy, homozygous light and heterozygous weights) with 18 pigs per treatment, further sub-divided into 6 replicates with 3 pigs per replicate. On 16th week of the experiment, one pig per replicate group was selected, fasted and sacrificed. Blood samples, sections of the gut (duodenum. jejunum and ileum) and tibia bone were collected for the assessment. Data generated were subjected to 1-way analysis of variance using SAS (2003) package. The results showed that total protein, albumin, total cholesterol, high density lipoprotein and aspartate aminotransferase were significantly influenced by weight asymmetry. Weight asymmetry had positive impact on duodenum villus height and ileum lamina propria depth of the pigs. Wet bone weight, bone length, medial thickness and lateral thickness of the tibia were also influenced by weight asymmetry. It was concluded that weight asymmetry had positive impact on some serological parameters, gut and tibia bone characteristics of pigs.

Keywords: Asymmetry; biochemical parameters; homogeneous morphology; serological parameters; tibia characteristics.

1. Introduction

Prospective benefits of good management practices in pig production system embrace better performance in terms of growth and reproductive potentials, prevention of diseases, reduced cost per unit gain, lower inputs for feed, manure handling, and maximize production output. The management of aggressions in pig farming entails the use of sedative drugs (Bostami *et al*. 2015). Aggressive behaviour in pigs is a territorial behavior required in the establishment of hierarchy in group housed pigs. Such hierarchy could have a long term effects especially among the less aggressive ones that could have limited access to feeding troughs and high propensity to stress, leading to large weight disparity among these sets of pigs (Marchant-Forde and Marchant-Forde, 2005). Avoiding weight variation at marketing is essential to minimize deductions of cost of live weight prices of pigs (Cheng *et al.* 2018). Different studies have been undertaken in order to proffer practical solution to aggressive behaviours among unfamiliar pigs reared together in the same pen, since aggression resurfaces at full peak once the acute effect of the chemical agent in drugs presently used in modern pig farms disappears (Rodenburg and Koene, 2007; Bostami *et al*. 2015). But, none of the practical techniques have proved to be a veritable management tool in curbing the underlying causes of aggressive behaviour among non-uniform weight pigs. This present study is aimed at exploring the serological potentials, tibia bone and gut morphology of growing pigs with similarity weights housed together.

2. Materials and Methods

The protocols of this present study conform to the animal welfare requirements for care and management of experimental animals, following the guidelines of the Animal Welfare Committee of Federal University of Agriculture, Abeokuta, Nigeria (FUNAAB, 2013). The study was conducted at the Piggery Unit of the Teaching and Research Farm of College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria in 2019. Fifty-four (54) Large White growing pigs were gotten from a reputable commercial farm within Abeokuta metropolis and grouped based on their body weight into 3 treatments (homogenous heavy weight, homogenous light weight and heterogeneous weight groups) with 6 replicates of 3 pigs per replicate. The initial mean weights of each category of pigs were 18.22±1.02 kg for homogeneous heavy weight group, 12.83±0.57 kg for homogeneous light weight and 14.00±1.08 kg for heterogeneous weight groups of growing pigs. As enunciated by earlier workers, three pigs serving as a replicate were placed together in a naturally ventilated experimental pen with floor dimension of 4 m by 3 m, equipped with concrete feeding and drinking troughs (Bostami *et al.* 2015;.Njoku *et al.* 2020). The homogenous heavy weight group is a group of pigs of the same age whose weight is significantly greater than the mean weight of the group. Homogenous light weight group involves pigs of the same age group whose weight is significantly less than the average weight of the group. Routine management practices were done on daily basis, with fresh water supplied *ad libitum* throughout the experimental period. The pigs were fed concentrate diet that contained 18.87% crude protein (CP), 4.84% ether extract (EE), and 6.40% crude fibre (CF) and 2541.99 kcal metabolizable energy/kg (ME/kg) to meet the requirement of NRC (1998) as shown in Table 1.

Table 1. Percentage composition of experimental diets of growing pigs

Ingredients	Percentage (%)
Maize	50
Palm kernel cake (14.50% CP)	10
Soya Bean Meal (44% CP)	18
Groundnut cake (53% CP)	9
Fish Meal (72% CP)	2
Wheat Offal	6.9
Bone Meal (36% CP)	3
Lysine	0.3
Methionine	0.25
*Premix	0.3
Salt (NaCl)	0.25
Total	**100**
Analyzed Nutrients	
Metabolizable energy (kcal/kg ME)	2834.59
Crude protein (%)	20.9
Ether extract (%)	4.56
Crude fibre (%)	4.68
Ash (%)	3.05

*To supply the following per kg diets; VitA 12600 IU; vit D3 2800 IU; vit E 49 IU; vit k 32.8mg; vitB1 1.4mg; vit B2 5.6mg; vit B6 1.4mg; vitB12 0.014mcg; Niacin 21mg; Pantothenic Acid 14mg; Folic Acid 1.4mg; Biotin 0.028mg; Chlorine chloride 70mg; Manganese 70mg; Zinc 140mg; Iron 140mg; Copper 140mg; Iodine 1.4mg; Celenium 0.28mg; Cobalt 0.7mg; Antioxidant 168mg.

2.1. Determination of serological indices and tibia bone characteristics

At the end of 16th week of the study, one pig whose weight is close to the mean replicate group was selected, fasted, stunned, slaughtered and bled by incision. Ten (10) ml of blood was collected into plane sample bottles without Ethylene Diaminetetra-Acetic Acid (EDTA) to assess serological parameters. The total protein was determined according to the method of Tietz and Norbert (1995). While the serum albumin, globulin and cholesterol were determined spectrophotometrically following the procedure of Douma and Briggs (1972) using commercial Bio-La-Tests (Pliva-LaChem Brno Ltd, Czech Republic). Creatinine was determined using enzymatic creatinine assays as described by Daly *et al.* (1996). Serum glucose was determined using a commercial glucose colorimetric assay kit

(Cayman®Chemical Company, Ann Arbor, USA) and protocol followed was according to the manufacturer's instruction.

The small intestine of the slaughtered pig was excised, thereafter, 5 cm length of duodenum, jejunum and ileum were cut from the middle of each segment and fixed in 10% buffer solution of formalin following the protocol of Van Nevel et al. (2005). Dehydration and embedment of the tissue in paraffin wax were carried out. Four-six (4-6) μm transverse sections were cut and mounted on glass slides, stained with haematoxylin. Ten (10) prominent villi and their adjoining crypts were assessed with light microscope, utilizing a calibrated eyepiece graticule as described by Halas *et al.* (2010).

The left tibia bones of the pigs were collected and the adhering muscles and connective tissues were manually removed after they were dipped in boiling water for 5 minutes. The tibia length, medial thickness, and lateral thickness were determined with the aid of Vernier calliper while wet tibia bone weight and dry tibia bone weight were determined with the aid of sensitive scale in line with the methods outlined by Sogunle *et al.* (2012). The bone breaking strength was determined using instron materials tester.

Data were processed by one-way analysis of variance using SAS (2003) package. Significantly ($p<0.05$) different means among variables were separated using New Duncan's Multiple Range Test as contained in the same statistical package.

3. Results

3.1. Effect of weight asymmetry on serological parameters of growing pigs

Total protein, albumin, total cholesterol, high density lipoprotein and aspartate aminotransferase were significantly influenced by weight asymmetry as depicted on Table 2 ($p<0.05$). Homozygous heavy weight and light weight pigs recorded similar total protein mean values (5.53±0.48 and 6.37±0.49 g/dl) which were significantly ($p<0.05$) lower than 7.77±0.33 g/dl documented for the heterogeneous weight pigs. Heterogeneous weight pigs had the highest albumin value of 4.43±0.24 g/dl, followed by 3.52±0.24 g/dl noted for homogeneous light weight pigs while the homogeneous heavy weight pigs had the least albumin mean value of 2.97±0.29 g/dl. Total cholesterol values of homogenous heavy and light weights pigs were comparable statistically (87.33±1.36 mg/dl and 85.33±3.16 mg/dl) and differed significantly from 94.67±1.71 mg/dl obtained for heterogeneous weight pigs. Heterozygous weight pigs had the highest significant HDL value of 50.10±3.72 mg/dl whilst the homogeneous light weight pigs recorded the lowest mean value (42.83±2.62 mg/dl). Homozygous heavy weight and heterozygous weight pigs had statistically similar AST values (36.17±1.83 μ/l and 37.67±1.12 μ/l) that differed significantly from 41.67±2.19 μ/l documented for homogeneous light weight pigs.

3.2. Effect of weight asymmetry on tibia bone characteristics and breaking strength of growing pigs

Wet tibia weight, tibia length, medial thickness and lateral thickness of the tibia were significantly influenced by weight asymmetry as shown in Table 3. The wet tibia bone weight differed significantly with the highest mean value of 109.73±4.23 g noted for heterogeneous weight pigs and the least value of 87.01±6.66 g documented for homogeneous light weight pigs. Tibia bone length of homogenous heavy weight pigs was the longest (15.90±0.38 cm) while that of homogeneous light weight pigs was the shortest (14.68±0.50 cm). The thickest medial value of 26.59±0.81 mm was noted for heterozygous weight pigs, followed by 23.61±2.14 mm noted for homogeneous heavy weight pigs and homogeneous light weight pigs recorded the lowest medial thickness value of 20.53±0.84 mm. Homogeneous heavy weight and heterogeneous weight pigs had comparable lateral thickness values (44.11±0.75 mm and 41.10±0.69 mm) that were significantly higher than 36.62±2.12 mm recorded for homogeneous light weight pigs.

Table 2.: Effect of weight asymmetry on serological parameters of growing pigs

Parameters	Homogeneous heavy weight	Homogeneous light weight	Heterozygous weight
Total protein (g/dl)	5.53±0.48[b]	6.37±0.49[b]	7.77±0.33[a]
Albumin (g/dl)	2.97±0.29[b]	3.52±0.24[b]	4.43±0.24[a]
Globulin (g/dl)	2.57±0.26	2.85±0.31	3.33±0.25
Glucose (g/dl)	93.67±2.99	94.17±3.38	93.67±3.45
Urea (g/dl)	5.67±0.15	5.27±0.51	5.43±0.35
Creatinine (g/dl)	0.83±0.08	0.63±0.08	0.77±0.13
Total cholesterol (mg/dl)	87.33±1.36[b]	85.33±3.16[b]	94.67±1.71[a]
Triglycerides (mg/dl)	100.00±1.46	102.33±3.06	99.83±2.20
High-density lipoprotein (mg/dl)	45.95±1.91[ab]	42.83±2.62[b]	50.10±3.72[a]
Low-density lipoprotein (mg/dl)	20.90±0.81	20.85±1.54	23.32±2.24
Very low-density lipoprotein (mg/dl)	20.10±0.55	20.77±0.45	19.28±0.65
Aspartate aminotransferase (μ/l)	36.17±1.83[b]	41.67±2.19[a]	37.67±1.12[b]
Alanine aminotransferase (μ/l)	23.33±1.89	19.42±0.97	24.83±1.82

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

Table 3. Effect of weight asymmetry on tibia bone characteristics and breaking strength of growing pigs

Parameters	Homogeneous heavy weight	Homogeneous light weight	Heterozygous weight
Wet tibia bone weight (g)	99.60±6.62[ab]	87.01±6.66[b]	109.73±4.23[a]
Dry tibia bone weight (g)	2.87±0.22	2.36±0.14	2.80±0.08
Ash weight (g)	31.53±3.86	30.76±1.36	31.83±2.21
Tibia bone length (cm)	15.90±0.38[a]	14.68±0.50[b]	15.38±0.23[ab]
Medial Thickness (mm)	23.61±2.14[ab]	20.54±0.84[b]	26.59±0.81[a]
Lateral thickness (mm)	44.11±0.75[a]	36.62±2.12[b]	41.10±0.69[a]
Tibia bone breaking strength (N)	17.41±1.55	17.81±0.31	15.88±1.35

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

Table 4: Effect of weight asymmetry on gut morphology of growing pigs

Parameters	Homogeneous heavy weight	Homogeneous light weight	Heterozygous weight
Duodenum (μm)			
Villus height	675.00±21.41	651.67±53.00	640.00±43.97
Apical width	29.17±0.83	32.33±1.05	34.17±3.00
Basal width)	103.33±6.15	102.50±5.72	104.17±6.64
Lamina propria depth	248.63±14.00	251.67±13.02	256.67±27.04
Jejunum (μm)			
Villus height	593.33±14.53[b]	655.67±37.66[ab]	683.33±21.40[a]
Apical width	40.83±2.71	39.00±2.45	42.50±1.71
Basal width)	117.50±6.30	119.43±6.34	103.33±8.82
Lamina propria depth	226.67±12.02	245.00±13.35	240.00±14.38
Ileum (μm)			
Villus height	643.33±15.63	681.67±29.52	683.33±26.29
Apical width	40.83±0.83	39.17±2.39	34.17±3.01
Basal width)	109.17±4.17	110.00±8.56	105.83±7.80
Lamina propria depth	223.33±10.54[b]	240.00±23.09[ab]	256.67±15.28[a]

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

3.3. Effect of weight asymmetry on gut morphology of growing pigs

According to the result obtained in gut morphology of growing pigs of different weight groups as presented in Table 4, showed significant differences in Jejunum villus height and ileum lamina propria depth of the small intestine. Heterogeneous weight group of pigs had the highest jejunum villus height (683.33±21.40 μm), followed by the value of 655.67±37.66 μm documented for homogenous light weight group of pigs whilst the least villus height of 593.33±14.53 µm was noted for homogeneous heavy weight pigs. Homogeneous light weight pigs had the highest ileum lamina propria depth of 240.00±23.09 µm whereas the least ileum lamina propria depth (223.33±10.54 µm) was documented for homogeneous heavy weight grouped pigs. Other gut morphological parameters considered in this present study were not statistically ($p>0.05$) influenced by weight asymmetry.

4. Discussion

Pigs are highly social animals whether they live in wild or intensively managed condition of commercial farm settings (Elena, 2017). The social organization is based upon dominance hierarchy that is established through agnostic interactions among group managed members. The social position of an individual pig in a group is determined by the age of the pigs, sex, body weight and size, degree of aggression, and social experiences (Puppe *et al*. 2008). The social interaction of intensively managed pigs is likewise swayed by the weaning age, the number of pigs housed together, group membership disruption, homogeneous age and weight, highly fixed and artificial group composition (Fels *et al*. 2014). The significant higher difference observed in the mean values of serum total protein and albumin of heterozygous weight pigs over their counterparts with homogeneous weights could be associated with higher social friction that could lead to stress in unevenly weight pigs managed together on the same pen. An increase in serum total protein may reflect increased protein synthesis or decreased protein loss (Uadia *et al*. 2016). WHO (1995) asserts that for optimal functionality of the body during stress, the body tends to adjust to the strain by modulating the body's anatomical, physiological and metabolic state. The elevation in serum total protein and albumin points to the fact establishing social hierarchy in heterozygous weight pigs takes more time and requires more energy expenditure compared to that of homogeneous weight pigs. The social friction could have led to depiction of fluids (water and blood volume) leading to high concentration of proteins in the serum (Sheri *et al.* 2010). The physical activity among the heterozygous weight pigs' aids digestion and bowel functions, resulting to higher serum protein synthesis among this set of pigs. Janssen and LeBlac (2010) enthused that physical exercise supports cardiovascular and respiratory functions, enhancing muscle tone, bone mass, slow down aging process, improves digestion, bowel transit time and prevents depression. Total cholesterol and high density lipoprotein were significantly higher among unevenly weight pigs compared to the result documented for uniformly weight groups. This observation gives credence to the assertion of Couillard *et al.* (2001) that physical activity in improved total cholesterol and high density lipoprotein levels while maintaining low density lipoprotein and triglycerides. The improved serum cholesterol and high density lipoprotein in heterozygous weight group could be linked with higher social challenges among this set of pigs that must have led to loss of body condition and fat. The higher aspartate aminotransferase noted in pigs with lower propensity of social conflicts (homogeneous light weight group) could indicate healthy liver functions of the experimental pigs. Leibowitz *et al*. (2012) noted that stress from social conflict could impact negatively on some body organs like heart tissues and skeletal muscles which must have resulted to the release of aspartate aminotransferase into the bloodstreams with a resultant increaser in serum aspartate aminotransferase. The increase in serum aspartate aminotransferase in homogeneous light weight pigs in this present is likely from injured heart tissue and skeletal muscle rather than the liver.

Heo *et al*. (2013) asserts that for small intestine to attain their optimum nutrient digestibility and absorptive capacity functions, longer villi heights are essential. This present study revealed that the villus height was longest in pig of different weight groups managed together and lowest in homogeneous heavy weight group. The statistical difference noted in the villus height of heterogeneous weight group could be associated with higher social activities among this set of pigs. The increase in social activity influences the demand for energy in order to meet up with the physiological needs of the pigs. Since, more energy is expended in exercise needed for establishment of social relationship among the pigs. The higher energy demand must have led to improvement in morphological and functional changes in the small intestine of pigs (Dong and Pluske, 2007). The improved changes in intestinal morphology include elongated villi, hypoplasia of crypt cells and decreased epithelial cell mitosis (Nabuurs *et al.*1993; Van Beers-Schreurs *et al.* 1995). Spreevwenberg *et al.* (2001) postulated that improved welfare indices and feed intake in pig leads to improvement in gut mucosal integrity affirmed by decrease in paracellular transport and an increase in villus height. Pluske *et al*. (1997) in their experiment established positive and linear correlation in feed intake and villus height in pigs. Moreover, the study of Verdonk *et al.* (2007) discouraged the adoption of restricted feeding in pig industry because of its negative impact in intestinal morphology of the pigs. Adequate feed intake hinders the damage of the barricade function of the tight junctions that indicates sufficient luminal nutrient supply to maintain the barricade function. This observation is line with this present study that showed that heterogeneous weight pigs had longest lamina propria depth compared to the values noted for other treatment groups. Hampson (1986) postulated that higher feed intake is essential for the incidence of crypt hyperplasia.

Genetic selection for fast growth rate in modern meat-type farm animals has led to skeletal abnormalities in this class of animal as a result of increased muscle to bone ratio (Kwiatkowska *et al*. 2017). The condition of the bone has been found to be directly related to the bioavalability of Calcium and Phosphorus at tissue level (Nkukwana *et al*. 2014). The quantity of ash generated from fiery bone and the force required in breaking the bone are two common indices used in assessing the bioavailability of Calcium and Phosphorus (Shaw *et al.* 2010). The present study indicated significant differences in wet tibia bone weight, tibia bone length, tibia medial thickness and lateral thickness. The significant increase in tibia wet weight of pigs with higher propensity of social challenges (heterozygous and homogeneous heavy weights pigs) could be linked with better mineralization due to higher physical activities that might have led improved nutrient digestion. Janssen and LeBlac (2010) states that physical exercise improves cardiovascular and respiratory rates, slows loss of muscular strength, thereby enhancing muscle tone, increased bone mass, aids nutrient digestibility and the function of bowel. The significant highest tibia length and lateral thickness in homogeneous heavy weight pigs must have resulted from the variation in the weight of pigs which depicts genetic relationship between body weight and bone morphology. This observation is in concordance with the study of Nkukwana *et al.* (2014) that noted positive correlation among body weight, dry bone weight and bone length.

5. Conclusion

It has been shown in this study that weight asymmetry influenced serum total protein, albumin, and cholesterol, high-density lipoprotein and aspartate aminotransferase of the pigs. Likewise, it improved tibia weight, length, medial and lateral thickness of the tibia bone. Hence, grouping of pigs on equal weight basis can be adopted as management practice in a commercial pig farm for enhancement of pig welfare.

6. Conflict of Interest

We hereby certify that there is no conflict of interest pertaining to the investigation and preparation of the manuscript.

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