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EFFECT OF WEANING REGIMES ON GROWTH PERFORMANCE, STRESS AND BEHAVIOURAL RESPONSES IN WEANLING PIGS

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ABSTRACT

Weaning is one of the most stressful events in pigs as this affects their health, growth performance and welfare especially during the first week post-weaning. Early weaning exposes weanling pigs to severe weaning stress. Therefore, this study assessed the effects of different weaning regimes on weanling pigs' growth, stress and behavioural responses. Seventy-two weanling pigs, allotted to three treatments in 6 replicates in a completely randomised design, were used for this study. The weanling pigs were weaned at four weeks (T1), six weeks (T2) and eight weeks (T3) and involved in the study immediately after weaning. Growth performance (feed intake, weight gain and feed conversion ratio – FCR), stress indices (corticosterone, white blood cell – WBC) and behavioural indices (feeding and aggressive behaviour) were measured for 6 weeks, twelve days and ten days post-weaning, respectively. Data were analysed using descriptive statistics and ANOVA at α 0.05. Feed conversion ratio of weanling pigs in T1 (2.42 ± 0.09) was significantly higher ($p < 0.05$) than in T2 (2.23 ± 0.15) and T3 (2.22 ± 0.10) groups. Corticosterone concentration in the weanling pigs ranged from 55.83 ng/ml (T1) to 48.31 ng/ml (T3) on day 3, from 45.58 ng/ml (T2) to 45.42 ng/ml (T1 and T3) on day 9, and from 46.52 ng/ml (T1) to 45.25 ng/ml (T2) on day 12. The white blood cell (WBC) count in the weanling pigs in T1 ($8.83 \pm 0.07 \times 10^3$ µl) was significantly higher ($p < 0.05$) than in T2 ($8.53 \pm 0.12 \times 10^3$ µl) and in T3 ($8.62 \pm 0.14 \times 10^3$ µl) on day 0. The values obtained for days 6, 9 and 12 demonstrated the same trend. Feeding behaviour showed that weanling pigs in T1 spent lesser time feeding than in T2 and T3 groups. The values ranged from 41.72 % (T1) to 70.31 % (T3). Weanling pigs in the T1 group showed more aggression towards pen mates for the first 3 days compared to T2 and T3 groups. The values ranged from 0.52 % (T3) to 9.24 % (T1). In conclusion, this study revealed that different weaning regimes have varying effects on the performance and welfare of pigs. It is important to carefully consider the weaning regimes implemented in pig production to minimize stress and promote healthy growth.

Key words: performance; weanling pigs; weaning time; weaning stress; feeding behaviour; aggressive behaviour

INTRODUCTION

The conventional age at which piglets are weaned is within 3-4 weeks. However, in some cases, piglets are weaned at varying times to as long as twelve weeks, thereby resulting into prolonged lactation, excessive weight loss and, thus, lengthens the time for their dam to attain optimal body condition for rebreeding (Jensen, 1986; Massacci *et al.*, 2020; Tang *et al.*, 2022).

When weaning is one of the most contentious topics in the pig industry, often economics, welfare and health overlap considerably. Whilst production and economic advantages are achieved by weaning piglets early, a reduction in lactation length means that the majority of piglets will have consumed little, if any, solid feed prior to weaning (Boudry *et al.*, 2004). Weaning age is also dependent on when piglets can develop their own immune systems without the reliance on maternal antibodies (Hueb *et al.*, 2019; Ding *et al.*, 2022).

Weaning is one of the most stressful events in the life of pigs, which affects animal health and growth performance, especially during the first week

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post-weaning (Campell *et al.*, 2013; Pluske 2013; Yin *et al.*, 2013). Although, weaning of piglets as early as 3 – 4 weeks provides additional benefits in that the sow is able to be mated sooner, therefore, producing more piglets/sow/year (Brooks and Tsourgiannis, 2003; Santos *et al.*, 2004) however, weaning piglets earlier than 5 weeks resulted in reduced feed intake and weight gain immediately after weaning, and this may eventually affect their growth rate throughout the growing phase (Brooks and Tsourgiannis, 2003). It is common for weanling pigs to stop growing or even lose weight during the first week after weaning, a phenomenon commonly referred to as the post-weaning growth check.

According to Weary *et al.* (2008), changes to the social and physical environments of pigs are more prominent in commercial systems than an outdoor system, and this can result in a pronounced behavioural and physiological response to the weaning process. Negative behavioural patterns, such as belly nosing and chewing on the tails or ears of other pigs, often occur as a result of maladaptation of weanling pigs to weaning (Blackshaw, 1981).

Therefore, this study was aimed at investigating the effect of different weaning time on growth, stress and behavioural responses of weanling pigs.

MATERIALS AND METHODS

Experiment site

The study was carried out at the Piggery Unit, Teaching and Research Farm, University of Ibadan, Nigeria. The laboratory analyses were carried out at the Animal Physiology Laboratory, Department of Animal Science, University of Ibadan.

Experimental animals, layout and design

Seventy-two weanling pigs (Large white x Landrace), allotted to three treatments in 6 replicates/treatments in a completely randomized design (CRD), were used in this study. The weanling pigs were weaned at four weeks (T1), six weeks (T2) and eight weeks (T3) and involved in the experiment immediately after weaning. A total of 18 pens were used for this experiment. Feeds and water were served twice a day at the hours of 08:00 and 14:00 throughout the 6 weeks of the experiment.

Behavioural Data Collection

Close circuit televisions (CCTV) were installed in the entire sties used in this study. The video cameras (AHDI Mega Pixel Cameras, CCTV Central, Mount Waverley, Victoria, Australia) with 3.6 mm focal lenses were mounted in the sties at strategic positions that provides a clear sight of the entire sty. Observations were continuously recorded using Analogue High Definition (AHD) Digital Video Recorder (DVR), which was connected to the cameras; the DVR was located in the monitoring room away from the pen, so that the activities of the operator will not affect the behaviour of pigs. Digital video data were viewed for collation of behaviour data. Recordings were done for four hours a day between 08:00 – 12:00 hours, for the first 10 days of the duration of the experiment. The behavioural parameters, measured in this experiment, were generated according to the ethogram in Table 1.

Data collection

The data were collected on growth performance, stress and behavioural indices.

Table 1. Behavioural ethogram of swine

Category	Definition
Feeding behaviour	Head in feeder or about 3 cm near the feeder (includes nosing the feeder)
Aggressive behaviour	
Biting	Nibbling, sucking or chewing ears, legs, feet or tail
The pursuit of one pig by another	The pursuit

Source: Adapted from Pluske and Williams (1996) and Bolhuis *et al.* (2005).

Body weight gain, feed intake and feed conversion ratio

The animals were fed twice a day at the hours of 08:00 and 14:00 daily. Quantity of feed served was weighed and fed to the pigs per replicate and the leftover feed was weighed using a table top weighing scale (Camry, 25kg). The feed intake was calculated by deducting the leftover from the amount served, as follows:

Feed Intake = Amount of feed served – Amount of feed leftover

Pigs in each replicate were weighed at the commencement of the experiment and on weekly basis using weighing scale. At the end of the experiment, the total weights gained were determined by deducting the initial body weight from the final body weight. Feed conversion ratio (FCR) was calculated as a ratio of feed consumed to the body weight change and it is expressed as:

$$FCR = \frac{\text{Average feed consumed}}{\text{Average weight gain}}$$

Blood collection

On the first day of the experiment, blood samples were collected from the anterior *vena cava* of the weanling pigs and subsequently at 3 days interval for 12 days. The blood samples for haematology were collected into bottles containing EDTA to prevent coagulation, while the blood samples for serum analysis were collected into plain bottles.

Serum corticosterone was determined using commercially available ELISA kit (Enzo Life Sciences, Cortisol ELISA kit, AD-901-071, Farmingdale, NY) in accordance with the manufacturers' instruction.

The parameters determined for haematology were: white blood cell counts, lymphocyte counts, monocyte counts, neutrophil counts and eosinophil counts, as described by Schalm *et al.* 1975.

Behavioural parameters

The behavioural data, according to the ethogram in Table 1, were deduced from the video recordings, which include feeding, biting pen mates and chasing pen mates. All these values were counted to measure the feeding and aggressive behaviour. The counting from the video recordings was done for one minute at each ten minutes, making a total of 24 counts in the four hours of each recording. The frequency of occurrence of each category of observation on the ethogram was then expressed as a percentile of the total observation according to Scott *et al.* 2006a, b.

Statistical analysis

A completely randomized design with the following model was adopted in this study:

$X_{ij} = \mu + \alpha_i + E_{ij}$, where

X_{ij} = any of the response variables; μ = the overall mean; α_i = effect of the i treatment (i = weaning at 4, 6 and 8 weeks) and E_{ij} = random error due to experimentation.

Performance and haematology data were subjected to analysis of variance (ANOVA) using the SAS software (2010) and means were separated with New Duncan Multiple Range Test, while the significance was assessed at $p < 0.05$. Corticosterone and behavioural data were analysed using descriptive statistics.

RESULTS

Growth performance of weanling pigs weaned at different weaning time

The growth performance of weanling pigs weaned at different weaning time was as shown in Table 2. There were no significant differences ($p > 0.05$) among the treatments for initial body weight, which ranged from 6.02 ± 0.27 kg in T3 to 4.31 ± 0.15 kg in T1 group. However, there were significant differences ($p < 0.05$) among the treatments for average weight gain, average feed intake, final weight and feed conversion ratio. The mean values for average feed intake showed that, T3 (12.81 ± 0.61 kg) and T2 (12.70 ± 0.39 kg) groups significantly ($p < 0.05$) differed from T1 group (10.94 ± 0.48 kg). The same trend was observed for average weight gain, where T3 (5.76 ± 0.29 kg) and T2 (5.69 ± 0.36 kg) groups significantly ($p < 0.05$) differed from T1 (4.53 ± 0.18 kg) group. Also, the mean values for the final weight showed that T3 (11.78 ± 0.31 kg) and T2 (11.18 ± 0.46 kg) groups significantly ($p < 0.05$) differed from T1 (8.84 ± 0.24 kg) group. In addition, T1 (2.42 ± 0.09) was significantly ($p < 0.05$) different from T2 (2.23 ± 0.15) and T3 (2.22 ± 0.10) groups in the feed conversion ratio.

Corticosterone concentration (ng/ml) in weanling pigs weaned at different weaning time

The results of the corticosterone concentration (ng/ml) in weanling pigs weaned at different weaning time (Figure 1.) show that animals in T1 group were more stressed after weaning compared to T2 and T3

groups. The corticosterone concentration for T1 was the highest on day 3 (55.83 ng/ml) compared to T2 (48.6 ng/ml) and T3 (48.31 ng/ml) groups.

White blood cell count (x 10^3 µl) of weanling pigs weaned at different weaning time

The results of white blood cell count (x 10^3 µl) of weanling pigs weaned at different weaning time (Table 3) show significant ($p < 0.05$) differences among the treatments from day 0 to 12. On day 0, WBC in T1 group (8.83 ± 0.07) was significantly ($p < 0.05$) higher than in T2 (8.53 ± 0.12) and T3 (8.62) groups. However, the values obtained for days 3, 6, 9 and 12 showed the same trend.

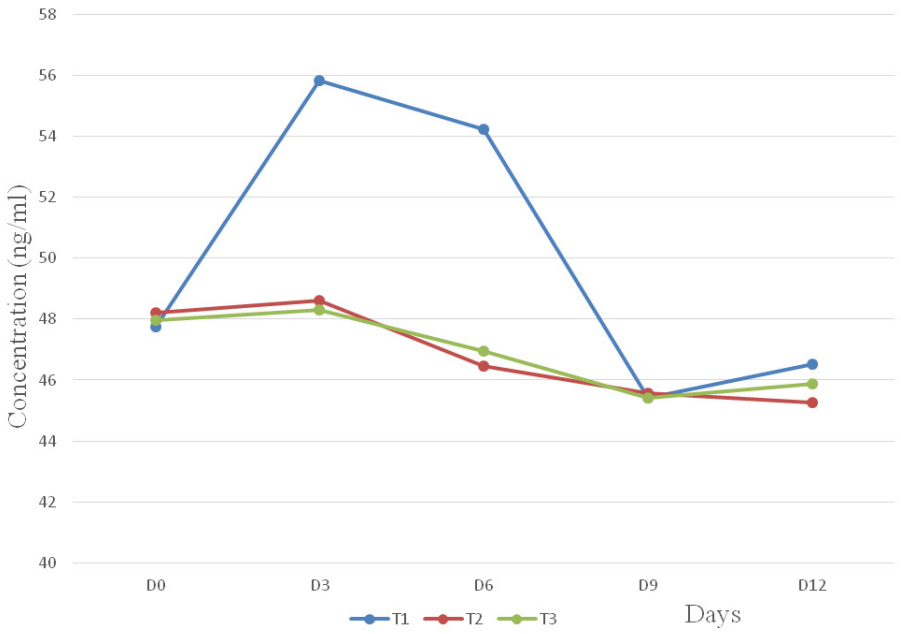
Lymphocyte count (%) of weanling pigs weaned at different weaning time

As Table 4 shows, no significant ($p > 0.05$) differences in the lymphocyte count (%) of weanling pigs weaned at different weaning time among the treatments from day 0 to 12 were observed.

Table 2. Growth performance of weanling pigs weaned at different weaning time

	Weaning time		
Parameters	4 weeks	6 weeks	8 weeks
Initial weight (kg)	4.31 ± 0.15	5.49 ± 0.42	6.02 ± 0.27
Average feed intake (kg)	10.94 ± 0.48^b	12.70 ± 0.39^a	12.81 ± 0.61^a
Average weight gain (kg)	4.53 ± 0.18^b	5.69 ± 0.36^a	5.76 ± 0.29^a
Final weight (kg)	8.84 ± 0.24^b	11.18 ± 0.46^a	11.78 ± 0.31^a
Feed conversion ratio	2.42 ± 0.09^a	2.23 ± 0.15^b	2.22 ± 0.10^b

^{a,b} Means in the same row with different superscripts are significantly different ($p < 0.05$). Treatment 1: Weaning at 4 weeks; Treatment 2: Weaning at 6 weeks; Treatment 3: Weaning at 8 weeks.



Treatment 1: Weaning at 4 weeks; Treatment 2: Weaning at 6 weeks; Treatment 3: Weaning at 8 weeks

Figure 1. Corticosterone concentration (ng/ml) in weanling pigs weaned at different weaning time

Table 3. White blood cell count (x 10^3 µl) of weanling pigs weaned at different weaning time

	Weaning time		
Day	4 weeks	6 weeks	8 weeks
0	8.83 ± 0.07[a]	8.53 ± 0.12[b]	8.62 ± 0.14[b]
3	11.08 ± 0.15[a]	9.80 ± 0.23[b]	9.10 ± 0.08[c]
6	9.98 ± 0.15[a]	9.20 ± 0.21[b]	8.98 ± 0.05[b]
9	9.69 ± 0.12[a]	8.78 ± 0.10[b]	8.64 ± 0.16[b]
12	8.71 ± 0.11[a]	8.49 ± 0.20[b]	8.53 ± 0.09[b]

[a,b,c] Means in the same row with different superscripts are significantly different ($p < 0.05$). Treatment 1: Weaning at 4 weeks; Treatment 2: Weaning at 6 weeks; Treatment 3: Weaning at 8 weeks.

Table 4. Lymphocyte count (%) of weanling pigs weaned at different weaning time

	Weaning time		
Day	4 weeks	6 weeks	8 weeks
0	44.33 ± 0.23	45.00 ± 0.27	43.33 ± 0.17
3	40.33 ± 0.41	42.00 ± 0.21	42.67 ± 0.52
6	41.67 ± 0.30	43.67 ± 0.42	43.33 ± 0.19
9	43.67 ± 0.33	44.33 ± 0.25	44.33 ± 0.42
12	43.67 ± 0.36	44.00 ± 0.14	45.00 ± 0.34

Treatment 1: Weaning at 4 weeks; Treatment 2: Weaning at 6 weeks; Treatment 3: Weaning at 8 weeks.

Table 5. Neutrophil count (%) of weanling pigs weaned at different weaning time

	Weaning time		
Day	4 weeks	6 weeks	8 weeks
0	48.00 ± 0.91	48.00 ± 0.46	49.33 ± 0.67
3	52.67 ± 0.74	50.33 ± 0.54	50.00 ± 0.16
6	50.67 ± 0.61	49.00 ± 0.53	48.67 ± 0.46
9	49.33 ± 0.57	48.67 ± 0.42	48.33 ± 0.59
12	49.67 ± 0.83	49.00 ± 0.34	48.00 ± 0.69

Treatment 1: Weaning at 4 weeks; Treatment 2: Weaning at 6 weeks; Treatment 3: Weaning at 8 weeks.

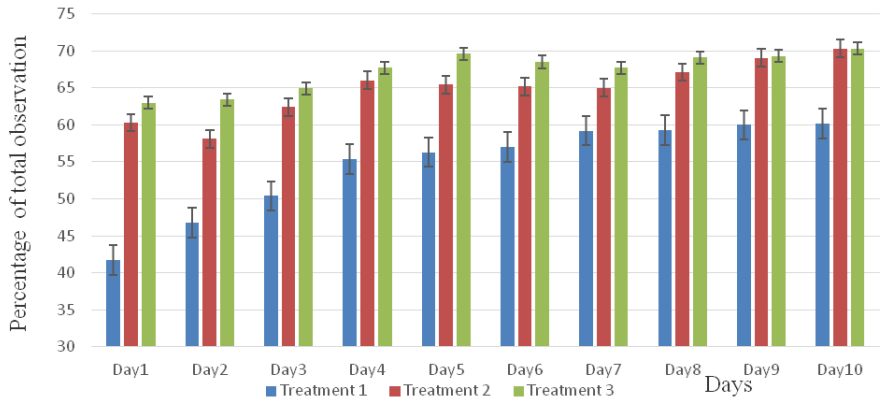
Neutrophil count (%) of weanling pigs weaned at different weaning time

No significant ($p > 0.05$) differences in the neutrophil count (%) of weanling pigs weaned at different weaning time were observed among the treatments from day 0 to 12 (Table 5).

Behavioural parameters

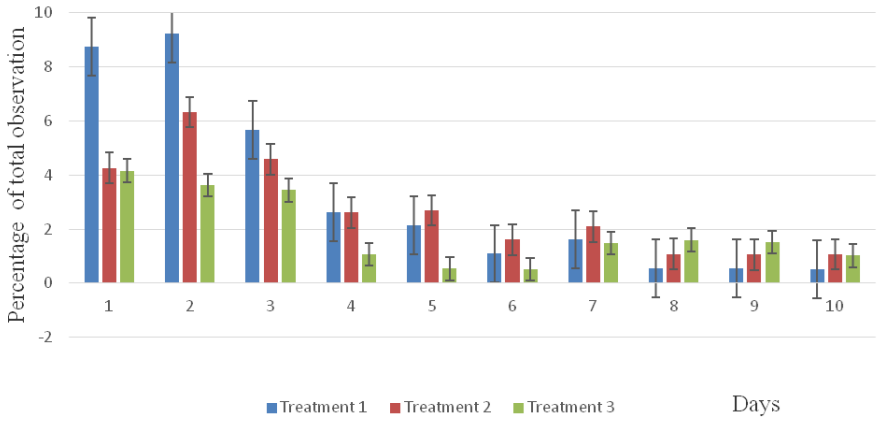
Feeding behaviour of weanling pigs weaned at different weaning time

The feeding behaviour of weanling pigs weaned at different weaning time is presented on Figure 2. The pigs



Treatment 1: Weaning at 4 weeks; Treatment 2: Weaning at 6 weeks; Treatment 3: Weaning at 8 weeks

Figure 2. Feeding behaviour of weanling pigs weaned at different weaning time



Treatment 1: Weaning at 4 weeks; Treatment 2: Weaning at 6 weeks; Treatment 3: Weaning at 8 weeks

Figure 3. Aggressive behaviour of weanling pigs weaned at different weaning time

weaned at 4 weeks of age spent lesser time feeding than those weaned at 6 and 8 weeks. T1 group (4 weeks) had the lowest value on day 1 (41.72 %), while the highest value – on day 10 (60.2 %). T2 group (6 weeks) had the lowest value on day 2 (58.10 %), while the highest value was recorded on day 10 (70.3 %). T3 group (8 weeks) had the lowest value on day 1 (63 %), while the highest value was on day 10 (70.31 %).

Aggressive behaviour of weanling pigs weaned at different weaning time

The Figure 3 shows that pigs weaned at 4 weeks of age showed more aggression towards their pen mates for the first 3 days, than those weaned at 6 and 8 weeks. T1 group (4 weeks) had the highest value on day 1 (9.24 %), while the lowest value – on day 10 (0.52 %). T2 group (6 weeks) had the highest value on day 2 (6.34 %), while the lowest value – on day 9 (1.06 %). T3 group (8 weeks) had the highest value on day 1 (4.16 %), while the lowest value – on day 6 (0.52 %).

DISCUSSION

Growth performance of weanling pigs weaned at different weaning time

The growth performance of weanling pigs weaned at different weaning time revealed that weaning time affects the performance of pigs. In this study, the weanling pigs were weaned at 4 weeks, 6 weeks and 8 weeks. The results of this study indicated that the average feed intake, average weight gain and final weight of weanling pigs weaned at 6 weeks and 8 weeks were significantly higher than those weaned at 4 weeks. Weanling pigs that consume less feed will grow slower, than those that consume feed at a faster rate, as reported by Kuller *et al.* (2004). The feed conversion ratio for weanling pigs weaned at 4 weeks was significantly higher than those weaned at 6 weeks and 8 weeks. This could be a result of a more developed gut of the weanling pigs weaned at 6 weeks and 8 weeks to handle solid feed. Brooks and Tsourgiannis (2003) reported that weaning piglets earlier than 5 weeks resulted in reduced feed intake and weight gain immediately after weaning. Also, various researchers (van Beers-Schreurs *et al.*, 1998; Berkeveld *et al.*, 2009) have reported a positive correlation between villus height and feed intake, indicating the importance of feed intake for proper intestinal function.

Corticosterone concentration (ng/ml) in weanling pigs weaned at different weaning time

Serum corticosterone was used as physiological indicator of stress in this study. As described by Moberg (2000), stress is a biological response mechanism, elicited when an organism perceives a threat to its homeostasis. Stress occurs when an animal is unable to physiologically or behaviourally cope with a challenge. When investigating the possible effects that weaning time have on weanling pigs' welfare, it is important to be able to identify, when the animals are unable to physiologically or behaviourally cope with the challenge i.e., when there is a biological cost to the animals (Moberg, 2000). Elevation of serum corticosterone concentration in stressful situation is an adaptation response of the organism to a changing environment (Jarvis *et al.*, 2006). In this study, the corticosterone concentrations (ng/ml) of weanling pigs weaned at 4 weeks, 6 weeks and 8 weeks were measured. Weanling pigs undergo weaning stress irrespective of the age at which they are weaned, however, the severity of the weaning stress on the animals depends on the age at which they are weaned. The results show that weanling pigs, weaned at 4 weeks, were more stressed after weaning compared to those weaned at 6 weeks or 8 weeks. Weanling pigs that are weaned early, probably experience a more pronounced stress response. The increased corticosterone concentration may be attributed to the stress of weaning, which was caused by maternal separation and abrupt change in nutrient source i.e. from milk to solid feed. Mason *et al.* (2003) reported that corticosterone, a physiological response of stress, is higher in weanling pigs, which consume little or no creep feed prior to weaning. Also, Campbell *et al.* (2013) reported that the removal of sow milk and environmental changes led to a drastic reduction in feed intake, which was caused by loss of appetite (anorexia).

White blood cell count (x 10^3 µl) of weanling pigs weaned at different weaning time

White blood cell count was also used as physiological indicator of stress in this study. Haematological parameters, such as white blood cell count, are good indicators of physiological and pathological changes in animals (Adenkola and Durotoye, 2004). In this study, the white blood cell count of weanling pigs, weaned at 4 weeks, was significantly higher than those weaned at 6 weeks and 8 weeks. This could be attributed to weaning stress (Buckham-Sporer *et al.*, 2008) and is suggested to be due to mobilisation of WBCs from their pool to the peripheral circulation, apparently due to their inhibitory role in circulating corticosterone, which is known to increase in animals under stress (Adenkola *et al.*, 2009). This is because increase in corticosterone release during stress in animals can enhance disease susceptibility and compromise the immune system. However, the mean white blood cell counts recorded in this study were similar to the reference value, as documented by Mitruka and Rawnsley (1977). Zahorec (2001) reported, that as glucocorticoid concentration increased, white blood counts also increased.

Neutrophil/Lymphocyte ratio of weanling pigs weaned at different weaning time

One of the useful stress indicator is the changes in WBC differential counts, particularly the neutrophil: lymphocyte ratio (N:L). In this experiment, on day 4, the lymphocyte counts reduced in all the treatments, but the reduction was more visible in weanling pigs weaned at 4 weeks, though not significant. However, the reverse

was the case for neutrophil counts, as they increased. As glucocorticoid concentration increases, lymphocyte (L) numbers tend to decrease, whereas number of neutrophils (N) tends to increase. Neutrophils are the first line of defence in an organism's immune system. When there is a threat to the immune system of an organism, the neutrophils are more produced due to their anti-inflammatory activities and the destruction of infiltrating micro-organisms. The results of this study agree with those of Zahorec (2001), who reported that as glucocorticoids concentration increased, neutrophil counts also increased, while lymphocyte count decreased.

Feeding behaviour of weanling pigs weaned at different weaning time

The result of this study showed that weanling pigs weaned at 4 weeks of age spent lesser time feeding, than those weaned at 6 and 8 weeks, and this caused a reduction in their weight gain compared to other treatments. This may be due to maternal separation and changes in nutrition source. However, the time spent for feeding gradually increased as days pass. The intake of solid feed increases naturally with age, but commercial rearing systems favour weaning well before the piglets achieve nutritional independence from the sow. Of course, the pronounced distress response typical, when piglets are weaned at a young age is not only due to these animals being more dependent upon the sow's milk; piglets are also likely more socially dependent upon the sow. Several studies have addressed the behavioural and performance consequences of early weaning in pigs, but only a few have focused on feed consumption, usually measuring feeding behaviour or intake in the period immediately following weaning. For example, average daily feed intake after weaning was higher for piglets weaned at 19 days, than at 9 days of age (Dritz *et al.*, 1996). Similarly, weanling pigs spent half the time eating during the first 48 h after weaning at 12 days than at 21 days of age (Gonyou *et al.*, 1998). Colson *et al.* (2006) found that the period of growth check was halved, when weanling pigs were weaned at 28 days versus 21 days of age, likely due to higher feed intake.

Aggressive behaviour of weanling pigs weaned at different weaning time

Aggression commonly occurs at weaning especially among piglets weaned at younger age. This study showed that weanling pigs, weaned at 4 weeks of age, were more aggressive than those weaned at 6 or 8 weeks, and this may be due to depression caused by maternal separation. However, the aggressiveness gradually reduced as days pass. Aggressive behaviour is one of the most obvious outcomes of separating piglets from their dams. Widowski *et al.* (2008) reported that typical piglets' responses to early abrupt weaning are high frequency of agonistic interactions, abnormal and stereotypic behaviours and distress vocalizations. Jarvis *et al.* (2008) attributed the aggression in these circumstances to the need to re-establish the litters' social order following the absence of the dam. Also, stress may alter cues emitted by the piglets, impairing recognition by littermates. For example, vocalizations of stressed piglets may differ qualitatively from those emitted by the non-stressed animal, as has been shown in the case of distress or pain (Taylor and Weary, 2000; Dupjan *et al.*, 2008).

CONCLUSION

The weaning process is a critical period in a pig's life that can significantly impact its growth, stress and behavioural responses. It is important to carefully consider the weaning regimes implemented in pig production to minimize stress and promote healthy growth. This study revealed that different weaning time has varying effects on the performance and welfare of pigs. Weaning piglets at 6 weeks gave the optimum result, improved their growth performance, reduced stress and behaviours indicative of compromised welfare.

AUTHOR'S CONTRIBUTIONS

Conceptualization: BANKOLE, T. O., ADEBIYI, O. A., EWUOLA, E.O., OLUYEMI, A. A.

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Investigation: BANKOLE, T. O., ADEBIYI, O. A., EWUOLA, E.O.

Data Curation: BANKOLE, T. O., ADEBIYI, O. A., EWUOLA, E.O., OLUYEMI, A. A., ABIOLA, O. J., ADEBIYI, F. G.

Writing-original draft preparation: BANKOLE, T. O., ADEBIYI, O. A., EWUOLA, E. O.

Writing-review and editing: BANKOLE, T. O., ADEBIYI, O. A., EWUOLA, E. O.

Project administration: BANKOLE, T. O., ADEBIYI, O. A., EWUOLA, E. O.

All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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