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Semen Characteristics and Specific Sperm Changes in Pubertal Boars Fed with Aidan (*Tetrapleura tetraptera*) Pod Meal

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Abstract
A total of eighteen crossbred (Large White X Duroc) weaner boars, aged 6 weeks, averaging 9.47kg, were used to study the impact of Aidan pod meal (APM) in a 16-week experimental period. The boars were divided into three treatment groups and assigned 0.0% (control), 2.5% or 5.0% APM per unit weight of feed. The experiment was randomized. Data were collected on semen quality and specific changes in morphometry and morphology of sperm. Mass motility, live and normal sperm proportions were reduced (P<0.05) at 5.0% APM. Sperm concentration and total viable sperm were reduced (P<0.05) at both levels (2.5% and 5.0%) of APM treatment. Sperm from APM-treated boars had longer (P<0.05) heads, which were wider (P<0.05) at 5.0% APM. The sperms were thicker (P<0.05) among boars fed with 5.0% APM than in the control diet. Abnormality of the sperm head, number of sperm with cytoplasmic droplet and clumping were reduced (P<0.05) in boars fed with APM. The number of sperm with broken mid-pieces increased in 5.0% APM application. It is concluded that application of Aidan pod meal significantly reduced the semen quality parameters of pubertal boars. Sperm heads were found to be longer and wider, with thick whole sperm, while most morphological sperm abnormalities were reduced, though with increased number of sperm having broken mid-piece at 5.0% APM feeding.
Keywords: Semen characteristics, sperm changes, pubertal boars, Aidan
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Introduction

Semen characteristics are very important to the pig farmers as not all boars in the herd will produce semen that would be used for a breeding programme. Factors such as nutrition, frequency of semen collection, genetics, circulating hormones, etc., influence semen traits (Adu and Egbunike, 2010). Althouse (1997) documented the minimum values for the quality of fresh, unextended boar semen. To fertilize an ovum sperm must have normal structures. The percentage of abnormally formed sperm depends on the level of stress that attends the process of sperm formation (Wolf, 2009, Gorski *et al.*, 2017). The fertility of a boar can be assessed by an objective semen quality evaluation, which revolves on morphological examination (Kondracki *et al.*, 2013).

The size and shape of a sperm equip it to effectively efface the *Zona pellucida* of the ovum. Semen samples with preponderance of sperm possessing major abnormalities in their morphology are not preferred for insemination programme, as male fertility is compromised (Gorski *et al.*, 2016). Sperm length has a positive correlation with rate of its motion

(Noorafshan and Karbalay-Doust, 2010). A sperm with comparatively longer tail tends to swim faster than the others (Fitzpatrick and Lupold, 2014), especially if it moves in a forward progressive fashion. Low sperm motility correlated with shorter tail length (Noorafshan and Karbalay-Doust, 2010).

It was opined by De Paz *et al*. (2011) that sperm head structure might be one of the factors that affect male fertility. Reduced fertility, deteriorated embryo quality, lowered sperm capacity to fuse with the ovum and miscarriages in the early period of pregnancy had been linked with sperm head defects (Chenoweth, 2005). Hirai *et al*. (2001) revealed that spermatozoa with higher fertility had narrower and shorter heads. Narrowed head dimension makes for a streamlined conformation (Gil *et al*., 2009). This work evaluated the gross changes in the semen and specific modifications in morphometry and morphology of sperm of pubertal boars exposed to dietary addition of Aidan (*Tetrapleura tetraptera*) pod meal (APM).

Materials and methods

Study Location

The study was carried out at the Piggery of Michael Okpara University of Agriculture, Umudike, Nigeria. Umudike lies on $05^0$29' N and 07^0 33'E, and 122m above sea level. Annual rainfall average is from 1700 to 2100 mm. Minimum and maximum temperature range from18-23^0 C and 26-36^0 C, respectively, relative humidity of 57-91% (NRCRI, 2022).

Sourcing and Preparation of Aidan pod meal

The pods were sourced from a local market in Aba, Abia State. Non-woody parts of dry pods were peeled off with a knife and milled. The meal was kept in air-tight bags and subsequently added at 0.0% (control), 2.50% and 5.0%, respectively, in the diets of the boars. See the information under Feeding of experimental animals.

Diet composition for the experimental pubertal boars is presented in Table 1

Table 1: Diet composition for the experimental pubertal boars fed APM

	Treatment		
Ingredients	**0.0% APM**	**2.5% APM**	**5.0% APM**
Maize	30.10	30.10	30.10
Groundnut cake	5.50	5.50	5.50
Palm kernel cake	20.00	20.00	20.00
Wheat offal	41.80	41.80	41.80
Bone meal	0.25	0.25	0.25
Oyster shell	1.50	1.50	1.50
Vitamin/mineral premix*	0.20	0.20	0.20
Methionine	0.05	0.05	0.05
Lysine	0.15	0.15	0.15
Salt	0.45	0.45	0.45
Total	**100**	**100**	**100**
APM (%)	0.00	2.50	5.00
Crude protein (%)	16.03	16.03	16.03
Digestible energy (Kcal/kg)	2817.65	2817.65	2817.65

* To provide per kg of diet: vitamin A (10 000 IU), vitamin D (20 000 IU), vitamin E (5 IU), vitamin K (2.5 mg), choline (350 mg), folic acid (1 mg), manganese (56 mg), iodine (1 mg), iron (20 mg), copper (10 mg), zinc (50 mg), cobalt (1.25 mg). APM = *Aidan* pod meal

Experimental Animals and Management

Crossbred (Large White x Duroc) 6-week-old weaner boars with average weight of 9.47kg were used for the study. They were stocked, allowed a two-week quarantine period. Ivermectin injectable solution (1%) was given subcutaneously at 0.25ml/12.5 kg body weight, to protect the animals from internal and external parasites. The boars were systematically divided

into three equal treatment groups. Each treatment was replicated 3 times with two boars in each replicate in a completely randomized design. They were reared in pens with hard and non-slippery concrete floor. The pens were kept clean daily.

Feeding of the Experimental Animals

The basal diet with the test ingredient (Table 1) was pre-weighed, soaked in water overnight and made available to the boars twice (morning and evening) daily. The boars were fed 5% of their body weight supplied in the morning and evening, while water was supplied *ad libitum (*Onyimonyi 2002, Ogunsipe et al., 2017) while the experiment lasted.

Semen collection and evaluation

Semen samples were collected via epididymal washings. At 28 weeks of age the boars were sacrificed by cervical dislocation and had their testes immediately harvested. The epididymides were excised. The cauda epididymis was cut. A smear of the semen was made on a glass slide, pre-heated to 37^0C for 15-20 minutes with a slide warmer, for semen study.

Sperm mass motility, sperm cell viability (live sperm proportion), abnormal sperm proportion and spermatozoa morphometries were evaluated according to El-Sherbiny (1987).

Sperm mass motility

The semen samples from each treatment group were evaluated for progressive motile sperm cells. This was done immediately after collection. A drop of the semen was smeared on a preheated glass slide and viewed under a light microscope at a lower magnification of x 10 and x 40 and scored subjectively in percentage.

Table 2 shows the descriptive and numerical scales for evaluation of sperm motility, according to Peter (2002).

Table 2: Descriptive and numerical scales for evaluation of microscopic pattern of sperm motility

Numerical scale	Descriptive scale	% Sperm motility	Wave pattern
0	Very poor	0 – 19	Immotile, no wave
1	Poor	20 – 39	Stationery and bunting. Weak movements; no waves
2	Fair	40 – 49	Oscillatory or rotary movements. Few waves and eddies
3	Good	50 – 79	Progressive rapid movement. Waves and eddies seen.
4	Very good	80 – 89	Vigorous rapid movement. Eddies seen
5	Excellent	90 – 100	Very vigorous rapid movement. Rapid waves and eddies

Source: Modified from Peter (2002)

Sperm cell viability (Live sperm proportion)

The proportion of sperm cells that were viable (alive) was determined by staining a drop of semen with Eosin-Nigrosin stain. The stained-glass slides were allowed to dry for 30 seconds before fixing with ethanol. The stained slides were viewed under a light microscope at x 100 magnification (oil immersion), and the proportion of viable sperm cells counted with a hand-held stopwatch manual counter. A total of 300 cells were counted and the percent viable sperm cells calculated. The sperm cells that were alive (viable) did not pick the stain while those that picked the stain were dead (El-Sherbiny, 1987).

Sperm concentration

The concentration of the sperm cells in the semen samples were evaluated using the haemocytometer. A dilution of 1: 200 was made using a red blood cell pipette. 10% buffered formalin solution was used as the semen diluting fluid to immobilize the sperm cells. The haemocytometer was charged with a drop of the semen solution and allowed for 2 minutes on a wet paper (to allow the sperm cells settle) before it was mounted on a light microscopic stage and viewed under x 40 magnification.

Sperm concentration per ml = Number of cells counted x $\mathrm{DF} \times 0.04 \times 10^6$

Note: DF = Dilution factor (Egbuka, 1995).

Abnormal sperm proportion

The abnormal sperm proportion was determined by the method described by El-Sherbiny (1987). A drop of the semen was stained using Eosin/Nigrosin stain and the mixture smeared on a glass slide and viewed under a lower magnification of x 40 to check for primary and secondary abnormal sperm cells, percentage of

the differential abnormalities such as head abnormalities, tail abnormalities, mid-piece abnormalities, cytoplasmic droplets and clumping (percentage covering of the microscopic field).

Spermatozoa morphometries

A drop of semen sample on a glass slide was stained with Eosin-Nigrosin stain. The stained slides were allowed to air-dry for 30 seconds before fixing with ethanol. The slides were subsequently viewed under an ocular morphometer (a type of micrometer) with an in-built metre rule with capacity to measure dimensions of a spermatozoon, at x100 magnification under oil immersion. About 7 spermatozoa were randomly measured by placing the meter rule over the head area, mid-piece and tail region. Mean head length, head width, mid-piece length and tail length were recorded (El-Sherbiny, 1987).

Data analysis

Data were subjected to analysis of variance according to Steele and Torrie (1980) and significantly different ($P<0.05$) means separated using Duncan's New Multiple Range Test (Duncan, 1955) with SPSS version 22

Results and Discussions

The semen quality traits of pubertal boars fed Aidan pod meal are presented in Table 3.

Table 3: Semen Quality of Pubertal Boars Fed Aidan Pod Meal

Parameter	0.0%APM	2.5%APM	5.0%APM	SEM
Live weight (kg)	46.85^{c}	49.97^{b}	53.11^{a}	0.99
Mass motility (%)	80.50^{a}	80.80^{a}	72.93^{b}	2.31
Live sperm proportion (%)	89.67^{a}	86.23^{a}	78.40^{b}	2.63
Sperm concentration (x10^{6}/ml)	244.98^{a}	152.35^{b}	132.42^{b}	21.10
Normal sperm proportion (%)	94.49^{a}	94.82^{a}	92.70^{b}	1.24
Total viable sperm (x10^{12})	220.93^{a}	114.22^{b}	75.69^{b}	26.45

$^{a-c}$ Means along the same row with different superscripts are significantly different
SEM = Standard error of the mean, APM = Aidan pod meal

Mass motility score, live sperm proportion and normal sperm proportion followed similar pattern and were significantly lowered at 5.0% APM. Mass motility in the range of 72.93-80.80% recorded in this study, is in line with 80% progressive motility which (Estienne and Harper, 2004) associated with most fertile ejaculate samples. It also compares with the ranges (82.32-86.43% and 56.67-81.67%) reported by Adu and Egbunike (2010) and Gbore (2009), respectively, in pubertal boars. Sperm motility is a function of sperm biological value, among other factors. Lower limits for progressive motility of 62% and 70%; below which farrowing rates and litter size would decrease, were recommended by Flowers (1998) and Althouse (2007), respectively. Live sperm proportion ranging from 78.40% to 89.67 % in this work compares with the ranges: 64.17-82.50% and 84.40-86.03% reported by Gbore (2009) and Adu and Egbunike (2010), respectively.

Sperm concentration and total viable sperm were significantly less in boars administered APM than in their counterparts without APM treatment. Sperm concentration ranging from 132.42 x 10^{6} to 244.98 x 10^{6} in this report is in line with the ranges (0.25-0.3 x 10^{9} /ml) submitted by Gbore (2009) in pubertal boars.

Bearing in mind the ultimate goal of semen quality assessment-to predict the potential fertility of a given sample and disclose the fertility of the male subject from whom it was sampled (Rodriguez-Martinez *et al.*, 1999), the significantly lower semen quality characteristics obtained in boars fed APM calls for concern. Several studies on conventional semen quality parameters revealed significant positive correlations between motility, viability and normal sperm proportion with fertility, measured with the litter sizes of inseminated sows (Sutkeviciene *et al.*, 2009).

Sperm morphometry parameters of pubertal boars fed Aidan pod meal are presented in Tabble 4.

Table 4: Sperm Morphometry of Pubertal Boars Fed Aidan Pod Meal

Parameter	0.0%APM	2.5%APM	5.0%APM	SEM
Head length	8.63[b]	8.91[a]	9.07[a]	0.17
Head width	4.33[b]	4.21[b]	4.56[a]	0.10
Length of mid-piece	11.03	11.08	11.12	0.02
Tail length	36.53	37.54	37.32	0.38
Total length	56.19	57.53	57.51	0.45
Thickness	1.00[b]	1.02[ab]	1.09[a]	0.02

SEM = Standard error of the mean, APM = Aidan pod meal

Total, tail/flagellum and mid-piece length were not significantly altered by APM administration. Sperm heads were however, significantly longer in boars fed APM than in those fed without APM. Sperm heads were wider (P<0.05) in boars fed 5.0% APM than in their peers fed 0.0% and 2.5% APM. In like manner, spermatozoa of the 5.0% APM-fed boars were significantly thicker than those of the boars fed control.

Morphometric traits of the spermatozoa recorded in this work were in line with the dimensions observed in bulls and domestic boars by Kondracki *et al*. (2009). The gross and specific implications of the dimensions of the sperm head on boar and by extension on sow fertility have been explained. For instance, Hirai *et al*. (2001) associating higher fertility with sperm having narrower and shorter heads clarified in agreement with Gil *et al*. (2009), that conformation to narrowness and shortness of head helps a sperm cell swim faster in its bid to access a waiting ovum at the ampullary-isthmic junction, where fertilization occurs.

Abnormalities in sperm morphology of pubertal boars fed Aidan pod meal are presented in Table 5.

Table 5: Sperm Morphological Abnormalities of Pubertal Boars Fed Aidan Pod Meal

Abnormality	0.0%APM	2.5% APM	5.0% APM	SEM
Abnormal head	1.49[a]	0.89[b]	1.11[b]	0.13
Abnormal tail	0.60	0.62	0.60	0.06
Cytoplasmic droplet	2.75[a]	1.20[b]	0.74[b]	0.51
Broken mid-piece	0.52[b]	0.49[b]	0.82[a]	0.09
Clumping	9.23[a]	5.43[b]	4.33[b]	1.01

SEM = Standard error of the mean, APM = Aidan pod meal

Percentages of abnormal head, cytoplasmic droplets, gel formation and clumping (percent covering of microscopic field) were similar (P>0.05) at the two levels of APM and lower (P<0.05) than in control. Proportion of sperm with broken mid-piece was, however, higher (P<0.05) in boars fed 5.0% APM. Abnormality of tail/flagellum was not influenced by the experimental additive.

Normal sperm proportion in this study (92.70-94.82%) shows that total abnormality would be in the range of 5.18-7.30%. This range of total abnormality fell in line with the recommended < 20% by Althouse (1997); he also recommended average cytoplasmic droplets of < 15%, both recommendations being tenable in boar semen samples meant for artificial insemination programmes.

The tendency of the spermatozoa to adhere to one another, forming a lump was significantly reduced by the experimental material. This would have eased the microscopic evaluation of the semen samples obtained from the boars so treated.

Conclusions and Recommendations

Application of Aidan pod meal significantly reduced the semen quality parameters of pubertal boars. Sperm heads were longer and wider with generally thick whole sperm while

most morphological sperm abnormalities such as abnormal head, cytoplasmic droplet, gel in semen and clumping, were reduced. There was an increased number of sperm with broken mid-piece only at 5.0% APM.
It is recommended that APM should be applied with caution in male porcine subjects which are to be used for breeding programmes, considering the effect of the meal on the semen of the pubertal boars.

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