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Evaluation of the Blood Profile of Pubertal Boars Fed *Tetrapleura tetraptera* Pod Pulp Meal

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Abstract

A total of 18, 6-week old crossbred (Large White x Duroc) weaner boars, averaging 9.47kg were used to evaluate the effect of the inclusion of Tetrapleura tetraptera pod pulp meal (TPM) on their haematological and serum chemistry profiles for a period of 16 weeks. The boars were randomly assigned to three treatment groups of 6 animals each; treatment one (control) had 0.00% TPM, while TPM was included in the basal diet at 2.50% and 5.00% to constitute treatments two and three, respectively. Each treatment was replicated thrice, with 2 animals in a replicate in a completely randomized design. Data were collected on haemoglobin concentration, packed cell volume; counts of erythrocytes, leucocytes, and platelets, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, serum glucose, total protein, albumin, globulin, aspartate aminotransferase, alanine aminotransferase, creatinine, urea, low-density lipoprotein, high-density lipoprotein, very low-density lipoprotein, triglycerides and total cholesterol. Results indicated significant ($P<0.05$) increase in erythrocyte counts, serum glucose and low-density lipoprotein at 5.00% TPM. Packed cell volume, haemoglobin, leucocytes, total protein and albumin increased progressively ($P<0.05$) with increase in TPM. Globulin, Alanine aminotransferase, aspartate aminotransferase, creatinine, very low-density lipoprotein and triglycerides were reduced significantly ($P<0.05$) at increasing levels of TPM. High-density lipoprotein and total cholesterol were increased significantly at 2.50% TPM compared to the other treatments. Urea level was lowest ($P<0.05$) at 5.00% TPM. It was concluded that TPM enhanced the haematology and serum protein; and moderated the liver and kidney metabolites, indicating a positive influence on hepatic and renal efficiencies.

Keywords: Pubertal boars, *Tetrapleura tetraptera*, Haematology, Serum chemistry, Lipid profile

1.0 Introduction

Blood profile is influenced by all that animals take in as feed, water and drugs. As a result, blood parameters are a veritable tool in the analysis of the impact of feed additives in the animals' systems (Olajide & Akinsoyinu, 2015). The disposition of animals to feed can be interpreted by changes in blood cell counts and serum or plasma metabolites (Aderemi, 2004). Hence, the health as well as overall physiological status of animals can be assessed from their haematology and serum indices (Khan & Zafar, 2005). Therefore, the researcher needs to be equipped with adequate knowledge of the implications of the changes in these parameters, as might be influenced by feed additives. Understanding of the potential health benefits and nutritional importance of phytochemicals has aroused the curiosity of both scientists and lay men (Arunsi *et al.*, 2020).

Consumption of herbs and herbal products as roots, leaves, fruits, flowers and stem barks; in the form of extracts or decoctions, is on the increase (Mulugeta, 2016). There is a gradual but consistent shift from orthodox to herbal drugs (Okereke *et al.*, 2017). However, herbs contain a world of active principles, which influence the body system in ways beyond what present knowledge can sufficiently enunciate (Kumar *et al.*, 2014). Indiscriminate application of these herbs portends danger for both animals and man. It is necessary to explore these herbs to elucidate the gross and specific implications of consuming them and recommend a margin of safety for man and his animals (Ezea, 2019).

Tetrapleura tetraptera (Schaum. & Thonn.) Taubert belongs to the family *Magnoliopsida: Rosidae: Fabales: Leguminosae*. It is synonymous with *Tetrapleura thonningii* Benth. (International Centre for Agroforestry [ICRAF], 2002) and *Adenanthera tetraptera* Schaum & Thonn. Its common names are: African Porridge Plant, Aidan/Aridan Tree (English).

Activity-directed fractionation of *T. tetraptera* pod pulp yielded scopoletin (Burkill, 1985), phenolic acids, fatty acids and soluble amino acids, aridanin (Adesina *et al.*, 1980), hentriacontane (Cole & Ross, 1977), carbohydrate residues, phenylpropanoids such as ferulic acid (Kyei and Allman, 2001), caffeic acid (Adelaja and Fasidi, 2012) and P-coumaric acid (Gmelin & Larsson, 1967); saponins (Adesina & Reisch, 1985). Mature pod pulp was reported to contain flavonoids such as 2', 4, 4' – trihydroxychalcone (isoliquiritigenin) (Maillard *et al.*, 1992), 2', 3, 4, 4' - tetrahydroxychalcone (butein) (Ngassapa *et al.*, 1993) and 4', 5, 7-trihydroxyflavanone (naringenin) (Fleischer *et al.*, 2006). Stem bark contained triterpene saponins (Note' *et al.*, 2009), oleanone-type saponins. Toxic constituents were also found: oxalate (8.14-16.6 mg/100g), tannin (16.5-35.7mg/100g), hydrogen cyanide (98-100mg/100g) and ascorbic acid (68mg/100g) (Dosunmu, 1997). Akin-Idowu *et al.* (2011) reported the phytochemicals in mg/100g dry weight of pod as: total polyphenol (38.05-2907.15), flavonoid (10.3-410.75), saponin (60.80-953.4), tannin (135.5-1097.5) and phytate (1021.0-5170.0). The need to assess and document the influence of the dry pod pulp meal of *Tetrapleura tetraptera* on the haematology and serum metabolites of pubertal boars informed this work.

2.0 Materials and Methods

2.1 Study Location

The research was carried out at the Piggery Unit of the Livestock Teaching and Research Farm, Michael Okpara University of Agriculture, Umudike, Abia State. Umudike lies on co-ordinates 05°29' N and 07° 33'E, and an altitude of about 122m above sea level. The average annual rainfall ranges from 1700 to 2100 mm. Minimum and maximum temperature are in the ranges 18-23° C and 26-36° C, respectively; while relative humidity is 57-91% (National Root Crops Research Institute [NRCRI], 2017).

2.2 Sourcing the Pods and Preparation of the Pulp meal

The pods were bought from New Market, Aba, Abia State. The two soft ‘wings’ of dry pods were peeled off with a sharp kitchen knife and subsequently crushed with a hammer mill for easy mixing with the other feed ingredients. The resulting meal was packed in air-tight bags until needed for addition to the pigs’ basal diets. *Tetrapleura tetraptera* pod pulp meal (TPM) was added as a spice at 0.0% (control), 2.50% and 5.0%, respectively, per kg of basal diet.

Table 1: Diet Composition for the Experimental Pubertal Boars fed TPM

Ingredients	Treatment		
	0.0% TPM	2.5% TPM	5.0% TPM
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
TPM (%)	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). TPM = *Tetrapleura tetraptera* pod pulp meal

2.3 Experimental Animals and Management

Crossbred (Large White x Duroc) 6-week old weaner boars weighing 9.47kg on the average were used for the experiment. They were stocked, allowed 2 week-quarantine period during which they were administered Ivermectin injectable solution (1%) via the subcutaneous route at 0.25ml/12.5 kg body weight, against internal and external parasites. The boars were tagged and systematically divided into three treatment groups of six animals. Each treatment was replicated thrice, two boars constituting a replicate in a completely randomized design. They were reared in pens with hard and non-slippery concrete floor. The pens were cleaned daily throughout the experiment.

2.4 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 while water was supplied *ad libitum*.

2.5 Blood Sampling and Analyses

At 22 weeks of age, two milliliters of blood samples each were taken from the jugular vein of the boars for haematology and serum chemistry studies, respectively. Samples for haematological studies were collected in bottles treated with ethylene diamine tetra-acetic acid (EDTA)-an anticoagulant, while those for serum chemistry were collected in EDTA-free bottles to allow for clotting and harvesting of sera.

Haematological indices were determined according to Jain (1986); while the serum chemistry indices were determined according to Schmidt and Schmidt (1963). Red blood cell osmotic fragility was tested according to Adenkola & Oluremi (2014). Total cholesterol, high-density lipoprotein (HDL) and triglycerides were determined using Randox commercial kit (Randox Co. UK.), while low-density lipoprotein (LDL) and very-low density lipoprotein (VLDL) were calculated using Friedwald equations. (Friedwald *et al.* 1972) as follows:

$$\text{LDL (mg/dl)} = \text{Total cholesterol (mg/dl)} - \frac{\text{Triglycerides (mg/dl)}}{5} - \text{HDL (mg/dl)}$$

$$\text{Very low-density lipoprotein (VLDL) (mg/dl) was calculated as: } \frac{\text{Triglycerides (mg/dl)}}{5}$$

2.6 Data Analysis

All data sets generated from the study were subjected to one-way analysis of variance (ANOVA) procedure and significantly different means separated using Fisher's least significant difference (F-LSD), using Statistical Package for Social Sciences (SPSS) version 20.

3.0 Results and Discussion

The haematological indices of pubertal boars fed *Tetrapleura tetraptera* pod pulp meal (TPM) are presented in Table 2.

Table 2: Haematological Indices of Pubertal Boars fed TPM

Index	Treatment			SEM
	0.0% TPM	2.5% TPM	5.0% TPM	
Red blood cells (x 10 ¹² /l)	7.42 ^b	4.46 ^c	8.05 ^a	0.55
Packed cell volume (%)	42.19 ^c	44.43 ^b	45.27 ^a	0.46
Haemoglobin (g/l)	11.56 ^c	11.83 ^b	12.47 ^a	0.14
White blood cells (x 10 ⁹ /l)	14.72 ^c	15.85 ^b	16.02 ^a	0.21
Platelets (x 10 ⁹ /l)	215.00 ^a	206.00 ^b	206.33 ^b	1.52
MCV(fl)	56.86 ^b	57.53 ^a	56.37 ^c	0.17
MCH (pg)	15.58 ^b	15.22 ^c	15.87 ^a	0.14
MCHC (g/l)	27.43 ^a	26.90 ^b	27.54 ^a	0.14

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

SEM = Standard error of the mean, TPM= *Tetrapleura tetraptera* pod pulp meal

MCV = Mean cell volume, MCH = Mean cell haemoglobin, MCHC = Mean cell haemoglobin concentration

Erythrocyte count was significantly higher (P<0.05) in boars fed 5% TPM than in those fed control, while packed cell volume, haemoglobin concentration and white blood cell counts increased significantly with increase in addition of TPM; while platelets were more abundant (P<0.05) in boars fed control than in their TPM-treated counterparts. There were significant but irregular changes in the red blood cell indices.

The values of haematological indices obtained in this study fell within the reference values documented by (Thorn, 2000) for erythrocytes (5-8 x 10⁶ /μL), packed cell volume (32-50 %), haemoglobin (10-16 g/dl), leucocyte count (11-22 x 10³ /μL) and mean corpuscular volume (50-68fl). Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were however, slightly lower than the reference values (17-21 g/dl) and (30-34g/), respectively, reported by the same author. Erythrocyte count, packed cell volume, haemoglobin concentration and leucocyte count obtained in this work were in line with the values reported for 25-week old Large White boars by Aladi *et al.* (2008).

Significantly higher RBC, PCV and Hb concentration in the pubertal boars fed 5% APM could mean that the bioactive components of the pod meal acted to stimulate and optimize the erythropoietic processes. The affinity of haemoglobin for oxygen has been documented (Balcerek *et al.*, 2020). Boars fed 5% APM had better/more efficient oxygen transport as a result of higher concentration of haemoglobin in their blood. This would have also conferred on them a health

advantage over their peers; which might have contributed to the better growth performance (Ezea, 2019). Lowered platelet count could mean a compromised clotting factor. This implies that any routine operation that might have involved an inversion of the blood would probably have resulted in haemorrhage. Table 3 shows the influence of *Tetrapleura tetraptera* pod pulp meal (TPM) on some serum chemistry parameters of the pubertal boars.

Table 3: Serum Chemistry Indices of Pubertal Boars fed TPM.

Index	Treatment			SEM
	0.0% TPM	2.5% TPM	5.0% TPM	
Glucose (mg/dl)	64.34 ^b	65.00 ^b	69.33 ^a	0.78
Albumin (mg/dl)	2.72 ^c	3.28 ^b	3.75 ^a	0.15
Globulin (mg/dl)	3.72 ^a	3.52 ^b	3.40 ^c	0.05
Total protein (mg/dl)	6.44 ^c	6.80 ^b	7.15 ^a	0.10
AST(u/l)	53.00 ^a	48.00 ^b	35.00 ^c	2.71
ALT(u/l)	32.00 ^a	30.00 ^b	25.00 ^c	1.06
Urea(mg/dl)	20.61 ^b	23.92 ^a	18.44 ^c	0.80
Creatinine(mg/dl)	1.55 ^a	1.38 ^b	0.88 ^c	0.10

^{a-c} Means on the same row with different superscripts are significantly ($P<0.05$) different.

SEM = standard error of the mean, TPM= *Tetrapleura tetraptera* pod pulp meal

AST = Aspartate aminotransferase, ALT = Alanine aminotransferase

Boars fed 5% TPM had more ($P<0.05$) serum glucose than those in other treatments. Total protein and albumin increased significantly ($P<0.05$) with increase in TPM across the treatments, while globulin was reduced progressively ($P<0.05$) with increase in TPM. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine were reduced ($P<0.05$) with increase in TPM across the treatments. The lowest ($P<0.05$) serum urea was obtained at 5% TPM. All the indices fell within the normal ranges submitted for pigs by (Research Animal Resources [RAR], 2009); except total protein, which was rather lower and ALT, which on the other hand was higher than the reference ranges. Protein quantity and quality are implicated in a case of low serum total protein and albumin (Murray *et al.*, 2000). High levels of ALT are attributed to necrosis of the hepatocytes, myocardial and skeletal muscle cells (Uthman, 2002).

Serum glucose was reduced significantly at the pre-pubertal phase of the boars (Ezea, 2019). At the pubertal stage however, serum glucose increased with increasing percentage addition of TPM. The trend in serum glucose level might have been age-related. It could be that the older the boars the more endowed they were to counter/suppress the hypoglycaemic effect of TPM (Dunshea & D'Souza, 2003). Another reason could be that the boars' systems might have mobilized more glucose from glycogenic amino acids (Berg *et al.*, 2002), since there was also a significant increase in serum total protein.

Increased serum total protein might be a pointer to better protein quality enhanced by addition of TPM to the feed mixture. Also, TPM addition might have encouraged secretion of proteolytic

enzymes-trypsin and chymotrypsin for better protein digestion (Srinivasan, 2005). Significant reduction in serum urea at 5% TPM could point to better quality feed as well as enhanced glomerular filtration rate, an index of renal efficiency. Reduced creatinine in a dose-dependent manner in this case cannot be linked to muscle mass since the boars fed TPM had more muscle mass than those fed control, as evidenced from the growth performance. It might therefore, be connected with the feed quality as imparted by TPM addition; which in turn would have enhanced more efficient renal function. On the other hand, however, reduced creatinine in serum is seen in conditions such as glomerulonephritis, acute tubular necrosis, congestive heart failure, polycystic kidney disease, shock and dehydration (Shivaraj *et al.*, 2010). The lipid profile of the experimental pubertal boars is presented in Table 4.

Table 4: Lipid Profile (mg/dl) of Pubertal Boars fed TPM

Index (mg/dl)	Treatment			SEM
	0.0% TPM	2.5% TPM	5.0% TPM	
HDL	50.93 ^b	79.75 ^a	52.30 ^b	4.69
LDL	7.35 ^b	6.68 ^c	7.94 ^a	0.21
VLDL	28.53 ^a	26.49 ^b	25.47 ^c	0.45
Triglycerides	142.65 ^a	132.43 ^b	127.28 ^c	2.26
Total cholesterol	86.81 ^b	112.92 ^a	85.71 ^b	4.43

^{a-c} Means on the same row with different superscripts are significantly ($P < 0.05$) different.

SEM = standard error of the mean, TPM= *Tetrapleura tetraptera* pod pulp meal

HDL = High-density lipoprotein, LDL = Low-density lipoprotein, VLDL = Very low-density lipoprotein

There were significant but irregular alterations in some of the serum lipid components. Low-density lipoprotein (LDL) was highest ($P < 0.05$) in boars fed 5.0% TPM. A possible explanation here is that the livers of the boars fed 5.00% TPM might have mobilized more cholesterol to the extra-hepatic tissues (coronary tissue and cerebral arteries) (Beitz, 2004) than in the rest of the treatments. Very low-density lipoprotein and triglycerides were reduced ($P < 0.05$) by application of TPM in the feed of the boars. Reduction in very low-density lipoprotein could mean that TPM addition moderated the transport of triglycerides to the extra-hepatic tissues (Ekor *et al.*, 2010). The triglyceride values in the TPM-treated boars seem to confirm the findings of Kuate *et al.* (2015), who observed hypo-lipidaemia in rats treated with hydro-ethanolic extract of Aidan pod pulp. TPM might have interfered with fat storage and perhaps lipid metabolism in the growing-finishing boars.

The significant reduction in serum triglycerides at the pubertal phase is of striking significance and implication. Being the storage form of lipids, triglycerides is found under the skin, around organs and muscles and within muscle fibres. For farmers whose interest is raising pigs from weaner to slaughter stage with minimal fat in carcass, TPM can be a veritable additive to reduce

fat accumulation. For boars that are meant for breeding, TPM may still be added to their feeds, though with utmost caution, considering its potential impact on their reproductive integrity.

4.0 Conclusion and Recommendations

It could be inferred from the foregoing that *Tetrapleura tetraptera* pod pulp meal (TPM) enhanced the haematology and serum protein; and moderated the liver and kidney metabolites, indicating a positive influence on hepatic and renal efficiencies. TPM should be added at 2.5% to growing-finishing pigs for improved haematopoiesis. Up to 5% of TPM could be added to pre-slaughter pig diets for low subcutaneous fat deposition as this is desirable for production of lean pork. Up to 5% of TPM could be added to pre-slaughter pig diets for enhanced hepatic and renal functions.

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