

## Liver enzymes and histo-morphology of pigs fed fermented and enzyme-supplemented cassava peels meal based diets

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**Target audience :** Animal Scientists, Veterinary Doctors, Students and Farmers.

### Abstract

Fresh cassava peels were collected, fermented for four days and sundried for 3-5 days. It was ground and used to replace maize in the grower and finisher diets. A group of 27 weaner gilts (Largewhite x Duroc), aged 8 weeks and weighing  $10.61 \pm 0.27$ kg were fed the diets. The weaner gilts were allotted to three treatments comprising T<sub>1</sub> (control), T<sub>2</sub> (fermented CPM) and T<sub>3</sub> (fermented CPM + maxigrain<sup>R</sup> enzyme) in a completely randomized design. The diets were given at 4% of their live body weights daily throughout the experiment. At week 28 of age, each was bled of 5mL fresh blood via venipuncture and serum was harvested for serum analyses for alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Aspartate aminotransferase (AST). Data were analysed using one way analysis of variance and where difference in means existed, it was separated with Duncan's Multiple Range Test. Results showed that the ALT of pigs on T<sub>2</sub> diet was significantly different [ $p < 0.05$ ] from those on T<sub>3</sub> and T<sub>1</sub> whereas the ALP of T<sub>3</sub> pigs was significantly different [ $p < 0.05$ ] from those of T<sub>1</sub> and T<sub>2</sub>. However, the AST of the pigs had no significant difference [ $p > 0.05$ ] among all the treatment groups. There were normal liver histo-architectures of pigs in all the treatment groups. Therefore, fermented cassava peels with or without enzyme supplementation has no deleterious effect on the liver serum enzymes and histo-architecture of pigs. Fermentation of cassava peels is therefore recommended to pig farmers as substitute for energy ingredient in the diet.

**Keywords:** Pigs, Fermented cassava peels, maxigrain<sup>R</sup> enzyme, Liver enzymes, Histo-morphology

### Description of problem

Necessity of nutrition for continuance of life points to food as critical substance that must be maintained free from dangerous levels of toxicants (1). The high demand for cereals due to increasing human population and their use by millers for compounding livestock feeds coupled with the need for livestock products have led to the use of unconventional feeds for animal production (2). These unconventional feed materials include

sorghum, spent grains and wheat offals (by-product of sorghum and wheat malting respectively) as well as cassava (3; 4). As a result of the increasing use of cassava in animal feeding there is greater exposure to dietary toxins from cyanogenic glycosides. It is generally accepted that the toxicity of cyanogenic glycosides is due entirely to the release of free cyanide (5).

Two types of cyanide toxicity have been recognized in human and animals: Acute

toxicity and chronic toxicity (6). The mechanism of acute toxicity is well understood (7; 8). In animal tissues, cyanide forms a stable coordination complex with ferric ion and as a result tends to keep this metal in the higher oxidation state ( $\text{Fe}^{3+}$ ). This reduces the efficacy of iron as an electron carrier in the electron transport chain, thus inhibiting cellular respiration. This explains why acute cyanide toxicity symptoms depend upon the rate of release of free cyanide into the animal tissue (9; 10). By contrast, the regular intake of small amount of cyanide in the diet does not result in death, but it is known to be responsible for the pathogenesis of several diseases, such as goitre (11; 10) renal problems (12; 13; 7), reproductive problems (12) and several neurological disorders (14).

Cyanogenic glycosides are phytotoxins which occur in at least 2000 plant species, of which a number of species are used as food in some areas of the world. Cassava and sorghum are especially important staple foods containing cyanogenic glycosides (15; 16; 17; 18, 19; 20). The potential toxicity of a cyanogenic plant depends primarily on the potential that its consumption will produce a concentration of HCN that is toxic to exposed animals or humans. Cyanogenic glycosides taken up intact with the food are partly hydrolyzed by the  $\beta$ -glucosidase activity of the bacteria of the gut flora of animals or humans (15; 16; 18; 19; 17; 20; 21). Also Obioha *et al.* (22) working on growing-finishing pigs reported that there was a progressive decline in average daily gain, feed efficiency and protein efficiency ratio from the zero CPM diet to the zero maize diet, but these comparisons were not significant. Liver weight and spleen weight (expressed as percentage of body weight) were slightly higher in the CPM diets than the control. Ikurior and Onuh (23) observed that daily gains of growing pigs fed cassava peel declined significantly ( $p < 0.05$ ) as level of inclusion increased.

When certain types of cells are damaged, they may leak enzymes into the blood, where they can be measured as indicators of cell damage. Aspartate aminotransferase (AST) is one of such enzymes (6). It is found in many tissues including the heart, muscle, kidney, brain, and lung. The amount of AST in the blood is directly related to the extent of tissue damage (24). Alanine aminotransferase (ALT) is produced mainly in the liver, and small amounts are found in the heart, muscle, and kidney. ALT catalyses the transfer of amino groups between L-alanine and glutamate to meet physiological needs (25). Measurement of ALT or ALT and AST helps in the diagnosis of cardiac or liver disease. Although serum levels of both ALT and AST become elevated whenever disease processes affect liver cells, ALT is the more liver-specific enzyme (26). Increase in the levels of ALT or AST or both may occur in situations of common bile duct stone e.g. transient biliary obstruction; medications e.g. acetaminophen; common liver disease causes e.g. alcohol abuse, cirrhosis, hepatotoxins, viral hepatitis, steatohepatitis (fatty liver); uncommon liver diseases e.g. autoimmune hepatitis. Alkaline phosphatase (ALP) is made mostly in the liver and by bone forming cells called osteoblasts with some made in the intestines and kidneys. The liver makes more ALP than other organs or bones and ALP test is used to detect liver or bone disorders (27; 28). All these form the basis of this present study to assess if it could negatively affect the seral biochemistry or histo-architecture of the liver.

## **Materials and methods**

### **Experimental site**

The experiment was carried out at the piggery unit of The Research Farm of The Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Oyo State, Nigeria. Ibadan is geographically located at latitude  $7^{\circ} 22' 39''$  N

and longitude 3° 54' 21" E. Ibadan has a tropical wet and dry climate, with a lengthy wet season. It has mean total rainfall of 1420.06 mm, mean maximum and minimum temperatures of 26.46 °C and 21.42 °C respectively and relative humidity of 74.55%.

### Source of ingredients, preparation and feed formulation

Fresh cassava peels for this experiment was sourced from Orile-Ilegun; an industrial layout in Ibadan, Oyo State, Nigeria. The maxigrain<sup>R</sup> enzyme was sourced from open market and has the following constituents: Amylase, xylanase, Beta-glucanase, cellulose, pectinase, protease, phytase and lipase. The fresh cassava peels which had not stayed for longer than 4-6 hours since peeled off were washed and immersed in clean bore-hole water in a plastic container (vat) and left at an ambient temperature of 26-30 °C for four days. Sign of fermentation which included foaming was looked out for. After four days, the cassava peels were separated from the broth and spread on a clean polythene sheet under the full glare of the sunlight for 3-5 days during which it dried to constant weight (29; 30). It was then used for onward compounding of the experimental diets thus:-

T<sub>1</sub> = Conventional maize-based diet (control).

T<sub>2</sub> = Diet with 40% maize-replaced fermented cassava peels.

T<sub>3</sub> = Diet with 40% maize-replaced fermented cassava peels supplemented with maxigrain<sup>R</sup> enzyme.

### Experimental animals, design and management

A group of 27 female weaner pigs (Largewhite x Duroc), aged 8 weeks each weighing 10.61±0.27kg, with good body conformation and having at least six pairs of teats, were used for this experiment. Measurement and recording of their body weights were carried out using weighing

balance. They were allotted to the above treatments using completely randomized design. Each treatment was replicated thrice. Close observations for deformity and other aberrations that could render them unfit for the experiment were looked out for and replacements made in their eventuality. The pigs were also prophylactically taken care of against endo- and ectoparasites using subcutaneous injection of ivermectin at 1mL/33kg body weight. Long acting oxytetracycline injection was also administered at 1ml/10kg body weight (i/m) which was repeated after 72 hours to help eliminate possible pathogenic microbes. Grower diet (Table 1) was introduced at 4% of pig's body weight daily (31; 32). The grower diets were given for the first eleven weeks after which they were replaced with finisher diet (Table 2) till the end of the experiment. Clean drinking water sourced from the borehole in the farm was supplied *ad-libitum* to the pigs.

### Data collection

Blood collection and analysis:- Blood was aseptically collected at week 28 of age for serum biochemical analysis. The bleeding was done in the morning before feeding after sterilizing the skin with methylated spirit. Using a sterile 5mL syringe and 21 gauge needle 5mL of whole blood was collected and dispensed into test tubes without anticoagulant and left for 10 minutes to coagulate. The supernatant serum decanted into sterile Bijoh bottles for the determination of ALT, AST and ALP. These serum metabolites were determined using diagnostic kits (Quimica Clinica Applicada, S. A. Spain). Alanine and aspartate aminotransferases were determined based on the colorimetric measurement of hydrazone formed with 2, 4 dinitrophenyl hydrazine (33) and ALP by phenolphthalein monophosphate method (34). Three pigs were selected (one from each treatment) and

slaughtered and the livers carefully dissected out for the histo-morphology preparation using the method of Humason (35).

### Statistical analysis

Data were subjected to analysis of variance (ANOVA). All means were separated ( $p>0.05$ ) and compared using Duncan's Multiple Range Test (36).

## Results and Discussion

### Liver serum enzymes and histo-architecture

Table 3 shows the liver serum of non-gravid pigs fed enzyme supplemented CPM-based diets. The ALT of pigs on  $T_2$  diet was significantly different [ $p<0.05$ ] from those on  $T_3$  and  $T_1$  whereas the ALP of  $T_3$  pigs was significantly different [ $p<0.05$ ] from those of  $T_1$  and  $T_2$ . However, the AST of the pigs had no significant difference [ $p>0.05$ ] among all the treatment groups. The liver serum enzymes particularly the transaminases – ALT and AST, are indicators of liver damage when they rise [37]. The treatment [ $T_3$ ] with maxigrain<sup>R</sup> enzyme supplementation demonstrated the highest values with respect to ALP and AST whereas ALT value was highest in  $T_2$  pigs and could be as a result of mild effect of HCN in the liver of pigs in this group. All the liver serum enzymes fell within normal ranges for pigs [38; 39]. The higher AST values in  $T_3$  and  $T_1$  could be due to increase in muscular activity of the pigs due to availability of more energy and protein in them. Increase in ALT is mostly found due to leaking damaged liver cells [40]. The lowest value of ALT was however recorded against  $T_3$  and could be due to the detoxifying action of the cocktail enzyme on HCN and other anti-nutrients as well as possible regulatory activity of the liver. There was, therefore, no impairment of the liver possibly because all the values fell within the normal ranges. The results of the liver function test:- AST, ALT and ALP, were not enough to draw conclusions on the status of

the liver. Hammad [41] in a research concluded that oral administration of whey proteins declined the values of ALT and AST in rats with fatty liver. Liver enzymes' levels are usually raised in acute hepatotoxicity but tend to decrease with prolonged intoxication due to damage to the liver [42]. The ALP specifically rises in cholestatic disease and necrosis due to secondary intra-hepatic biliary obstructions and as part of the nodular regeneration process [43]. But in this study, it was within the normal range [39], suggesting no biliary obstruction or cholestasis. Figures 1, 2 and 3 suggested no hepatic lesions and this is in consonance with the report of Nnoli *et al.* (44) that other organs and small intestine showed no pathological changes in acute cyanide poisoning of a man.

### Conclusions and Applications

1. The biochemical effects of cyanide from fermented and enzyme supplemented cassava peels on the activities of the transaminases and alkaline phosphatase were not detrimental to the pigs using serum liver enzymes and liver histo-architectures as reference parameters. This, therefore, establishes that fermented cassava peels can be used as non conventional source of energy in the feed of monogastrics particularly pigs, without detrimental consequences.
2. Fermented and enzyme supplemented cassava peels will serve to minimize cost of feed and by extension increase profit in animal enterprise.
3. Further work should focus on growth, reproduction, effect on other visceral organs, and blood profile of pigs and other monogastric animals

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**Table 1:- Dietary composition of pig's grower diets**

	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Maize	40.00	-	-
CPM	-	40.00	40.00
PKC	20.00	29.50	29.50
BDG	14.00	10.00	10.00
GNC	12.50	11.00	11.00
BLM	5.00	5.00	5.00
Palm oil	4.00	4.00	4.00
Bone meal	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00
Methionine	0.20	0.20	0.20
Lysine	0.75	0.75	0.75
Premix	0.40	0.40	0.40
Salt	0.15	0.15	0.15
Total	100	100	100
C.P. (%)	20.82	20.47	20.47
ME (kcal/kg)	2871.50	2759.23	2759.23

**Table 2:- Dietary composition of pig's finisher diets**

	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Maize	40.00	-	-
CPM	-	40.00	40.00
PKC	22.50	23.50	23.50
BDG	10.00	10.00	10.00
W/O	14	9.00	9.00
SBM	-	4.00	4.00
BLM	5.00	5.00	5.00
Palm oil	4.00	4.00	4.00
Bone meal	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00
Methionine	0.20	0.20	0.20
Lysine	0.75	0.75	0.75
Premix	0.40	0.40	0.40
Salt	0.15	0.15	0.15
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>
C.P. (%)	17.02	17.36	17.36
ME (kcal/kg)	2822.88	2710.23	2710.23

Provided the following/kg diet: Vitamin A-8,000 IU, Vitamins D3 -3,000 IU, Vitamins E-8 IU, Vitamin K -2mg, Vitamin B1- 1 mg, Vitamin B2-0.2 mg, Vitamin B12-5 mg, Nicotinamide -10 mg, Selenium- 0.1 mg, Ca Pantothenate - 5 mg, Folic acid -0.5 mg, Choline Chloride -150 mg, Iron -20 mg, Manganese -80 mg, Copper -8 mg, Zinc -50 mg, Cobalt -0.225mg, Iodine -2 mg Antioxidant - 0.1ppm

Key:- CPM = Cassava peels meal, PKC = Palm kernel cake, W/O = Wheat offal, BDG = Brewer's dried grain, SBM = Soybean meal, BLM = Blood meal, C.P. = Crude protein, ME = Metabolizable energy.

**Table 3: Liver serum biochemical parameters of non -gravid pigs fed with CPM-based diets**

Parameter	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	* Normal
ALT (µL)	30.67±1.20 <sup>ab</sup>	35.33±1.86 <sup>a</sup>	27.00±2.52 <sup>b</sup>	21.7 -46.5
ALP (µL)	47.33±2.40 <sup>b</sup>	58.67±3.38 <sup>b</sup>	117.00±14.16 <sup>a</sup>	41 -176.1
AST (µL)	37.67±2.73	36.33±0.67	41.67±1.76	15.3 -55.3

ab:- means on the same row with different superscripts are statistically different (p<0.05)

\* Merck's Manual (1998).



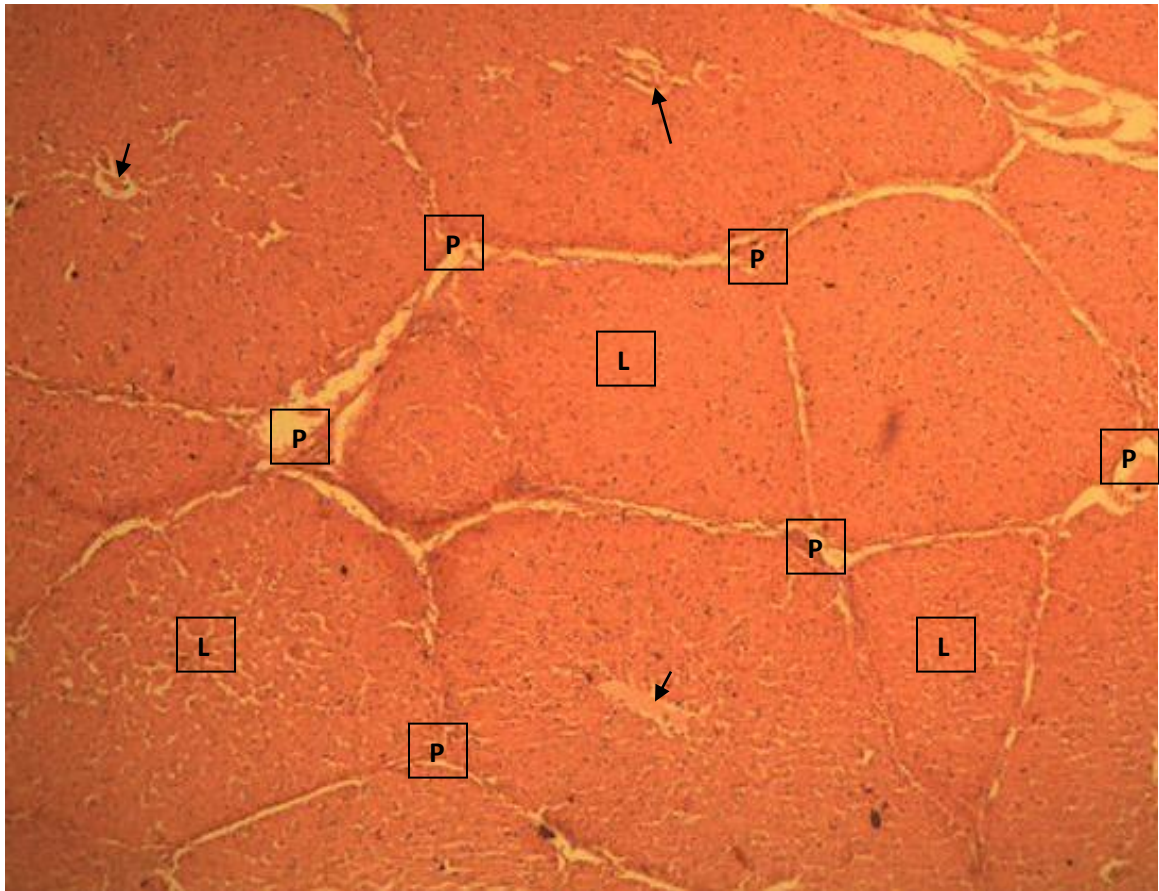


Figure 1: T<sub>1</sub> liver: Sections of the swine liver showing the normal hepatic histo-architecture. It shows a well demarcated lobulation of the hepatic lobules (L), each made up of hepatocytes arranged in radiating manner around the central veins (arrow). At the periphery of the lobules are the portal triads (P). H&Ex40

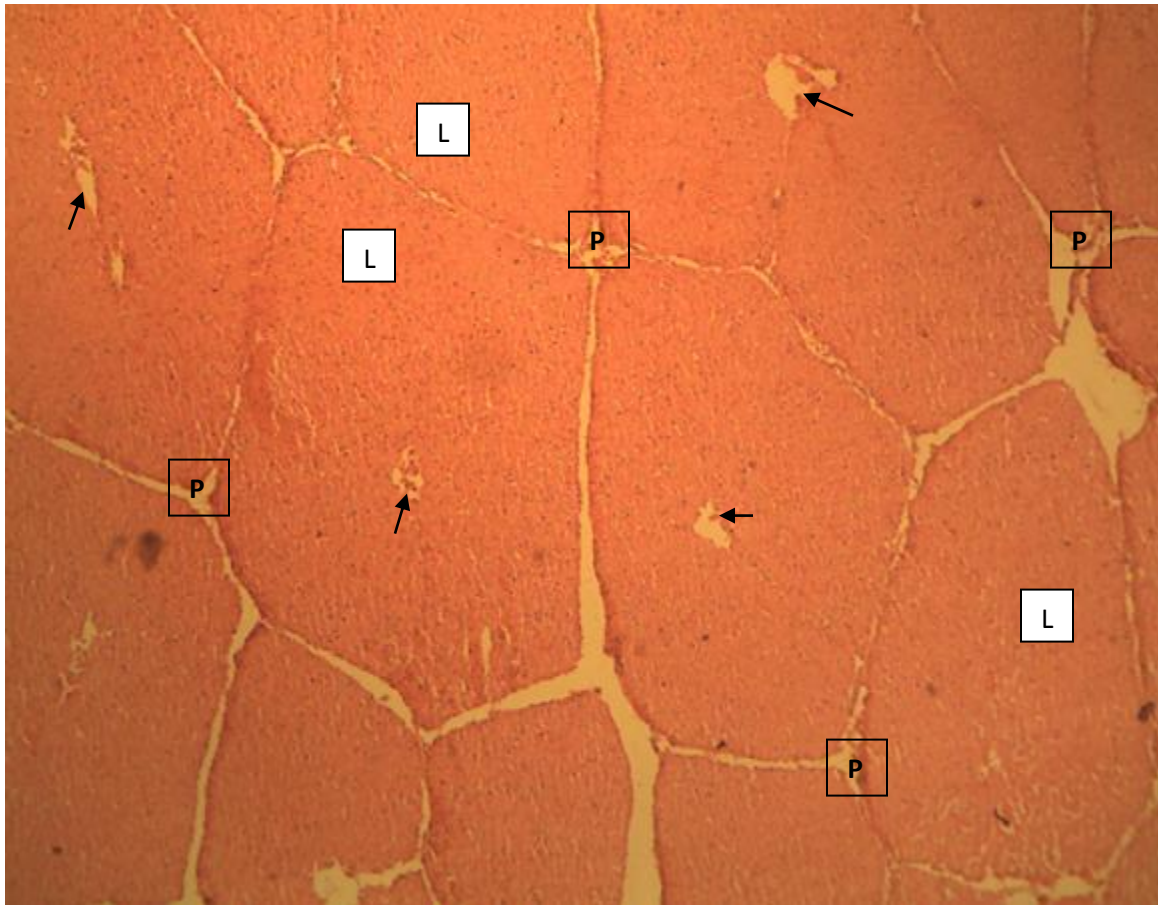


Figure 2: T<sub>2</sub> liver: Sections of the swine liver showing normal histo-architecture. It shows a well demarcated lobulation of the hepatic lobules (L), each made up of hepatocytes arranged in radiating manner around the central veins (arrow). At the periphery of the lobules are the portal triads (P).

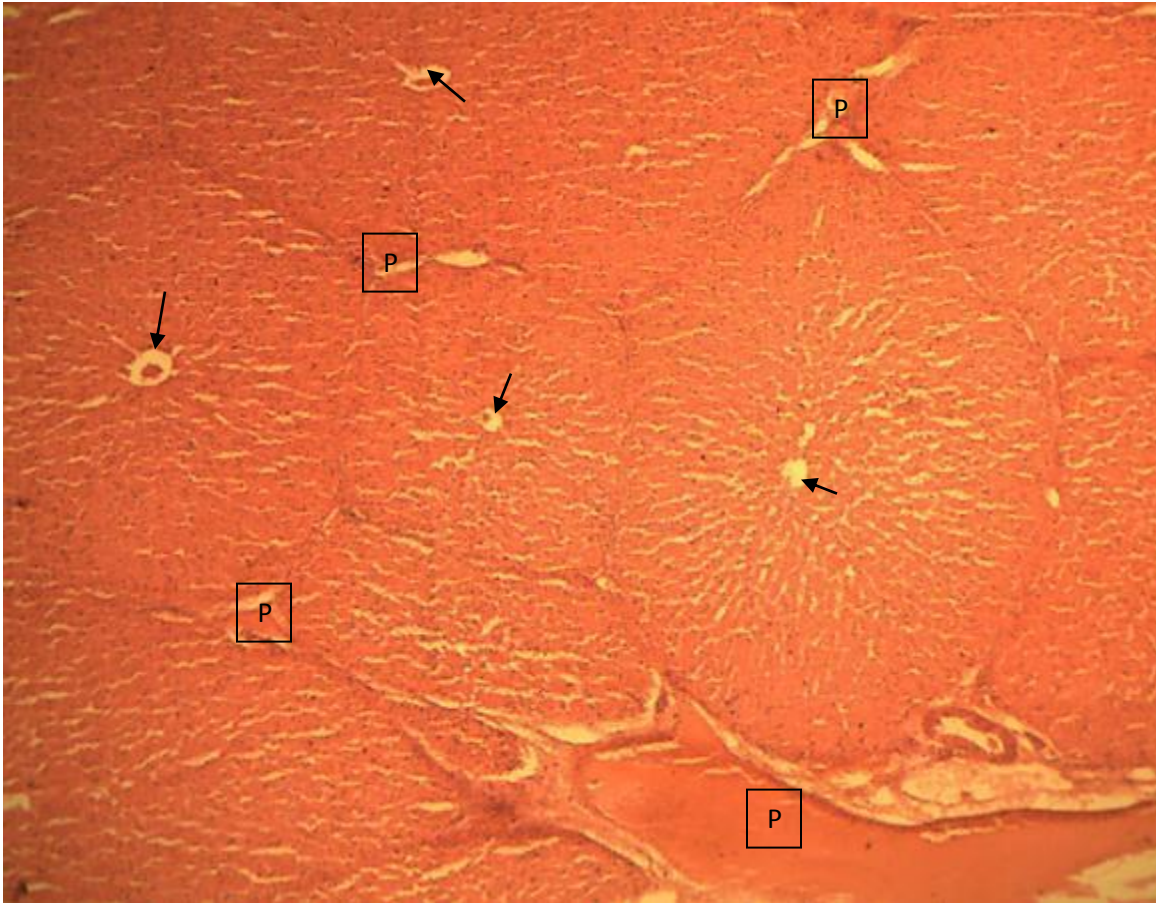


Figure 3: T3 liver: Sections of the swine liver showing the normal hepatic histo-architecture. It shows a well demarcated lobulation of the hepatic lobules, each made up of hepatocytes arranged in radiating manner around the central veins (arrow). At the periphery of the lobules are the portal triads (P).