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A Gate Way to Food Security in Africa

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64 GROWTH PARAMETERS OF RATS FED VARYING RATIONS OF EDIBLE CHITOSAN-STARCH FILMS PACKAGING

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Abstract

This study aimed at assessing the growth parameters of rats fed varying rations of edible chitosan-starch films supplemented diets. Commercial rat diet was supplemented with 5 – 40 % rations of chitosan-starch films. Twenty-five rats were randomised into five groups and fed the supplemented diets for a period of 28 days. Proximate composition of chitosan, edible chitosan-starch films and chitosan-starch film supplemented diets were determined using the AOAC methods. Weekly feed intake, weekly weight-gain and organ weight ratios were determined. Histopathology of the liver, kidney and intestine were carried out. Chitosan was significantly ($p<0.05$) lower in all proximate components except for carbohydrate and fibre, chitosan-starch film was significantly higher in moisture, fat and ash compared to the supplemented diet and the control. Rats placed on 5 % edible chitosan-starch film had the highest feed intake and weight gain while those placed on 40 % had the least. The kidney and intestine body-weight ratios were significantly ($p<0.05$) lower in the rats on the supplemented diets compared to the control, not different in the liver body-weight ratio. The liver showed normal intact hepatic cells, while the kidney and intestine showed degeneration of the glomeruli, capsular space, and connective tissue inflammation in rats fed diets above 5 % of edible chitosan-starch film. This study suggests that edible chitosan-starch films may be toxic to albino rats at levels higher than 5 % diet inclusion.

INTRODUCTION

Chitosan-based edible films are increasingly being used for food packaging. Chitin is converted into chitosan after de-acetylation. It is a D-glucosamine and N-Acetyl-D-glucosamine linear amino polysaccharide, and its solubility in acetic and hydrochloric acids helps it form films (Mitelut *et al*., 2015). Chitosan-based films are produced from a combination of lipids, proteins, polysaccharides, and proteins (Han, 2014), appropriate modifications to their features enhances their antibacterial, barrier, antioxidant, mechanical, optical, and thermal stability qualities. Edible chitosan films are commonly considered as a biodegradable, renewable polymers and are discovered to be digested and biodegradable with outsounding preservation properties, they are used to package a variety of foods like; meat, fruit, and vegetables. Although edible films are produced from natural resources the risk of prolonged usage is unknown (Douglas *et al*., 2015), there is however a dearth of literature on the histoarchitectural organ changes of rats fed edible chitosan-starch film supplemented diets.

OBJECTIVES

The objectives are:

- determine the proximate compositions of chitosan, edible chitosan-starch films and edible chitosan-starch film supplemented diets
- determine the feed intake and weight-gain of albino rats fed edible chitosan-starch film supplemented diets
- determine the organ-weight ratio of the liver, kidney and intestine of albino rats fed edible chitosan starch film supplemented diets
- determine the histopathology of the liver, kidney and small intestine of albino rats fed edible chitosan-starch films

MATERIALS AND METHODS

Source of Material

Chitosan was purchased from Beijing, Wisapple Biotech, Co., Ltd. Cassava was sourced from the Ultra-Modern Market Minna, Niger State, Nigeria

Experimental animals

Albino rats weighing between 80-100 g were purchased from Ahmadu Bello University animal house in Zaria, Nigeria. This experiment was approved by the Ethical Committee of the Federal University of Technology, Minna with Assign number 000033.

Starch extraction

Starch extraction from cassava tubers was carried out using the methods of Kaur et al., (2016) without modification.

Preparation of edible chitosan-starch films

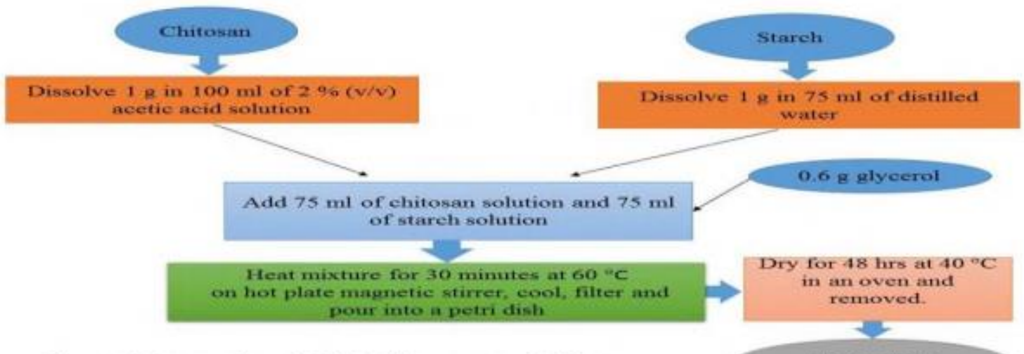


Figure 1: Preparation of Edible Chitosan-starch Films.
Source: (Ossamulu *et al.,* 2019).

Proximate composition

Proximate composition of chitosan, edible chitosan-starch film and chitosan-starch film supplemented diets were determined by the methods described by AOAC, (2012).

Histopathology

Histopathology was carried out as described by Fadia *et al*. (2022)

Data Analysis

All analyses in triplicate and were subjected to analysis of variance (ANOVA) followed by post-hoc Duncan test for comparison of the mean using SPSS version 23.0.

Results and Discussion

The proximate compositions of chitosan, edible chitosan-starch film and chitosan-starch film supplemented diets are shown in Table 1. The moisture content of edible chitosan-starch film was significantly ($P<0.05$) higher than the moisture content in chitosan. The supplemented diets were significantly ($P<0.05$) high in moisture content than the control diet. The higher the film supplemented in the diets, the higher the moisture content, the same trend was observed for ash and lipid content. However, a different pattern was observed for fibre, protein and carbohydrate contents. The higher the film supplemented in the diets the lower the fibre, protein and carbohydrate contents.

Figure 2 shows the feed intake by albino rats, the feed intake of the experimental rat groups and that of the control were not significantly different (P>0.05) at the first week, although there was a significant difference (P<0.05) in the feed intake at week 2, 3 and 4 of albino rats placed on the control and supplemented diets. Group B rats placed on 5 % film had the highest feed intake in all the weeks while group E rats placed on 40 % edible film had the least feed intake.

Figure 3 shows the body weight-gain, there was a significant change (P<0.05) in the weekly body weight-gain in the rats placed on the supplemented diets compared to those fed the control diets. Group B rats fed 5% edible chitostan-starch film had the highest body weight-gain in all the weeks.

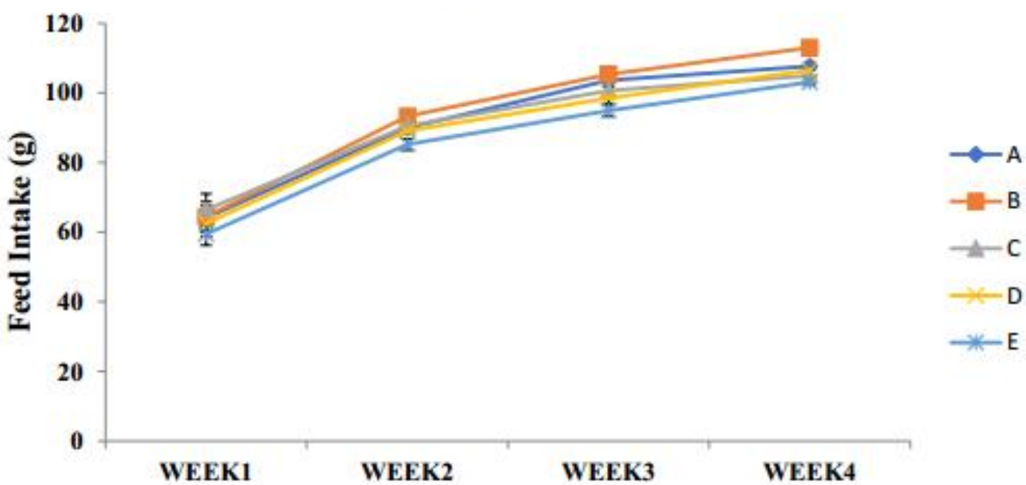
Figure 4 shows the kidney body-weight ratio, liver body-weight ratio and intestine body-weight ratio of albino rats placed on edible chitosan-starch film supplemented diets. The result showed that there was significant difference (P<0.05) in the kidney body-weight ratio and in the intestine body-weight ratio. However, there was no significant difference (P>0.05) in the liver body-weight ratio of the albino rats.

Plate I – XV shows the histoarchitecture of the liver, kidney and intestine of rats placed on edible chitosan-starch film supplemented diets and those of the control. There was no change observed in the liver of rats placed on edible chitosan-starch film supplemented diets when compared to that placed on the control diet. There was a degeneration of the glomeruli, capsular space in the kidney and connective tissue inflammation in the intestine of rats fed the supplemented diet.

Table 1: Proximate Compositions of Chitosan, Edible Chitosan-Starch Film and Chitosan-Starch Film Supplemented Diets.

Parameters (%)	*Chitosan-starch film supplemented diets					Edible Chitosan-Starch Film	Chitosan
	A	B	C	D	E		
Moisture	9.67 ± 0.17^{a}	12.67 ± 0.17^{b}	14.17 ± 0.44^{c}	17.00 ± 0.29^{d}	17.33 ± 0.73^{de}	18.67 ± 0.73^{e}	11.00 ± 0.00^{a}
Ash	6.00 ± 0.50^{b}	7.50 ± 0.29^{c}	8.83 ± 0.33^{d}	11.67 ± 0.73^{e}	13.83 ± 0.17^{f}	12.33 ± 0.33^{e}	4.33 ± 0.33^{a}
Fat	10.10 ± 0.29^{b}	13.50 ± 0.00^{c}	14.33 ± 0.44^{cd}	15.33 ± 0.44^{d}	14.17 ± 0.17^{c}	25.33 ± 0.44^{e}	8.50 ± 0.29^{a}
Fibre	13.23 ± 0.44^{d}	8.00 ± 0.29^{b}	7.67 ± 0.33^{b}	6.33 ± 0.17^{a}	6.17±0.67a	7.50 ± 0.00^{b}	10.17 ± 0.33^{c}
Protein	13.73 ± 0.12^{e}	12.69 ± 0.06^{d}	12.45 ± 0.10^{cd}	12.28 ± 0.00^{c}	11.58 ± 0.10^{b}	0.82 ± 0.06^{a}	4.32 ± 0.06^{f}
Carbohydrate	47.27 ± 0.20^{d}	45.64 ± 0.23^{d}	42.55 ± 1.17^{c}	37.39 ± 0.73^{b}	36.92 ± 0.41^{b}	35.35 ± 0.78^{a}	61.68 ± 0.34^{e}

Values are Mean ± Standard Error of Mean of triplicate determinations.
Values with different superscripts between the samples are significantly different ($p<0.05$).
*A: Control (100% rat chow), B: 5% film, C: 10% film, D: 20% film, E: 40% film.



Diet Groups
A= Control (100% rat chow)
B= Rats fed 5% film
C=Rats fed 10% film
D= Rats fed 20% film
E= Rats fed 40% film

Figure 2: Feed intake by albino rats placed on edible chitosan-starch film supplemented diets.

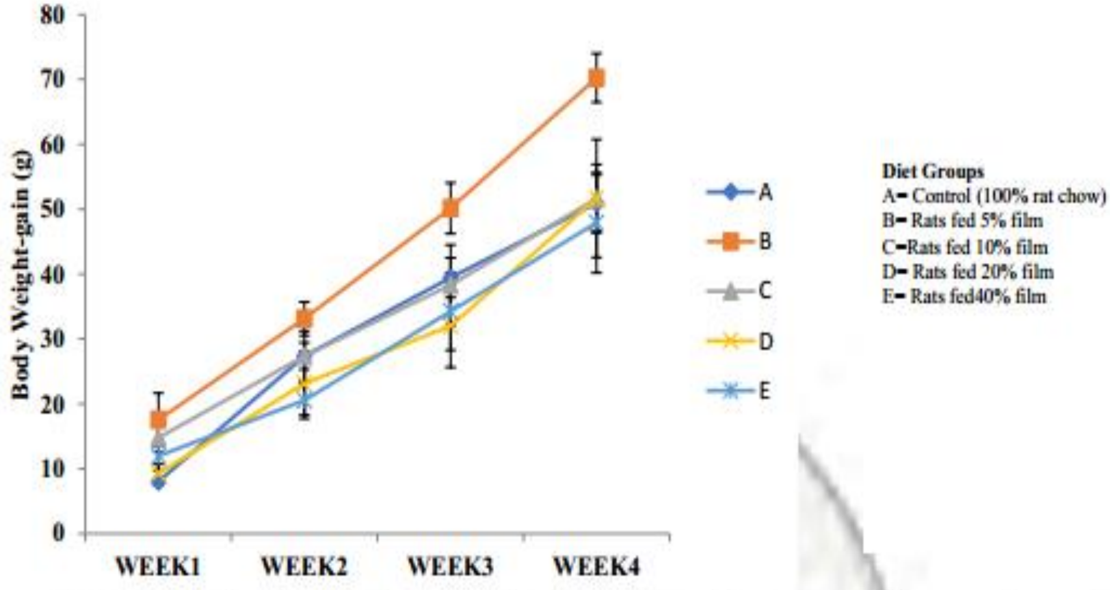


Figure 3: Body weight-gain of albino rats placed on edible chitosan-starch film supplemented diets.

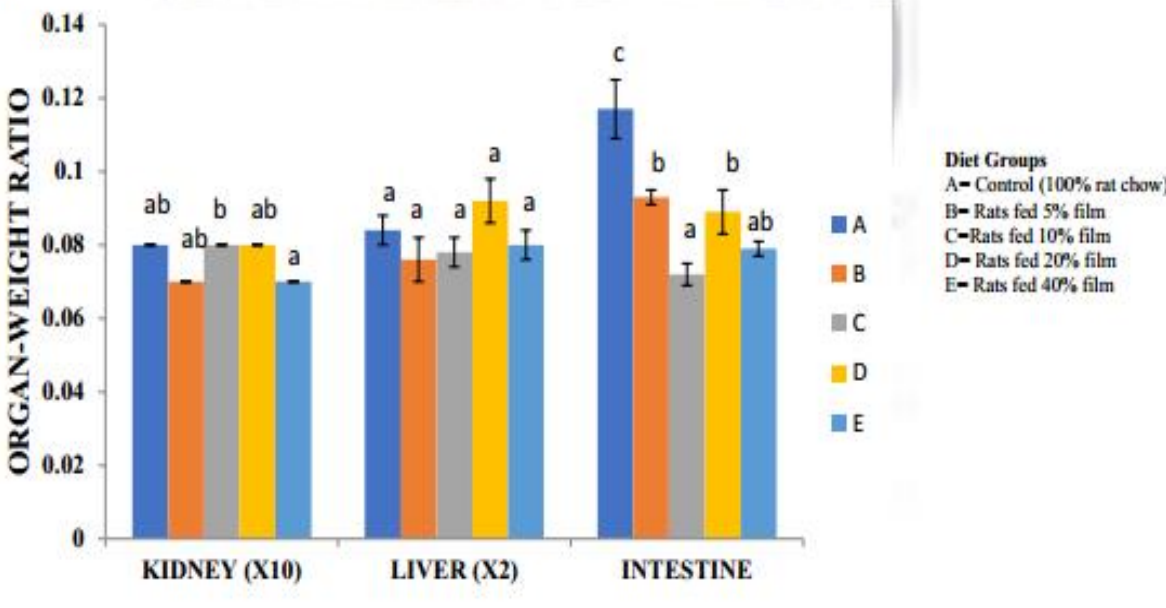


Figure 4: Organ-weight ratio of the kidney, liver and intestine of albino rats placed on edible chitosan-starch film supplemented diets.

LIVER

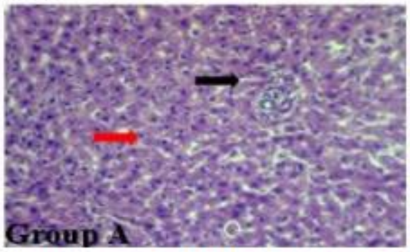


Plate 1: Photomicrograph of the Liver tissue showing normal histological architecture with intact hepatic cells

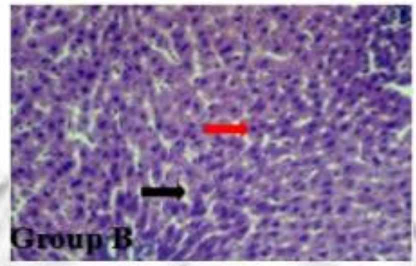


Plate 2: Photomicrograph of the liver tissue showing unaltered hepatic cell

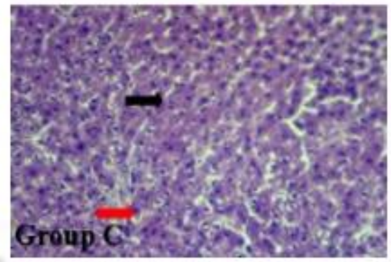


Plate 3: Photomicrograph of the Liver tissue showing unaltered hepatic cells

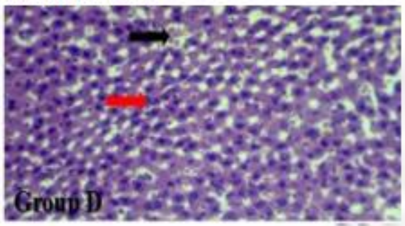


Plate 4: Photomicrograph of the Liver tissue showing normal histological features

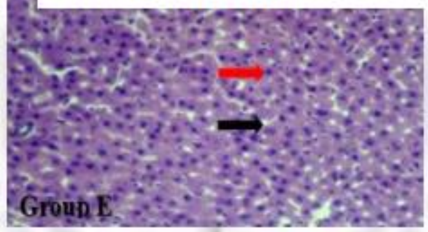


Plate 5: Photomicrograph of the Liver tissue showing unaltered hepatic cells

Plate 1-5: Photomicrograph of the Liver Tissue (Mg x 40; Eosin/Haematoxylin)
Black Arrow: Hepatocytes, **Red Arrow**: Hepatic sinusoids

KIDNEY

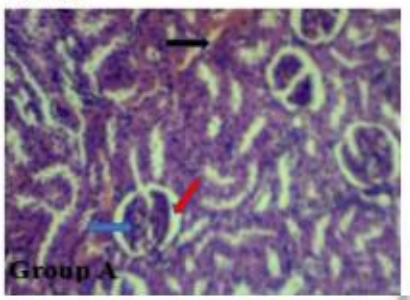


Plate 6: Photomicrograph of the kidney tissue showing normal histological architecture with intact glomeruli and capsular space.

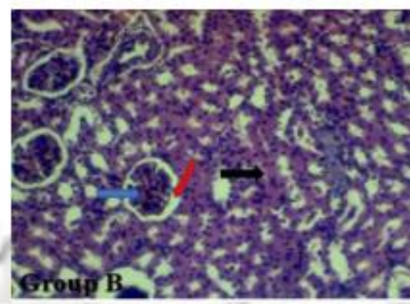


Plate 7: Photomicrograph of the kidney tissue showing normal glomeruli and capsular space.

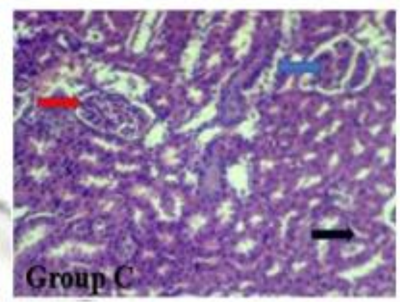


Plate 8: Photomicrograph of the kidney tissue showing normal capsular and glomeruli degeneration.

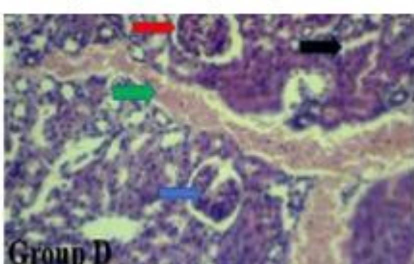


Plate 9: Photomicrograph of the kidney tissue showing normal capsular and

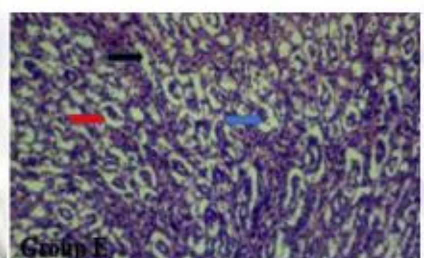


Plate 10: Photomicrograph of the kidney tissue showing degeneration of capsular

Plate 6-10: Photomicrograph of the Kidney Tissue (Mg x 40; Eosin/Haematoxylin)
Blue Arrow: Glomerulus, **Red Arrow**: Capsular space, **Green Arrow**: Area of Inflammation, **Black Arrow**: Distal convoluted tubules.

INTESTINE

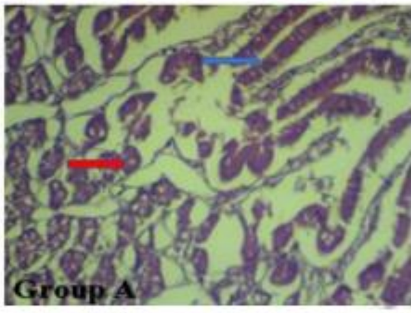


Plate 11: Photomicrograph of the intestinal tissue showing normal histological architecture with intact epithelial cells

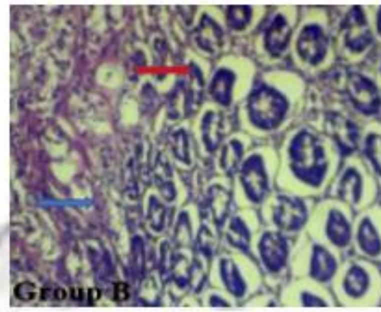


Plate 12: Photomicrograph of the intestinal tissue showing unaltered histological architecture.

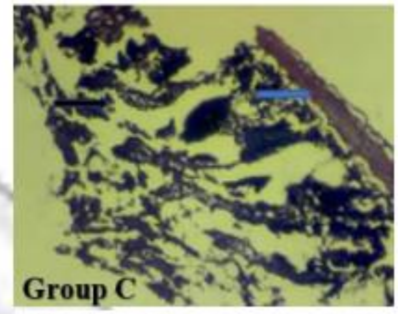


Plate 13: Photomicrograph of the intestinal tissue showing slight connective tissue degeneration.

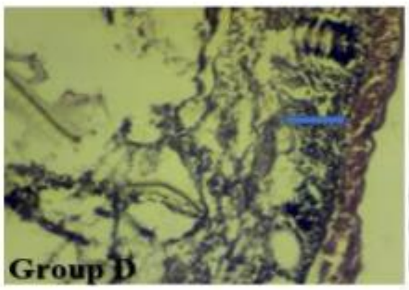


Plate 14: Photomicrograph of the intestinal tissue showing connective tissue inflammation.

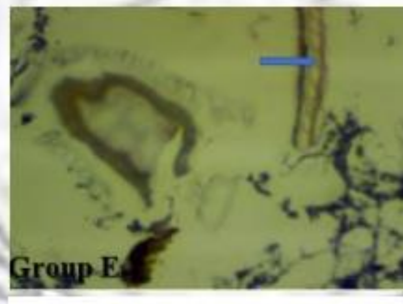


Plate 15: Photomicrograph of the intestinal tissue showing connective tissue inflammation.

Plate 11-15: Photomicrograph of the Instestine Tissue (Mg x 40; Eosin/Haematoxylin). Blue arrow: Smooth muscle fibre, Red arrow: Lamina propria (Connective tissue)

CONCLUSION

Chitosan was significantly lower in all proximate components except for carbohydrate and fibre, chitosan-starch film was significantly higher in moisture, fat and ash compared to the supplemented diet and the control. The kidney and intestine body-weight ratios were significantly lower in the rats on the supplemented diets. Group B rats placed on 5 % edible chitosan-starch film supplemented diets had the highest feed intake and weight-gain in all the weeks. Also, noticeable changes were observed in the histoarchitecture of the kidney and intestine of albino rats placed on diets supplemented with more than 5 % edible films, this was in agreement with our earlier report on its safety based on haematological and biochemical indices (Akanya *et al*., 2022). Therefore, edible chitosan-starch films may be considered toxic to albino rats at levels higher than 5 % diet inclusion.

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