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TABLE OF CONTENTS

PRELIMINARY PAGES	8
SUSTAINABLE CROP PRODUCTION PRACTICES FOR CLIMATE RESILIENCE FOOD AND NUTRITION SECURITY	22
AGRICULTURALECONOMICS EXTENSION AND RURAL SOCIOLOGY	28
1 ADOPTION INDEX OF MAIZE PRODUCTION TECHNOLOGIES AND CORRELATION MATRIX IN SMALLHOLDER SYSTEMS	29
2 RURAL WOMEN AND AGRO – PROCESSING: A CASE STUDY OF RURAL WOMEN PARTICIPATION IN GROUNDNUT PROCESSING IN KATSINA STATE	35
3 PROFITABILITY OF DRIP IRRIGATED MAIZE PRODUCTION FOR IMPROVED FOOD SECURITY IN MAIDUGURI SEMI-ARID REGION OF BORNO STATE, NIGERIA	41
4 PROFITABILITY ANALYSIS OF POULTRY EGG PRODUCTION IN IBADAN METROPOLIS, OYO STATE NIGERIA	49
5 ADOPTION OF MODERN BEEKEEPING TECHNOLOGIES IN SELECTED LOCAL GOVERNMENT AREAS OF BENUE STATE NIGERIA	56
6 ADOPTION OF RECOMMENDED COCOYAM PRODUCTION TECHNOLOGIES AMONG FARMERS IN ENUGU STATE NIGERIA	64
7 GENDER ANALYSIS OF FARMING HOUSEHOLDS' ACCESS TO LIVELIHOOD RESOURCES IN SELECTED LOCAL GOVERNMENT AREAS OF NIGER STATE, NIGERIA	74
8 INNOVATIVE APPLICATION IN MANAGEMENT OF PROBLEMATIC SOIL (ACIDIC SOIL) UNDER MAIZE PRODUCTION IN NIGER STATE, NIGERIA	85
9 EFFECTS OF RISK MANAGEMENT STRATEGIES ON POVERTY STATUS OF RICE FARMERS IN NIGER STATE, NIGERIA	92
10 EFFECTS OF FARMER-HERDER'S CONFLICT ON THE FARMERS PRODUCTIVITY IN ADAMAWA STATE, NIGERIA	98
11 EFFECTS OF ADOPTION OF IMPROVED BEEHIVE TECHNOLOGIES ON INCOME AND WELFARE	

STATUS OF BEEKEEPERS IN EKITI STATE, NIGERIA	105
12 SOCIO ECONOMIC EVALUATION OF THE IMPACT OF WAPP FISH CULTURE PROGRAMME IN IBEJU-LEKKI LGA IN LAGOS STATE, NIGERIA	112
13 ASSESSMENT OF OUT-MIGRATION AMONG ARABLE CROP FARMERS IN KOGI STATE, NIGERIA: GENDER DYNAMIC APPROACH	120
14 THE EFFECT OF AGRITECH STARTUPS ON PARTICIPATION AND POVERTY STATUS OF ADOPTERS IN OGUN STATE, NIGERIA	129
15 OUTCOMES OF WORK-FAMILY CONFLICT AMONG UNIVERSITY EMPLOYEES: CORRELATES OF TIME, STRAIN AND JOB SATISFACTION	136
16 MICROBIOLOGICAL QUALITY OF OVEN ROASTED PLANTAIN (<i>MUSA PARASIDIACA</i>)	141
17 A REVIEW OF FOOD SECURITY AND POVERTY STATUS OF WOMEN FARMERS UNDER IFAD-VCDP IN NIGER STATE, NIGERIA	146
18 COMPARATIVE ANALYSIS OF THE EFFECT OF ANCHOR BORROWERS PROGRAMME (ABP) ON FOOD SECURITY STATUS OF RICE FARMERS IN EBONYI AND KEBBI STATES, NIGERIA	153
19 EFFECTS OF LAND DEGRADATION ON CEREAL CROP PRODUCTION IN RURAL AREAS OF NIGER STATE, NIGERIA	160
20 FUNCTIONAL PROPERTIES OF CASSAVA SEED PROTEIN CONCENTRATE	167
21 EFFECTS OF INSURGENCY ON CROP FARMING ACTIVITIES OF RURAL WOMEN IN ADAMAWA STATE, NIGERIA	172
22 ADOPTION OF BIO-FORTIFIED FOOD CROP IN NIGERIA: A REVIEW	178
23 DETERMINANTS OF THE ADOPTION OF IMPROVED BEEHIVE TECHNOLOGIES IN BENUE STATE, NIGERIA	182
24 FUNGI ASSOCIATED WITH MILLET GROWN IN ZONE A AGRO-GEOGRAPHICAL ZONE OF NIGER STATE NORTH CENTRAL NIGERIA	196
25 GENDER ANALYSIS OF FARMING HOUSEHOLDS' ACCESS TO LIVELIHOOD RESOURCES IN SELECTED LOCAL GOVERNMENT AREAS OF NIGER STATE, NIGERIA	203

LEAF MEAL	381
54 PROXIMATE COMPOSITION OF RAW AND PROCESSED FULL-FAT LEBBECK (ALBIZIA LEBBECK) SEEDS	389
55 GINGER (ZINGIBER OFFICINALE) AS FEED SUPPLEMENT: INFLUENCE ON GROWTH PERFORMANCE AND HEALTH OF GROWING RABBITS – A REVIEW	393
56 ASSESSMENT OF MIXTURES MEAL OF BREWERS DRIED GRAINS AND SORGHUM BREWERS DRIED GRAINS ON GROWTH AND NUTRIENT DIGESTIBILITY OF WEANER RABBITS	399
57 MODELLING OF PHENOTYPIC TRAITS AS DETERMINANTS OF BREEDING POTENTIALS OF CATTLE UNDER LOW EXTERNAL INPUT	405
58 ASSESSMENT OF MATING PROFILE OF RED SOKOTO BUCKS ADMINISTERED VARYING DOSAGE OF ETHANOLIC EXTRACT OF TIGER NUT (CYPERUS ESCULENTUS)	411
59 EFFECTS OF CRUDE OR SYNTHETIC ENZYMES ON THE DIGESTIBILITY OF BROILER FINISHER CHICKENS FED GROUNDNUT-COWPEA SHELL BASED DIETS	416
60 GROWTH PERFORMANCE AND EGG PRODUCTION OF JAPANESE QUAILS FED DIFFERENTLY PROCESSED (LEUCAENA LEUCOCEPHALA) BASED DIETS	421
61 EFFECTS OF THE METHANOL LEAF EXTRACT OF NEWBOULDIA LAEVIS ON OESTROGEN LEVELS DURING PREGNANCY IN RABBIT DOES IN KADUNA STATE, NIGERIA	427
62 GROWTH AND BODY MORPHOMETRIC PARAMETERS OF BROILER CHICKENS ORALLY ADMINISTERED VARYING LEVELS OF LEMONGRASS EXTRACT, AT FINISHER PHASE	434
63 THERMOREGULATORY RESPONSES IN PERIPARTURIENT SAHELIAN AND WEST AFRICAN DWARF GOATS DURING THE HOT-DRY SEASON IN THE NORTHERN GUINEA SAVANNAH ZONE OF NIGERIA	442
64 GROWTH PARAMETERS OF RATS FED VARYING RATIOS OF EDIBLE CHITOSAN-STARCH FILMS PACKAGING	448
65 EFFECT OF BREED AND SEX ON PELT GROWTH OF NEW ZEALAND WHITE AND CHINCHILLA GIGANTAS RABBITS	458
66 WATER QUALITY ASSESSMENT OF THE PROPOSED KWADNA RESERVOIR WITHIN GIDAN KWANU MAIN CAMPUS, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGER STATE, NIGERIA	

465

67 PHYLOGENY BETWEEN CLARIAS GARIEPINUS AND HETEROBRANCHUS BIDOSALIS INFERRED FROM SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) DNA MARKERS	472
68 EFFECTS OF PHOTOPERIOD AND FEEDING RATE ON THE GROWTH PERFORMANCE AND FEED UTILIZATION OF CLARIAS GARIEPINUS	477
69 IMPACT OF COVID-19 INDUCED LOCKDOWN ON AQUACULTURE PRODUCTION IN MINNA, NIGER STATE, NIGERIA	482
70 EVALUATION OF ADAPTIVE PLASTICITY IN WILD SAROTHERODON GALILAEUS AND COPTODON ZILLI IN CONCRETE POND	489
71 EFFECT OF PROCESSED SELECTED MEDICINAL PLANTS DIETS ON HAEMATOLOGICAL PARAMETERS OF CLARIAS GARIEPINUS (BURCHELL, 1822)	497
72 ASSESSMENT OF INORGANIC FERTILIZER ON FRESHWATER FISH CULTURE IN NIGERIA	505
73 UTILIZATION OF MAIZE BRAN CHEMICAL HYDROLYSATE USING MINERAL ACID IN THE PRATICAL DIETS OF CLARIAS GARIEPINUS	511
74 SENSORY AND PROXIMATE COMPOSITION OF 'BISCUIT' PRODUCED FROM SOME LESS VALUED DRIED FISH POWDER	517
75 PROXIMATE AND SENSORY ASSESSMENT OF AADUN PRODUCED FROM MAIZE AND GROUNDNUT PASTE	524
76 NUTRI-CEREALS AS POTENTIAL FUNCTIONAL INGREDIENTS: CHARACTERIZATION AND VALORIZATION	531
77 MICROBIOLOGICAL QUALITY OF DEVELOPED WHEAT BISCUIT FORTIFIED WITH EGGSHELL CALCIUM	532
CROP SCIENCE/PRODUCTION	537
78 EVALUATION OF SINGLE AND MIXED VIRUS-INOCULATED BAMBARA GROUNDNUT LANDRACES FOR NODULATION AND NITROGEN FIXATION	538
79 EFFECTS OF JATROPHA CURCAS LEAF EXTRACT ON THE GROWTH CHARACTERISTICS AND NEMATODE ASSOCIATED WITH TOMATO (SOLANUM LYCOPERSICUM)	545

64 GROWTH PARAMETERS OF RATS FED VARYING RATIIONS 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 outstanding 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.

pg. 448

Proceedings of the 2nd ICAAT, 2022

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).

pg. 449

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±0.67 ^a	7.50±0.00 ^b	10.17±0.33 ^c
Protein	13.73±0.12 ^c	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.

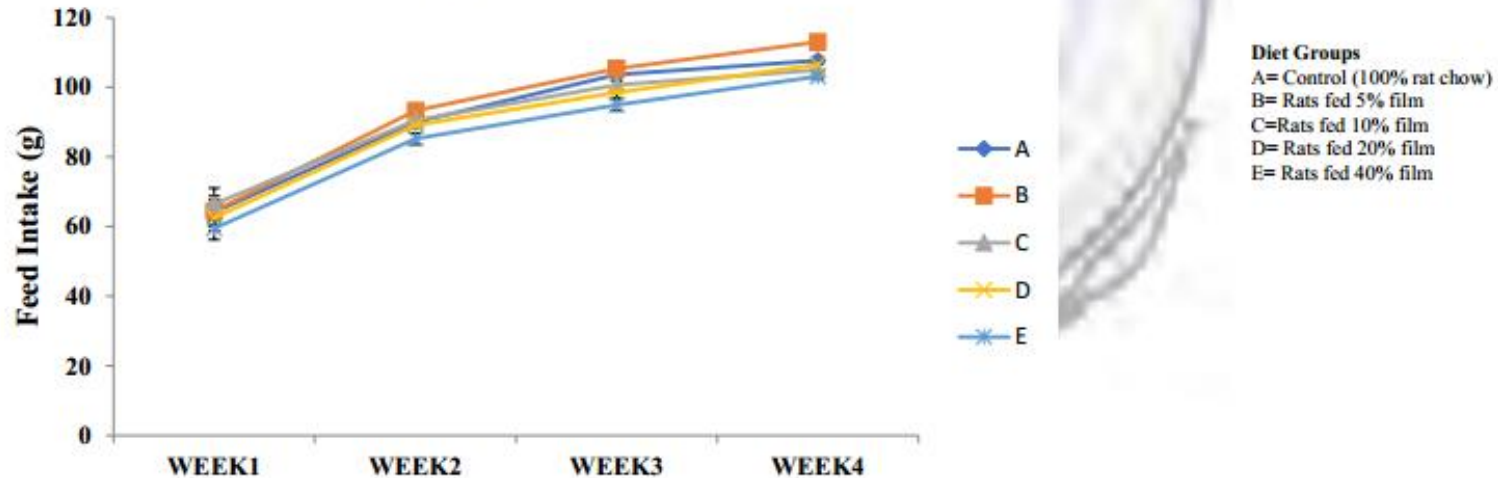


Figure 2: Feed intake by albino rats placed on edible chitosan-starch film supplemented diets.

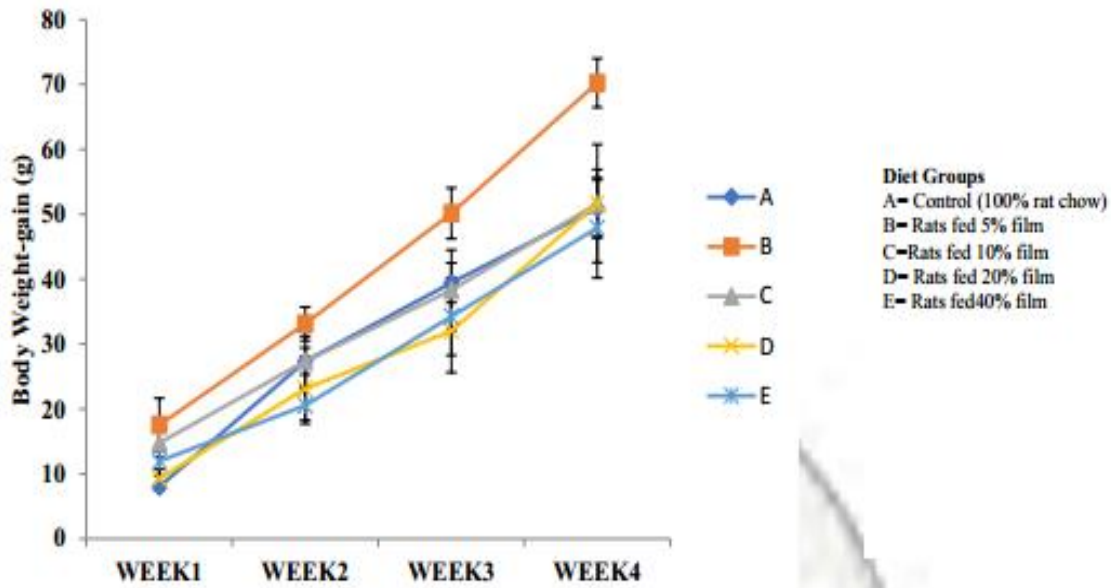


Figure 3: Body weight-gain of albino rats placed on edible chitosan-starch film supplemented diets.

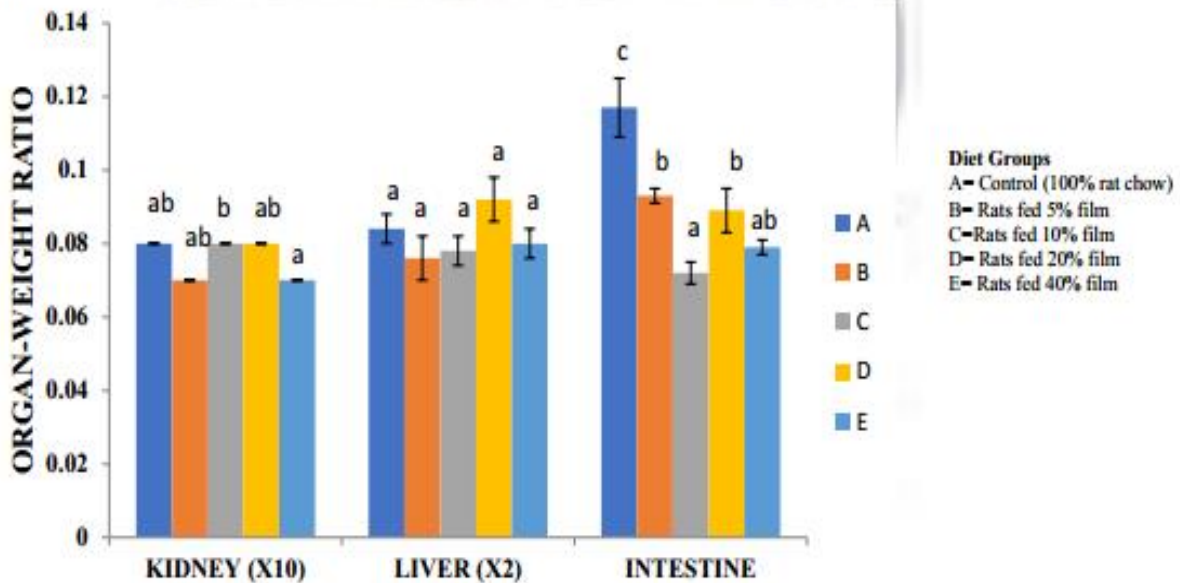


Figure 4: Organ-weight ratio of the kidney, liver and intestine of albino rats placed on edible chitosan-starch film supplemented diets.

LIVER

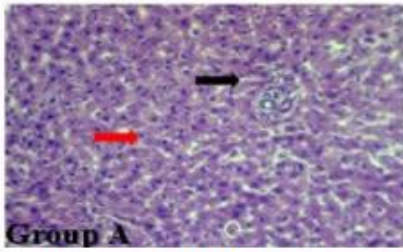


Plate 1: Photomicrograph of the Liver tissue showing normal histological architecture with intact hepatic cells

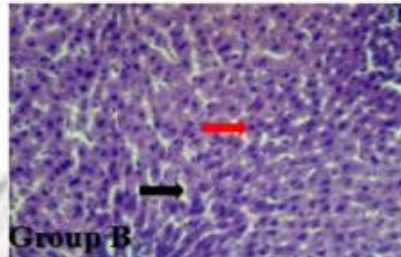


Plate 2: Photomicrograph of the liver tissue showing unaltered hepatic cell

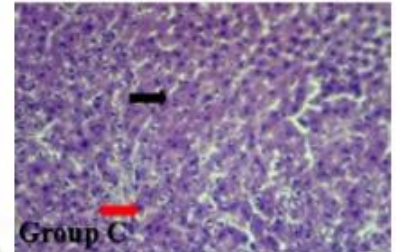


Plate 3: Photomicrograph of the Liver tissue showing unaltered hepatic cells

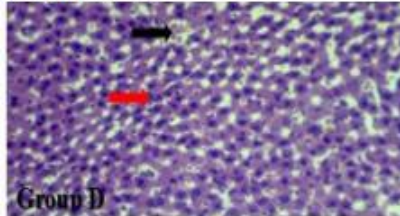


Plate 4: Photomicrograph of the Liver tissue showing normal histological features

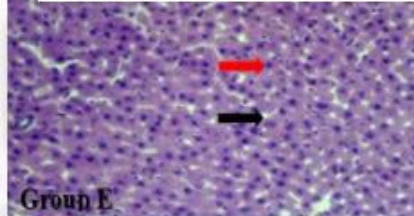


Plate 5: Photomicrograph of the Liver tissue showing unaltered hepatic cells

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

KIDNEY

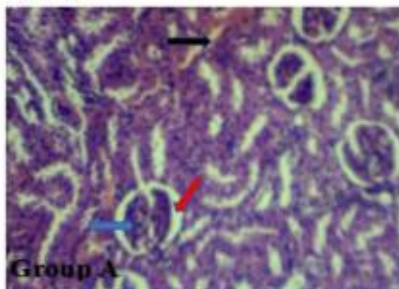


Plate 6: Photomicrograph of the kidney tissue showing normal histological architecture with intact glomeruli and capsular space.

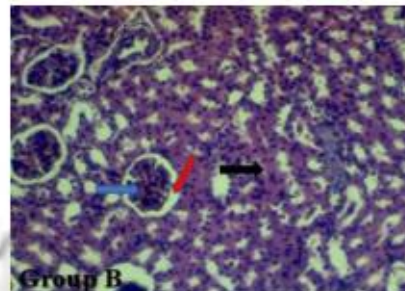


Plate 7: Photomicrograph of the kidney tissue showing normal glomeruli and capsular space.

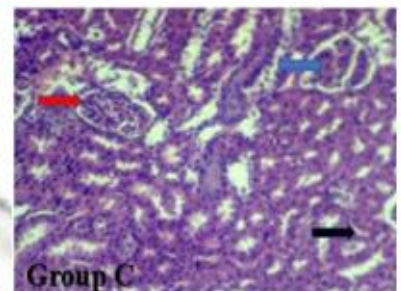


Plate 8: Photomicrograph of the kidney tissue showing normal capsular and glomeruli degeneration.

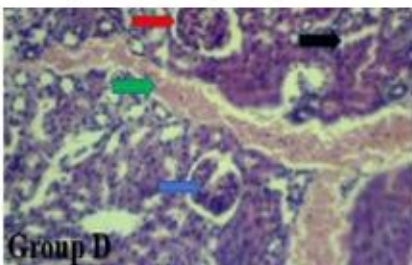


Plate 9: Photomicrograph of the kidney tissue showing normal capsular and

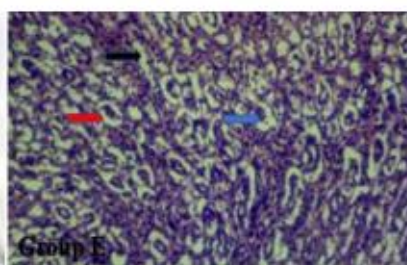


Plate 10: Photomicrograph of the kidney tissue showing degeneration of capsular

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

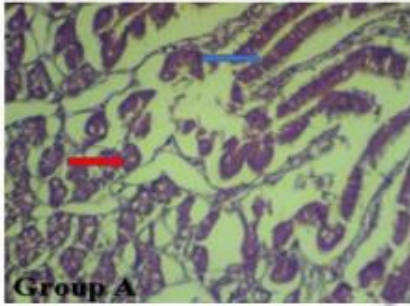


Plate 11: Photomicrograph of the intestinal tissue showing normal histological architecture with intact epithelial cells

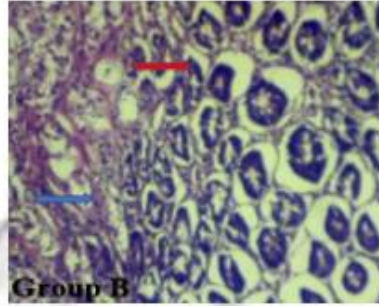


Plate 12: Photomicrograph of the intestinal tissue showing unaltered histological architecture.

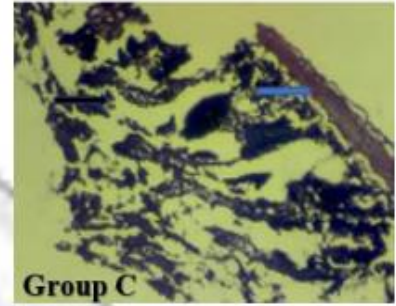


Plate 13: Photomicrograph of the intestinal tissue showing slight connective tissue degeneration.

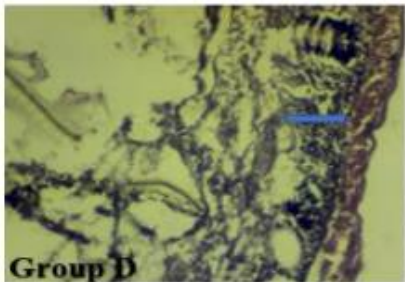


Plate 14: Photomicrograph of the intestinal tissue showing connective tissue inflammation.

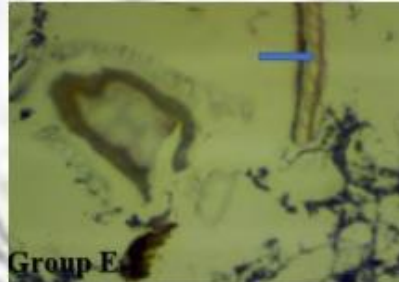


Plate 15: Photomicrograph of the intestinal tissue showing connective tissue inflammation.

Plate 11-15: Photomicrograph of the Intestine 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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