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Effects of fruit and vegetable smoothie formulations on serum electrolytes, haematological indices and faecal bacteriology: An *in vivo* experiment in female Wistar albino rats

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Abstract:

Background: The global demand for fruits and vegetables consumed as smoothies is increasing, driven by a growing population and heightened awareness of their health benefits. However, the nutritional significance and health effects of smoothies remain underexplored. Chemotherapeutic agents are widely used in managing various illnesses but are associated with the dysregulation and destruction of healthy cells, raising concerns in clinical settings. This study aimed to assess the potential of smoothie formulations in regulating serum electrolytes, haematological indices, and faecal bacteriology in an experimental animal model.
Methodology: Thirty female Wistar albino rats (*Rattus norvegicus*) were divided into 5 groups of 6 rats each, using a permuted-block randomized design, with the 5th group serving as the positive control. Smoothie formulations of banana/watermelon (BNWM), watermelon/cucumber (WMCC), pineapple/orange (PPOR), and banana/cucumber (BNCC) were prepared at 10g/100ml concentrations. The smoothies were administered to the rats orally at 1ml/kg body weight daily for 30 days, while the control group received no smoothie formulations. Faecal count and bacterial identity were carried out twice during the experiment using standard bacteriological techniques. Serum electrolytes and haematological indices were determined by standard methods on blood samples collected through cardiac puncture from anaesthetized rats on day 30 of the experiment. Experimental data were expressed as mean±SEM and percentages. Comparisons between groups were performed using Student's *t*-test and ANOVA. Significance difference was defined at $p<0.05$.
Results: Residual feed intake (RFI) values ranged from 33.8±5.09 to 36.9±3.66, with groups 3–5 rats showing progressive weekly increases while groups 1 and 2 rats fluctuated. Residual water intake (RWI) was highest in the control group (80.8±22.66ml) compared to smoothie-fed groups (59.2–67.8ml). Body weight reductions occurred across all groups but were not statistically significant ($p=0.1507$). Faecal bacteriology showed variable trends, with group 2 rats exhibiting a marked increase, group 3 rats with slight decrease, and group 1 rats having no detectable growth at the second sampling. Overall, Gram-negative isolates (51.2%) were slightly more frequent than Gram-positive (48.8%), with *Vibrio* spp (19.5%), *Staphylococcus* spp (17.1%), and *Bacillus* spp (14.6%) being most common. Electrolytes remained within normal ranges, although bicarbonate was lower in smoothie-fed rats (18.7–22.7mEq/L) compared to control (24.3mEq/L). Hematological indices showed significantly elevated white blood cells, lymphocytes, mean corpuscular volume, and platelets in smoothie-fed groups relative to the control, while haemoglobin, packed cell volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration showed non-significant variations.
Conclusion: This *in vivo* animal experimental findings suggest that smoothie formulations have potential in promoting weight management, maintaining electrolyte balance, enhancing haematological parameters, and reducing the pathogenic impact of gut microbiota without inducing symptoms. Further research is recommended to explore the incorporation of smoothies as dietary supplements, particularly for health conditions requiring electrolyte normalization and immune support, offering a safer alternative to synthetic therapies.

Keywords*:* Albino rats, faecal bacteriology, smoothie formulations, electrolytes, haematological indices

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Effets des préparations de smoothies aux fruits et légumes sur les électrolytes sériques, les indices hématologiques et la bactériologie fécale: une expérience *in vivo* chez des rats albinos femelles Wistar

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Résumé:

Contexte: La demande mondiale de fruits et légumes consommés sous forme de smoothies est en hausse, stimulée par la croissance démographique et une sensibilisation accrue à leurs bienfaits pour la santé. Cependant, l'importance nutritionnelle et les effets des smoothies sur la santé restent peu étudiés. Les agents chimiothérapeutiques sont largement utilisés dans la prise en charge de diverses maladies, mais ils sont associés à une dysrégulation et à la destruction des cellules saines, suscitant des inquiétudes en milieu clinique. Cette étude visait à évaluer le potentiel des préparations de smoothies dans la régulation des électrolytes sériques, des indices hématologiques et de la bactériologie fécale dans un modèle animal expérimental.
Méthodologie: Trente rats albinos Wistar femelles (*Rattus norvegicus*) ont été répartis en cinq groupes de six rats chacun, selon un dispositif randomisé en blocs permutés, le cinquième groupe servant de témoin positif. Des préparations de smoothies banane/pastèque (BNWM), pastèque/concombre (WMCC), ananas/orange (PPOR) et banane/concombre (BNCC) ont été préparées à des concentrations de 10g/100 ml. Les smoothies ont été administrés aux rats par voie orale à raison de 1 ml/kg de poids corporel par jour pendant 30 jours, tandis que le groupe témoin n'a reçu aucune préparation de smoothie. La numération fécale et l'identité bactérienne ont été réalisées à deux reprises au cours de l'expérience à l'aide de techniques bactériologiques standard. Les électrolytes sériques et les indices hématologiques ont été déterminés par des méthodes standard sur des échantillons de sang prélevés par ponction cardiaque chez des rats anesthésiés au 30e jour de l'expérience. Les données expérimentales ont été exprimées en moyenne ± écart-type et en pourcentages. Les comparaisons entre les groupes ont été réalisées à l'aide du test '*t*' de Student et d'une ANOVA. La différence de significativité a été définie à $p<0,05$.
Résultats: Les valeurs de la consommation alimentaire résiduelle (RFI) variaient de 33,8±5,09 à 36,9±3,66, les rats des groupes 3 à 5 présentant une augmentation hebdomadaire progressive, tandis que les rats des groupes 1 et 2 fluctuaient. La consommation d'eau résiduelle (RWI) était la plus élevée dans le groupe témoin (80,8±22,66 ml) par rapport aux groupes nourris au smoothie (59,2 à 67,8ml). Français Des réductions de poids corporel ont été observées dans tous les groupes, mais n'étaient pas statistiquement significatives ($p=0,1507$). La bactériologie fécale a montré des tendances variables, les rats du groupe 2 présentant une augmentation marquée, les rats du groupe 3 une légère diminution et les rats du groupe 1 n'ayant aucune croissance détectable lors du deuxième prélèvement. Globalement, les isolats Gram négatifs (51,2%) étaient légèrement plus fréquents que les Gram positifs (48,8%), les *Vibrio* spp (19,5%), *Staphylococcus* spp (17,1%) et *Bacillus* spp (14,6%) étant les plus courants. Les électrolytes sont restés dans les plages normales, bien que le bicarbonate était plus faible chez les rats nourris au smoothie (18,7–22,7mEq/L) que chez le témoin (24,3mEq/L). Les indices hématologiques ont montré une augmentation significative des globules blancs, des lymphocytes, du volume globulaire moyen et des plaquettes dans les groupes nourris au smoothie par rapport au groupe témoin, tandis que l'hémoglobine, l'hématocrite, l'hémoglobine corpusculaire moyenne et la concentration corpusculaire moyenne en hémoglobine n'ont montré aucune variation significative.
Conclusion: Ces résultats expérimentaux in vivo sur des animaux suggèrent que les préparations à base de smoothies pourraient favoriser la gestion du poids, maintenir l'équilibre électrolytique, améliorer les paramètres hématologiques et réduire l'impact pathogène du microbiote intestinal sans induire de symptômes. Des recherches complémentaires sont recommandées pour explorer l'intégration des smoothies comme compléments alimentaires, notamment pour les pathologies nécessitant une normalisation électrolytique et un soutien immunitaire, offrant ainsi une alternative plus sûre aux thérapies synthétiques.

Mots-clés: Rats albinos, bactériologie fécale, préparations à base de smoothies, électrolytes, indices hématologiques

Introduction:

Smoothies are a popular and convenient means of consuming various fruits, vegetables, and other nutrient-rich ingredients in liquid form. It is a versatile platform for combining vitamins, minerals, fibers, antioxidants, and essential fatty acids for a valuable, balanced diet (1). It serves as health enthusiasts, easy meal replacement, providing hydration, energy, and nourishment, aiding in weight management, and improving immune function (2). The high fiber content from fruits and vegetables can help regulate blood sugar levels, promote satiety, and enhance digestive health. Antioxidants from ingredients like berries can combat oxidation, stress, and inflammation, supporting cardiovascular health and reducing the risk of chronic diseases (3).

The versatility of smoothies has gained popularity as a healthful beverage convenience option and plays a vital role in the gut microbiome. Recent studies have highlighted the profound effect diet has on the gut microbiome, a community of microorganisms that plays a crucial role in digestion, metabolism, and immune function (4).

In albino rats, research into the con-

sumption of smoothies has shown promising results in modulating the gut microbiome (5). The fiber content in smoothies, especially from fruits and vegetables, acts as prebiotics, feeding beneficial bacteria in the gut. This can increase microbial diversity, improving gut health and potentially enhancing the rats overall physiological functions.

A balanced microbiome in these rats has been associated with improved digestion, reduced inflammation, and better nutrient absorption, thus demonstrating the potential therapeutic benefits of smoothies for gut health. The present study aims to investigate the effects of smoothie consumption on serum electrolytes, haematological indices, and faecal bacteriology in experimental albino rats.

Materials and method:

Study setting:

This experimental study was conducted at the Animal House of the Physiology Department, University of Calabar, Calabar, Nigeria. The facility is equipped for housing and handling laboratory animals under controlled environmental conditions suitable for in vivo experiments. All procedures involving the animals were carried out in accordance with institutional guidelines and the principles outlined in the Guide for the Care and Use of Laboratory Animals (6).

Fruits and smoothie preparations:

Five different fruits; banana (*Musa paradisiaca*), watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*), pineapple (*Ananas census*) and orange (*Citrus sinensis*), were purchased fresh within Ekpo Abasi Junction fruit market in Calabar south and were aseptically wrapped using sterile foil paper. The identities of the fruits were confirmed by the Faculty Botanist, Professor Samuel Udoh, of the Department of Plant Science and Biotechnology, University of Cross River State (UNICROSS), Nigeria before being transported to the Department of Microbiological Laboratory, UNICROSS, for analysis.

The fruits were washed using running tap water several times, peeled with a sterile knife, and diced into smaller pieces. Ten g of each fruit was weighed using a digital compact scale (Atom A123) weighing balance and 20 g of the combined fruits were blended in 100 ml distilled water with a Moulex juicer blender (Model JB-70B) for 5 minutes.

Four different fruit combinations; banana/watermelon (BNWM), watermelon/cucumber (WMCC), pineapple/orange (PPOR), and banana/cucumber (BNCC), were used to prepare different types of smoothies. The prepared smoothies were emptied into sterile bottles and administered orally within 5 minutes of preparation to the experimental rat groups.

Albino rats for the study:

Thirty, 74-day old female Wistar albino rats (*Rattus norvegicus*), were purchased from the Faculty of Basic Medical Science, University of Calabar (UNICAL) on April 12, 2024. The rats were randomly divided into 5 groups of 6 rats each. The individual groups were housed in a standard laboratory cage placed in the well-ventilated laboratories with an average temperature of (27±2^{o}C), 85% relative humidity with an alternative 12 hours of natural light and 12 hours of dark or night cycle. The laboratory cages were properly disinfected with Morigad veterinary Lysol solution a few days before the arrival of the experimental animals.

Animals were adequately cared for in compliance with the principles of laboratory animal care (6). The cages were fitted with feeders containing fresh commercially prepared growers' mash (Vital Feeds, Grand Cereals Limited, Ibadan, Nigeria) and clean drinking tap water daily. The animals were allowed to acclimatize in the experiment site for seven days before each experiment commenced.

Experimental design:

The experimental design used was a permuted-block randomized design with four treatment groups; banana/watermelon (BNWM), watermelon/cucumber (WMCC), pineapple/orange (PPOR), and banana/cucumber (BNCC), and a control group. Rats in each treatment group was given smoothies based on body weight (1ml/kg) daily within the 30 days of experimental trials, except for the control group (group 5). The control group (group 5 rats) was fed with 100% commercially prepared growers mash (Vital Feeds, Grand Cereals Limited, Ibadan, Nigeria) and clean drinking water, in place of smoothie from the beginning to the end of the experiment.

General observations of animals:

The experimental animals were adequately observed for abnormalities in behavioral and physical changes such as eyes, skin, posture, fur, and response to handling. Time started, and a lasting period in such situations was recorded and documented. Residual daily feed/ water intake, changes in body weight, mortality, and morbidity were also examined.

Growth examination study:

The growth examination study was carried out based on the method described by Pugalenthi et al., (7).

Residual feed-intake (RFI):

Daily feed consumption was ascertained by subtracting the initial quantity (g) of feed, known as residual feed intake (RFI), from the leftover (LOF); $\mathrm{RFI} - \mathrm{Leftover}\ \mathrm{x}100$

Residual water-intake (RWI):

The residual daily water intake was calculated by subtracting the initial volume (mL) of water (IVO) from the leftover volume of water (LVO); $\text{RWI} - \text{leftover x}100$

Bacteriological analysis of faecal sample of albino rats:

The faecal samples of the rats were taken twice at intervals of four weeks during the experimental period. A sterile spatula was used to pick the rat faeces (pallets) into a universal bottle and transported to the University of Cross River State (UNICROSS) Microbiology laboratory for analysis within an hour. Approximately 1g weight of the faecal pallet was dissolved in 9ml sterile normal saline and shaken vigorously and intermittently for 30 minutes until the pallets were completely dissolved. Taking 1ml of the pallet solution, a ten-fold serial dilution down to 10^{-10} was done.

Bacterial cell count, isolation and identification:

Bacterial cell count was carried out using a standard bacteriological technique; a pour-plating method was employed for easy morphological characterization. A 1ml of 10^{-7} and 10^{-8} of the pallet solutions were placed in a clean Petri dish; then, 20ml of prepared semi-solid, molten Nutrient or MacConkey agar (RDM-NA-01 or RDM-MCA-02) were added. The solution was also cultured using *Salmonella-Shigella* (SS agar) (CA92121UAS) and thiosulfate-citrate-bile-salt (TCBS agar) (CM1 025) as differential media for the identification of *Salmonella-Shigella* and *Vibrio* species respectively.

The Petri dishes were gently swirled after adding the semi-solid molten agar until the contents were properly mixed. The plates were allowed to set before incubation at 37^{o}C in a humidified incubator for 24 hours, after which different morphological characterizations were observed, and emergent colonies were counted and recorded. The purification of discrete colonies was done by three successive sub-cultures and re-isolation on nutrient agar, characterized by standard bacterial techniques as described in Cheesbrough (8).

Haematology and serum electrolyte determination:

Blood sample was collected from the rats according to the method described by Oguwike et al., (9). At the end of the 30 days trial, all the rats were anesthetized with urethane at 6mg/kg (0.6 ml) per rat. About 2ml of blood was collected by cardiac puncture into ethylene-diamine tetra acetic acid (EDTA) bottle for electrolyte determination and 2ml into lithium heparin bottle for haematological analysis.

The haematological parameters such as white blood count (WBC), neutrophils, lymphocytes, monocytes, eosinophils, basophils, atypical lymphocyte (ALY), large immature cell (LIC), red blood count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelet (PLT) were determined by the procedure described by Sastry (10). The electrolyte parameters; Sodium (mmol/l), potassium (mmol/l), chloride (mmol/l) and bicarbonate (mEq/L) were determined using Audiom (AZ) automated electrolyte machine.

Statistical analysis:

Experimental data were expressed as mean±SEM and as percentages. Comparisons between 2 groups were performed using Student's *t*-test, and between more than 2 groups using ANOVA, with Bonferroni multiple comparisons. Statistically significant difference was defined as $p<0.05$. Graphs were generated using ORIGIN® version 6.1 and Microsoft Excel™.

Results:

Residual feed and water intake:

The mean residual feed intake (RFI) values were 35.9±3.27, 33.8±5.09, 36.9± 3.66, 35.5±5.94, and 36.1±6.25 for rats in groups 1–5, respectively. Across the experimental period, rats in groups 1 and 2 showed more fluctuations in RFI compared to the other groups. In contrast, rats in groups 3–5 demon strated a progressive increase in RFI over the weeks, with the highest values observed in week 4 of the trial (Table 1, Fig 1).

The residual water intake (RWI) values across the experimental groups are presented in Table 2. The mean RWI was highest for rats in group 5 (80.8 ± 22.66 ml), followed by rats in group 3 (67.8 ± 5.51 ml), while rats in groups 1, 2, and 4 recorded lower mean intakes (59.6±5.59 ml, 62.7±9.32 ml, and 59.2±11.00 ml, respectively). Across the four weeks of the experiment, fluctuations in intake were observed in rats for most groups rather than a consistent progressive increase (Table 2, Fig 2). Figs 1 and 2 showed that RFI and RWI of the rats varied among the group with no commensuration between both within the experimental period.

Table 1: Residual feed intake (RFI) for all the rats in the experimental and control groups

Rat groups	Σ of RFI for Week 1	Σ of RFI for Week 2	Σ of RFI for Week 3	Σ of RFI for Week 4	Mean ± SE
Group 1	32.9	29.6	36.4	44.8	35.93±3.27
Group 2	34.4	24.8	28.0	47.8	33.75±5.09
Group 3	30.0	32.5	38.5	46.4	36.85±3.66
Group 4	25.0	27.7	38.1	51.2	35.50±5.94
Group 5	20.8	34.1	38.1	51.2	36.05±6.25

Group 1=Banana and Watermelon (BNWM), Group 2=Watermelon and Cucumber (WMCC), Group 3=Pineapple and Orange (PPOR), Group 4=Banana and Cucumber (BNCC), Group 5=Control; SE = Standard error

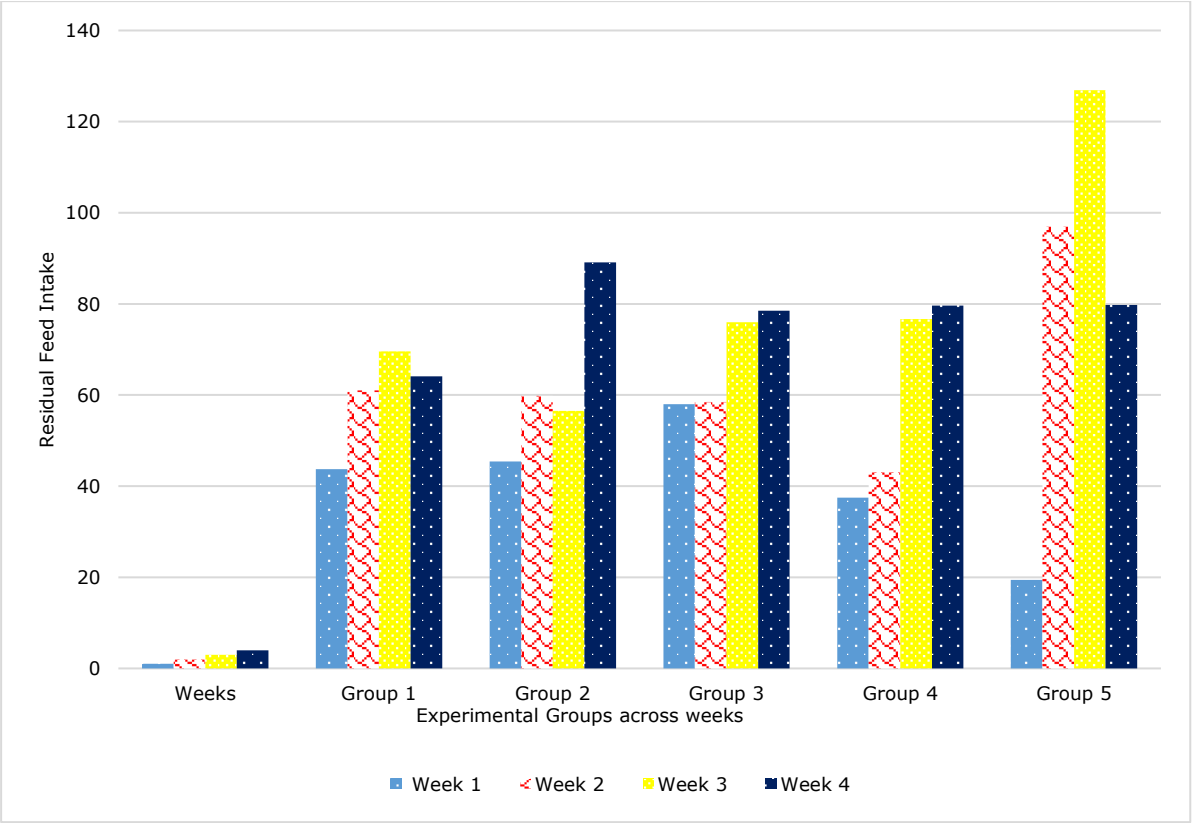

Fig 1: Variations in residual feed intake within the experimental period

Table 2: Residual water intake (RWI) for all rats in the experimental and control groups

Rat groups	Σ of RWI for Week 1	Σ of RWI for Week 2	Σ of RWI for Week 3	Σ of RWI for Week 4	Mean ± SE
Group 1	43.7	61	69.6	64.1	59.6±5.59
Group 2	45.4	59.8	56.5	89.1	62.7±9.32
Group 3	58	58.5	76	78.5	67.8±5.51
Group 4	37.5	43.1	76.7	79.6	59.2±11.00
Group 5	19.4	97	126.9	79.8	80.8±22.66

Group 1=Banana and Watermelon (BNWM), Group 2=Watermelon and Cucumber (WMCC), Group 3=Pineapple and Orange (PPOR), Group 4=Banana and Cucumber (BNCC), Group 5=Control; SE = Standard error

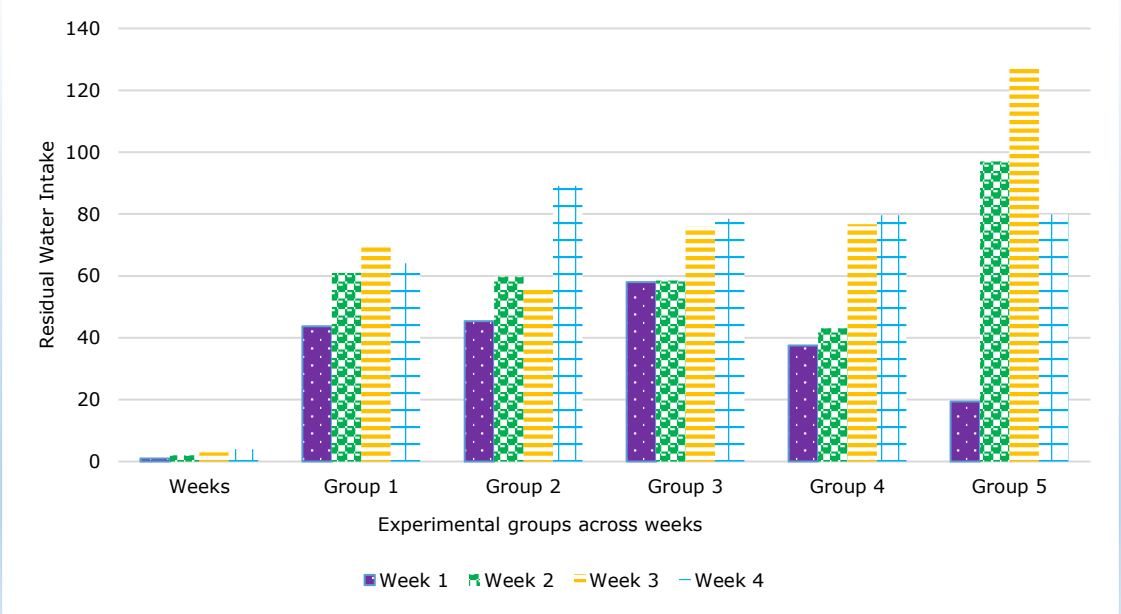

Fig 2: Variations in residual water intake within the experimental period

Table 3: Body weight changes for all the rats in the experimental and control groups

Rat groups	Σ of body weight change for Week 1	Σ of body weight change for Week 2	Σ of body weight change for Week 3	Σ of body weight change for Week 4	Mean± SE	*p* values
Group 1	27.2	12.7	5.3	8	13.3± 4.88	0.0302
Group 2	8.8	9.7	5.9	8.3	8.2± 0.81	0.5006
Group 3	9.8	8.2	7.7	9.6	8.8±0.52	0.7717
Group 4	8.8	7.2	7.8	22.3	11.5±3.61	0.1982
Group 5	14.9	15.8	7.2	23.7	15.4±3.37	0.4344
***p* values**	0.1507	0.1806	0.7287	0.2553		

Group 1=Banana and Watermelon (BNWM), Group 2=Watermelon and Cucumber (WMCC), Group 3=Pineapple and Orange (PPOR), Group 4=Banana and Cucumber (BNCC), Group 5=Control; SE = Standard error

Body weight of rats:

The general body weight assessment revealed varied reductions across all experimental groups, including the control. In group 1, the reduction rate was progressive throughout the four weeks of the experiment, with weekly cumulative changes of 27.2g, 12.7g, 5.3g, and 8.0g, respectively. The mean (±SE) body weight changes were 13.3±4.88g, 8.2± 0.81g, 8.8±0.52g, 11.5±3.61g, and 15.4± 3.37g for groups 1–5, respectively.

Statistical analysis showed no significant differences in the mean body weight change among the groups (p=0.1507), although rats in group 1 exhibited the largest variability over time. Weekly comparisons indicated that body weight changes did not differ significantly between groups in weeks 1–4 (p-values ranging from 0.1507 to 0.7287) (Table 3).

Bacteriological analysis of the faecal contents of the rats:

The faecal bacteriology of each experimental group was assessed at two intervals within the 30-day experimental trial. The highest bacterial cell count during the first sampling was 2.0–3.9×10⁷ CFU/ml for group 3 while the lowest was 1.3–1.9×10⁷ CFU/ml for group 2. During the second sampling, the highest bacterial cell count was 6.0–7.6×10⁷ CFU/ml for group 2, while the lowest was 1.0–4.0×10⁷ CFU/ml for group 3. Among the experimental groups (groups 1–4 fed with smoothie formulations), variable trends in faecal bacterial counts were observed.

There was no detectable bacteria growth for rats in group 1 during the second sampling. Group 2 rats exhibited marked increase (1.61 ×10⁷ CFU/ml at first sampling and 6.8×10⁷ CFU/ml at second sampling). Group 3 rats showed slight decrease (2.95×10⁷ CFU/ml at first sampling and 2.51×10⁷ CFU/ml at second sampling), and group 4 rats showed moderate increase (2.95×10⁷ CFU/ml at first sampling and 3.52×10⁷ CFU/ml at second sampling). In the control group (group 5 not fed with smoo-

thie formulations), the mean bacterial count increased from 3.45×10^7 CFU/ml to 6.65×10^7 CFU/ml between the two samplings (Table 4).

Frequency of bacteria isolates from the rats in both experimental and control groups:

Across all the groups, a total of 41 bacteria were isolated, with frequency of Gram-negative bacterial isolates higher at 51.2% (n =21) compared to 48.8% (n=20) for Gram-positive bacteria (Fig. 3). Albino rats in groups 2 and 3 (smoothie-fed) had the highest number of isolates, with 9 each (21.95%). Rats in groups 4 and 1 (smoothie-fed) recorded 8 (19.51%) and 7 (17.10%) isolates, respectively. The control group (group 5 rats non-smoothie fed) also had 8 isolates (19.51%) (Fig 4).

Table 4: Mean (± standard deviation) bacterial cell count (CFU/ml)

Rat groups	First Sampling			Second Sampling		
	Colony forming unit per mL (Cfu/mL)	Mean	S/E	Colony forming unit per mL (Cfu/mL)	Mean	S/E
Group 1	7.0 - 6.9	6.95	0.070 ± 0.05			
Group 2	1.9 - 1.32	1.61	0.410 ± 0.29	7.6 - 6.0	6.8	1.131 ± 0.8
Group 3	3.9 -2.0	2.95	1.344 ± 0.95	4.0 - .02	2.51	2.107 ± 1.5
Group 4	3.1 - 2.8	2.95	0.212 ± 0.15	4.02 -3.02	3.52	0.707 ± 0.5
Group 5	3.5 -3.4	3.45	0.071 ± 0,05	7.10 -6.2	6.65	0.637 ± 0.5

SE – Standard error

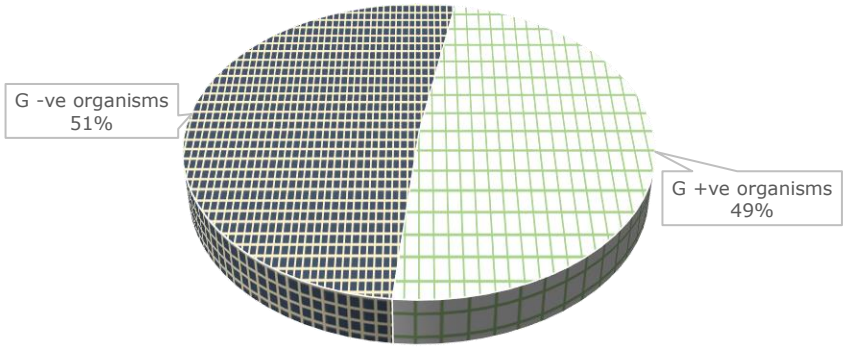

Fig 3: Frequency of faecal Gram-positive and Gram-negative bacterial isolates from the rats

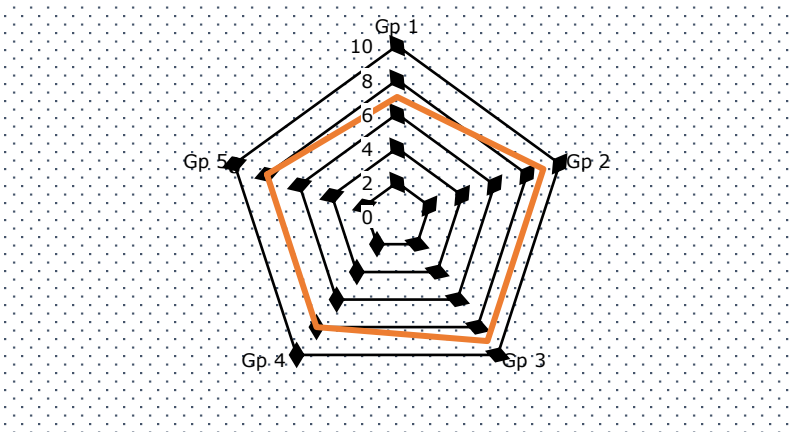

Fig 4: Frequency distribution of bacterial isolates across the different rat groups
Connecting light brown line indicates the number of isolates for each group

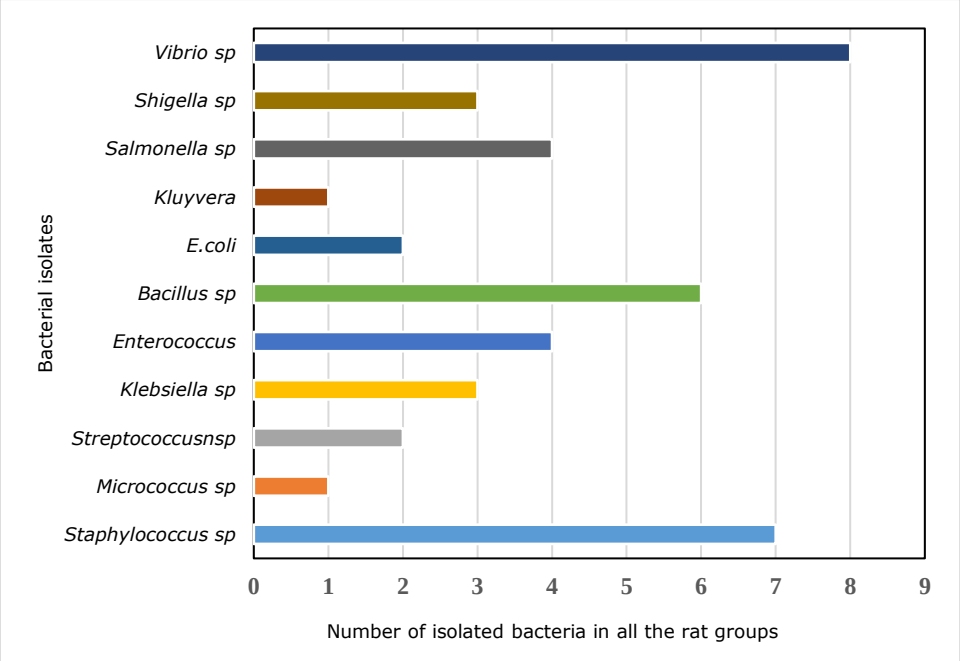

Fig 5: Frequency of different faecal bacterial species isolated from all the rats in the study

Eleven genera/species of bacteria were isolated, *Vibrio* spp were the most frequent, with 8 isolates (19.5%), followed by *Staphylococcus* spp with 7 isolates (17.1%) and *Bacillus* spp with 6 isolates (14.6%). *Enterococcus* spp and *Salmonella* spp were each with 4 isolates (9.8 %), while *Klebsiella* spp and *Shigella* spp were each with 3 isolates (7.3%). *Streptococcus* spp and *E. coli* were each with 2 isolates (4.9%), while *Micrococcus* spp and *Kluyvera* spp were each with 1 isolate (2.4%) (Fig. 5).

Bacillus spp, *Vibrio* spp, and *Shigella* spp were isolated in higher frequency during the second sampling period. *Micrococcus* spp, *Streptococcus* spp, *Enterococcus* spp and *Salmonella* spp were isolated exclusively during the first sampling period while *Kluyvera* spp and *E. coli* were isolated exclusively during the second sampling period (Fig 6).

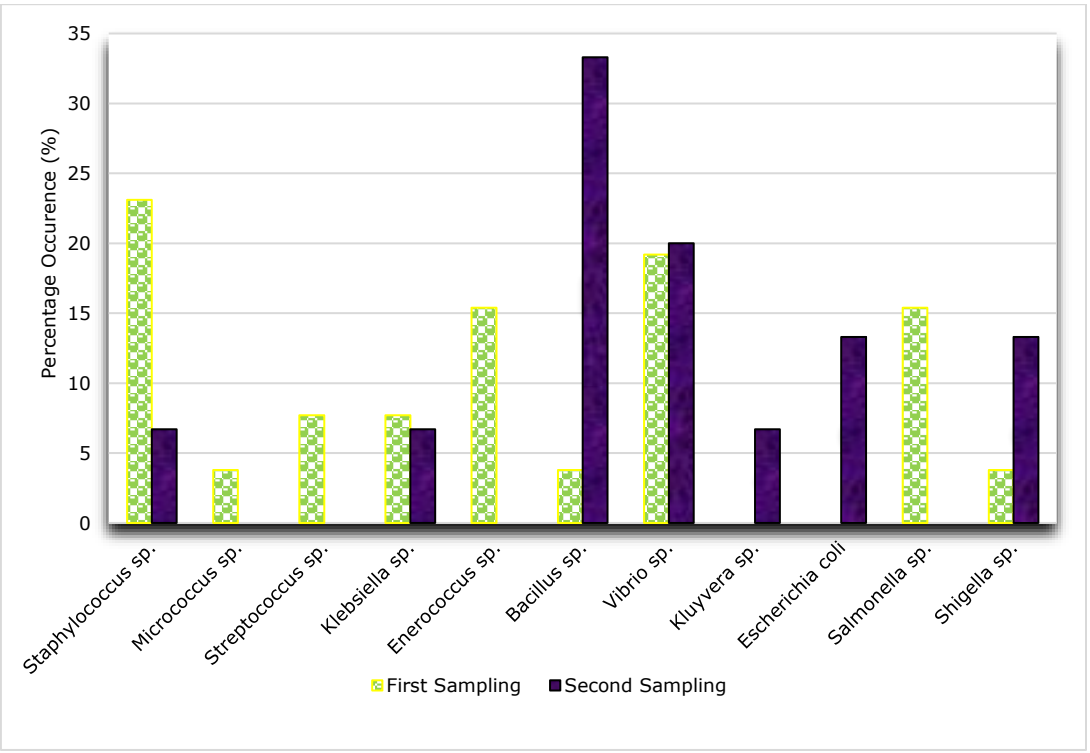

Fig 6: Faecal bacterial isolates from first and second sampling during the study period

Serum electrolytes of rats administered with different smoothie formulations:

Table 5 shows smoothie-dependent variations in the mean values of Na^+, K^+, Cl^-, and HCO_3^-. The electrolytes (Na^+, K^+, and Cl^-) tested were within their respective normal reference ranges across all experimental groups. The mean HCO_3^- level in the control group (24.33±0.33 mEq/L) fell within the normal reference range for rats (23–28 mEq/L), whereas the mean HCO_3^- level in groups 1–4 (18.7–22.7 mEq/L) were comparatively lower than the normal range.

Haematological parameters of rats administered with different smoothie formulations:

The haematological indices of rats fed with the different smoothie combinations showed variable responses compared to the control group (Table 6). Significant (p<0.05) increases were observed in mean total WBC counts (8.8±2.1, 10.4±1.0, 10.6±3.4, and 10.8±4.2×10³/µL for groups 1–4, respectively) relative to the control (4.3±0.8×10³/µL). The mean lymphocyte concentrations were also significantly elevated in the smoothie-fed groups (69.0±0.3, 68.2±0.1, 75.3±1.2, and 69.7±5.1×10³/µL) compared to control (47.5 ±7.4×10³/µL).

The mean haemoglobin (Hb) levels (23.37±8.5, 15.43±0.3, 15.13±0.4, and 13.03± 0.8 g/dL) were higher than control values (12.87±0.6 g/dL), although the differences did not reach statistical significance (p=0.31). Similarly, the mean packed cell volume (PCV) were higher for rats in groups 1–3, but lower for group 4, compared to the control, with a near-significant difference (p=0.0504).

For red cell indices, the mean MCV values were significantly higher for rats in groups 2–4 (63.7±0.3, 62.2±1.2, and 61.7± 0.7 fL) and in the control (61.7±0.9 fL), compared to group 1 (42.8±1.2 fL) (p<0.0001). The mean MCH and MCHC values did not differ significantly across groups (p=0.489 and p= 0.278, respectively). The mean platelet counts, however, were significantly increased in smoothie-fed groups relative to the control (p< 0.0001).

There were no significant changes observed in mean monocytes (p=0.701), basophils (p=0.999), eosinophils (p=0.212), or LIC (p=0.146) levels. Eosinophils were absent in group 2 rats (WMCC smoothie), and LIC was undetected in group 1 rats (BNWM smoothie). The different smoothie combina- tions fed to rats in groups 1–4 significantly (p<0.05) increased the mean values of monocytes (17.7± 0.4, 18.5±0.2, 14.2±0.5, and 19.3±4.7×10³/ µL respectively), basophils (0.12±0.1, 0.4± 3.1, 0.4±3.9, and 0.3±0.3×10³/µL), Hb (23.4 ±8.0, 15.4±0.3, 15.1±0.4, and 13.0±0.7× 10³/µL), and MCH (24.6±5.2, 22.1±0.1, 22.1 ±0.1, and 22.7±0.5 × 10³/µL), compared to the controls.

The MCV was increased only in groups 2, 3, and 4 (63.7±0.3, 62.2±1.2, and 61.7± 0.7 × 10³/µL) and also in control (61.73±0.9), compared to group 1 (42.83±1.2). The mean values of RBC were within normal range of 6.5–9.5 × 10⁶/µL for rats in both experimental and control groups (p=0.6474). Eosinophils were absent in group 2 rats fed with WMCC smoothie combination, while LIC was not detected in group 1 rats fed with the BNWM smoothie combination (Table 6).

Table 5: Mean value (± standard error) of electrolytes of rats fed with different formulation of smoothies

		Electrolyte parameters			
Rat groups	**Different Combination of Smoothie**	**Sodium Na^+ (mmol/L)**	**Potassium K+ (mmol/L)**	**Chloride CL - (mmol/L)**	**Bicarbonate HC03 (mEq/L)**
Group 1	BNWM	137.0 ± 0.3	5.2 ± 0.1	103.3 ± 0.3	18.7 ±0.3
Group 2	WMCC	138.0 ±0.9	4.4 ± 0.1	103.3 ± 0.3	21.3 ± 0.3
Group 3	PPOR	138.33 ± 0.3	4.1 ± 0.1	103.3 ± 0.3	21.7 ± 0.2
Group 4	BNCC	138.67 ±0.3	3.7 ± 0.3	101.3 ± 0.3	22.7 ±0.3
Group 5	Control	140.0 ± 0.1	4.9 ± 0.1	101.7 ± 0.3	24.3 ± 0.3
***p* values**		0.00268	0.0000041	0.000027	0.000001

Table 6: Mean values (± standard error) of the haematological parameters of Albino rat (*Rattus norvegicus*) fed with different combinations of smoothies and the controls

Haematological parameters	Smoothies combinations for experimental group				Control group	*p* values
	BNWM	WMCC	PPO	BNCC		
	Group 1	Group 2	Group 3	Group 4	Group 5	
WBC (× 10^3/µL)	8.8 ± 2.1	10.4 ± 1.0	10.6 ± 3.4	10.8 ± 4.2	4.3 ±0.8	0.2655
Neutrophils (× 10^3/µL)	12.7 ± 0.1	12.2± 2.0	10.1 ± 0.7	10.6 ± 0.8	17.9 ± 2.9	0.02003
Lymphocytes (× 10^3/µL)	69.0 ± 0.3	68.2 ± 0.1	75.3 ±1.2	69.7 ± 5.1	47.5 ±7.4	0.0007
Monocytes (× 10^3/µL)	17.7 ± 0.4	18.5 ± 0.1	14.2 ± 0.5	19.3 ± 4.7	15.4 ±4.4	0.70063
Eosinophil (x 10^3/µL)	0.27 ± 0.1	-	0.1 ± 0.1	0.03 ± 0.1	0.2 ±0.1	0.21187
Basophils (× 10^3/µL)	0.12 ± 0.1	0.4 ± 3.1	0.4 ± 3.9	0.4 ± 0.3	0.55 ±0.1	0.99995
ALY (× 10^3/µL)	0.20 ± 0.1	0.23 ± 0.1	0.07 ± 0.1	0.18± 0.3	0.14 ± 0.1	0.0000
LIC (× 10^3/µL)	-	1.003 ± 0.5	0.9 ± 0.1	0.47 ± 0.2	1.43 ± 0.1	0.14569
RBC (x 10^6/µL)	9.05 ± 2.5	7.18 ± 0.1	6.86 ± 0.1	6.19 ± 0.3	6.69 ± 1.8	0.6474
Hb (× 10^3/µL)	23.37± 8.5	15.43 ± 0.3	15.13 ± 0.4	13.03 ± 0.8	12.87 ± 0.6	0.314
PCV (%)	47.53± 5.0	46.83 ± 2.7	44.6 ± 2.3	38.13 ±1.9	37.3 ± 1.2	0.0504
MCV (× 10^3/µL)	42.83± 1.2	63.73 ± 0.3	62.17 ±1.2	61.73 ±0.7	61.73 ± 0.9	0.0000
MCH (× 10^3/µL)	24.6± 5.2	22.1 ± 0.1	22.7 ± 0.5	16 ± 5.6	21.4 ± 0.5	0.4888
MCHC (× 10^3/µL)	34.7 ± 0.8	34.25 ± 0.6	36.33 ± 0.3	34.6 ± 0.3	31.1 ± 3.5	0.27766
PLT (× 10^3/µL)	554 ± 3.1	557 ± 5.5	635.3 ± 2.9	600.3± 2.8	828.3 ± 4.7	0.0000

WBC- White Blood Cells, ALY – Absolute Lymphocyte Count, LIC – Leukocyte (White Blood Cell) Count, RBC – Red Blood Cell Count, Hb – Haemoglobin, PCV – Packed Cell Volume (Haematocrit), MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular Haemoglobin Concentration, PLT – Platelet Count. BNWM - banana/watermelon, WMCC- watermelon/cucumber, PPOR- pineapple/orange BNCC-banana/cucumber

Discussion:

Vital processes such as hydration, nerve function, muscle contraction, anaemia, kidney diseases, and others are associated with electrolytes and haematological imbalance, a significant occurrence among immuno-compromised and aged individuals. Smoothie made of nutrient-rich ingredients supplies the needed vitamins, minerals, fibers, antioxidants, and essential fatty acids. The administration of banana/watermelon (BNWM), watermelon/cucumber (WMCC), pineapple/orange (PPOR), and banana/cucumber (BNCC) smoothies to experimental Wistar albino rats resulted in varying but generally positive effects on residual feed intake (RFI), residual water intake (RWI), and body weight changes (BWC).

The findings from this study showed a significant mean RFI ($p<0.05$) and higher mean value of RWI (80.8) in the control group compared to the experimental groups. There was a significant weight reduction ($p<0.05$) for group 2 (WMCC) and group 3 (PPOR) and this aligns with findings of Ezeigwe et al., (11). This implies that a smoothie combination made of watermelon/cucumber (WMCC), and pineapple/orange (PPOR) contributed to the weight reduction of the experimental animals in the above-mentioned groups and entails that the combinations can be a good regimen in the management of bodyweight at certain doses. We observed a progressive significant difference in daily food intake (DFI) to daily water intake (DWI) from week two to week four of the experiment. However, this had no reflection on the overall BWC of individual rats. This may mean that the rate of feed versus water intake (DFI to DWI) does not have a direct interpretation of an increase in body weight

Haematological analysis showed variations across groups. Although the smoothie-treated groups tended to have higher haemoglobin, red blood cells, mean corpuscular haemoglobin, and packed cell volume values compared to the control, these differences were not statistically significant ($p>0.05$). However, significant differences were observed in other parameters, such as neutrophils ($p=0.020$), lymphocytes ($p=0.0007$), absolute lymphocyte count (ALY) ($p=0.0000$), mean corpuscular volume (MCV) ($p<0.0001$), and platelets ($p<0.0001$), indicating that the smoothies had measurable effects on certain haematological indices. Whereas in a study by Barrientos et al., (12) on effects of *Citrus sinensis* (orange) and *Lycopersicon esculentum* Miller (tomato) juices on the haematological parameters of *Rattus albus* (Albino rat), a decreased level for all parameters was observed in both smoothie-treated groups and control group. According to Ezeigwe et al., (11), blood indices (MCV, MCH, and RBC) showed no significant differ-

ences when administered lime juice and could imply administration with lime juice at different dosages used does not have any negative impact due to its antioxidant content and it supports haematological stability without altering red blood cell parameters.

Electrolytes are involved in several body processes such as acid-base balance (pH), controlling fluid levels, blood clotting process, nerve conduction, muscle contraction and acting as co-factors or coenzymes (13). In this study, the control group (group 5) without smoothie supplementation had a bicarbonate (HCO_3^-) level of 24.3±0.3 mEq/L, which falls within the normal reference range for rats (23–28 mEq/L). In contrast, groups 1–4 rats showed comparatively lower HCO_3^- values (18.7–22.7 mEq/L), suggesting a trend toward reduced buffering capacity. Since bicarbonate is the major buffer for maintaining pH homeostasis, lower levels may predispose animals to metabolic acidosis, which can affect normal cellular function. In addition, one-way ANOVA showed significant differences ($p<0.05$) in some electrolyte parameters among the groups. Deviation from the normal may lead to metabolic alkalosis. Also, excessive bicarbonate in the blood may result in shallow breathing (hypoventilation), dizziness, confusion, muscle cramps, etc. Electrolytes must be kept at a level that is suitable for normal biochemical and physiological functions (14).

The results of the faecal bacteriology revealed that group 1 recorded the highest bacterial cell count (6.95×10^7 CFU/ml) during the first faecal sampling, while rats in groups 2 (6.8×10^7 CFU/ ml) and 5 (6.65×10^7 CFU/ ml) showed the highest counts during the second sampling. This indicates that bacterial proliferation varied across treatment groups, with certain smoothie combinations supporting higher microbial loads in the gut compared to others. Such variations may reflect differences in the substrates provided for microbial growth.

A total of 41 bacterial isolates in 11 genera/species were identified. *Vibrio*, *Staphylococcus*, and *Bacillus* species were the most frequent in occurrence with 8 (19.5%), 7 (17.0%), and 6 (14.6%) isolates, respectively. *Micrococcus* and *Kluyvera* species were the least, with frequency of 1 (2.4%) isolate each during the experimental period. A comparison of bacterial isolates between the smoothie-treated groups (groups 1–4) and the control (group 5) showed that both have shared and distinct species. *Staphylococcus* spp, *Bacillus* spp, *Vibrio* spp, *Salmonella* spp, *Shigella* spp, and *Enterococcus* spp were consistently present across most groups, including the control. In contrast, additional organisms such as *Micrococcus* spp and *Kluyvera* spp (group 1), *Klebsiella* spp (group 2), *E. coli* (groups 1 and 3), and *Streptococcus* spp (groups 2 and 4) were restricted to the smoothie-fed groups.

These findings suggest that while some bacterial species were common across all groups, smoothie supplementation promoted the emergence of additional organisms and supports the hypothesis that smoothie consumption may positively modulate the gut microbiota of albino rats. Additionally, the isolation of these bacteria may also reflect potential exposure of the animals to contaminated water, food, and poor hygiene conditions. The study by Oguwike et al., (9) on the consumption of uncooked fermented *Pentaclethra macrophylla* Bent and its bacteriological effects on the gastrointestinal tract of male albino Wistar rats highlighted the presence of organisms such as *Staphylococcus* spp, *Micrococcus* spp, and *Bacillus* spp, with higher frequency of occurrence in different parts of the animals.

From this study, a nutrient-dense smoothie combination was found to maintain normal electrolyte ranges in albino rats and to increase the concentrations of hematological parameters, both of which are vital for immune modulation and the overall health of the experimental animals. Precautionary measures should be observed when handling laboratory animals to minimize the risk of zoonotic infections.

Conclusion:

The findings from this study demonstrated that smoothie formulations made from different fruit combinations have beneficial effects on serum electrolyte balance, haematological parameters, and faecal bacteriology in *Rattus norvegicus*. The smoothies effectively maintained electrolyte concentrations within normal ranges, enhanced hematological indices critical for immune function, and contributed to weight management in the experimental animals. Additionally, the presence of isolated bacterial strains suggests that smoothie consumption may positively modulate gut microbiota, potentially reducing the pathogenicity of harmful organisms.

These findings highlight the potential of smoothies as a natural dietary supplement for promoting health, supporting immune function, and managing weight. Further research is recommended to explore their therapeutic applications in human health, particularly for conditions associated with electrolyte imbalances and immune suppression.

Contributions of authors:

EMO conceived and designed the study, supervised the research process, and critically revised the manuscript for intellectual content; AMA conducted the experimental

work, coordinated data collection, drafted sections of the manuscript, and served as the corresponding author; EGA assisted in data analysis, literature review, and preparation of the initial draft of the manuscript; NGC contributed to interpretation of findings, manuscript editing, and provided technical input during revisions; AJO conducted part of the experimental work, results interpretation and provided input during manuscript editing; and AEA contributed to interpretation of findings, data analysis and provided inputs during revisions. All authors approved the manuscript submitted for publication.

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