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Original research paper

EFFECT OF ISOLATED PROBIOTIC *LACTOBACILLUS PLANTARUM* N24 ON GROWTH AND HAEMATOLOGICAL PERFORMANCE IN MALE ALBINO RATS

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Abstract: Lactic Acid Bacteria (LAB) are known probiotic microorganisms whose presence in food such as yoghurt is immensely beneficial. We investigated the potential of *Lactobacillus plantarum* N24 on physical and haematological parameters in Wistar albino rats. Standard microbiological techniques were used to isolate LAB from raw and fermented cow milk. The LAB was screened for strain-level identification using 16S rRNA gene sequencing. Yoghurt was prepared with probiotics using *L. plantarum* N24. *In vivo* assessment of three Groups (G1-G3) of Wistar albino rats (n=9), G1: rats fed with animal feeds, G2: rats fed with animal feeds and prepared probiotic yoghurt, G3: rats fed with animal feeds and live probiotic commercial yoghurt) and were monitored for weight gain, certain haematological parameters and *Lactobacilli* count. Data were analysed using descriptive statistics and ANOVA at *a p*-value of 0.05. This study revealed appreciable weight gain from 11.00±1.00 g on day 7 to 40.00±1.15 g on day 28. A significant improvement in feed intake and feed efficiency ratio was observed in the animals in G2 compared to other groups. The PCV (%), WBC ($x10^9$/L), RBC ($x10^6$/ML) and *Lactobacilli* count ($x10^6$ CFU/mL) were significantly better in G2 (49.7±2.4, 9.1±0.07, 8.71±0.20, 22.0±16.0) than in G1 (39.3±1.5, 7.67±0.19, 8.50±0.50, 0.46±0.04); and G3 (46.3±1.5, 7.30±0.33, 8.63±0.20, and 3.57±0.70, respectively) indicating the potential benefits of *L. plantarum*N24 as probiotics. *L. plantarum* N24 was a suitable probiotic candidate and could be used as a supplement to promote growth and increase or maintain the beneficial gut microflora.

Key words: *Lactobacillus plantarum N24, probiotics, yoghurt, microflora*

INTRODUCTION

Lactic acid bacteria (LAB) are generally considered commensals in the human gastrointestinal system and can provide health advantages when ingested in sufficient quantities (Han, Lee, Lee, & Paik 2020; Guine, Florença, Barroca, & Anjos, 2020). Additionally, they play a significant role in preserving or controlling the gut's microbial environment by lowering intestinal disorders. These microbes are also significant human-derived microorganisms and include members of the genera *Lactobacillus, Enterococcus, Streptococcus, Pediococcus, Lactococcus*, and *Bifidobacteria* group (Vaughan, Heilig, Ben-Amor, & De

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Vos, 2005; Zoetendal, Vaughan, & De Vos 2006; Jang, Lee, & Paik, 2019; Behera, El Sheikha, Hammami, & Kumar 2020). More so, *Salmonella, Shigella, Escherichia coli, Vibrio cholera,* and *Campylobacter* are among the intestinal pathogens with which lactic acid bacteria frequently interact. This interaction may lead to the production of antibiotic substances that inhibit these pathogens. Certain strains of *Lactobacillus* are being researched for their probiotic qualities and are suggested as a preventative treatment for children's severe diarrhoeal illnesses (Delcaru et al., 2016). According to Melia et al. (2017), *Listeria monocytogenes* bacteria can be inhibited by *L. fermentum* L23, while several probiotic *Lactobacillus* strains that have been identified from different milk sources can suppress *E. coli* (Melia, Yuherman, Jaswandi & Purwati 2018), *E. coli* O157, and other bacteria (Purwati, Kurnia, & Pratama, 2019). Products made by the actions of bacteria, yeast, or moulds are known as fermented foods.

According to Lee and Lucey (2010), yoghurt is made by fermenting milk with a starting culture containing *Streptococcus thermophilus* and *L. delbrueckii* ssp. *Bulgaricus*. When the bacteria ferment lactose, lactic acid is produced. Yoghurt's distinctive flavour and texture are enhanced by fermentation and can be consumed by those who are lactose intolerant. Probiotics such as *Lactobacillus* have been linked to numerous beneficial effects on growth performance, and strengthening innate immunity to prevent disease (Safari, Adel, Lazado, Caipang & Dadar, 2016). Studies have shown improved haematological indices and health status when probiotics were administered (Soltani, Kane, Taheri-Mirghaed, Pakzad & Hosseini-Shekarabi 2019; Shehzadi et al., 2023). Blood parameters are crucial for monitoring stress reactions and evaluating human and animal health. Erythrocyte status and oxygen-carrying capacity are specifically known to be indicated by haematological parameters including red blood cell (RBC), packed cell volume (PCV), and white blood cell (WBC). These indices were also checked on experimental animals, such as Wistar albino rats, to ascertain their normal range. Any change from this range pointed to problems with the animals' bodies and health. This study investigated how oral administration of locally isolated *L. plantarum* N24 strains affects the haematological variables and overall health of rats. It also sought to determine how effective probiotics are at promoting growth and its persistence and survival within the gastrointestinal tract.

MATERIALS AND METHODS

Sample collection and bacterial isolation

Raw and fermented cow milk was obtained from the Dairy and Research Farm of the University of Ibadan, and the local market in Ibadan, South-Western Nigeria. Lactic acid bacteria (LAB) were isolated from raw and fermented milk using the de Man, Rogosa, and Sharpe agar (MRS; Difco Laboratories, Detroit, MI) as previously described by Aforijiku and Onilude (2019). The presumeptive LAB was characterised using multiple biochemical parameters and physiological properties and was further subjected to molecular confirmation.

Molecular Characterisation of LAB Isolates

The DNA of the presumptive *L. plantarum* was extracted using a GeneJET Genomic DNA Purification Kit (Thermo-Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. PCR amplification of DNA fragments encoding 16S rRNA was carried out using a GeneAmp 9700 PCR System Thermocycler (Applied Biosystem Inc., USA) as previously described by Aforijiku et al. (2021). The amplicon was separated by gel electrophoresis and purified using 60 µL of 20% (w/v) PEG 8000, 2.5 M sodium chloride. The purified amplicon was sequenced in a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using a Big-Dye terminator v3.1 cycle sequencing kit according to the manufacturer's protocol. Bio-Edit and MEGA 6 bioinformatics tools were used for analysis. The NCBI-BLAST program was used for similarity checks against the GenBank database.

Bacterial strains, product preparation and inoculation

During the experimental inoculation, 24-hour-old broth cultures of *L. plantarum* N24, *L. plantarum* N17, *L. brevis*i N10, and *L. casei* N1, previously identified (Aforijiku, Fakorede & Adediran, 2021) were centrifuged at 8000 rpm for 10 min and pellets were washed with phosphate buffer Saline (PBS) at pH 7.2.

Finally, the pellets were further re-suspended in PBS-pH7.2 and plated to obtain 10^6 CFU/ml. One millilitre of the sample containing *L. plantarum* N24 (10^6 CFU/ml) was used to produce yoghurt utilising the protocol of Rahman, Gul and Farooqi (1999). The commercial yoghurt was made from cow milk using the commercially available yoghurt starter culture Yoghourmet® containing (*Bifidobacterium longum, L. rhamnosus, L. casei, L. helveticus, L. bulgaricus, L. acidophilus*, and *Streptococcus thermophilus*), (Lallemand Company, Canada) as described by the manufacturer's procedures. Moreover, 1 ml (10^6 CFU/ml) of the produced probiotic yoghurt and commercial yoghurt were administered to the rat during the experiment.

Determination of proximate composition

The proximate composition including moisture, ash, protein, fat and carbohydrate of the probiotic and commercial yoghurt were evaluated using the recommended protocol of the Association of Official Analytical Chemists (AOAC, 1990)

Moisture content

The moisture content was determined using the oven method. The weight reduction was measured and documented as the moisture content, following Eq. 1.

$$\%\ Moisture = \frac{weight\ of\ water\ sample}{weight\ of\ sample} x\ 100 \quad (1)$$

Ash content

The ash content was evaluated using direct heating. The weight of the resulting ash was determined by the Eq. 2.

$$\%\ Ash = \frac{weight\ of\ ash}{weight\ of\ sample} x\ 100 \quad (2)$$

Protein content

The crude protein content was evaluated using the macro Kjeldahl method. The nitrogen content in the sample was then determined with the Eq. 3.

$$\%\ Nitrogen = \frac{molar\ mass\ of\ nitrogen\ X\ acis\ cocerntration\ (0.02M)\ X\ volume\ made\ X\ titre\ value}{Sample\ weight\ (2g)\ X\ 10\ X\ 100} \quad (3)$$

Finally, the percentage of crude protein in the sample was calculated using Eq. 4.

$$\%\ crude\ protein = \%\ Nitrogen\ X\ 6.38 \quad (4)$$

Fat content

The fat content was analysed with the Soxhlet solvent extraction technique. The percentage of the fat content was calculated with the Eq. 5.

$$\%\ Fat = \frac{weight\ of\ extracted\ fat}{weight\ of\ sample} x\ 100 \quad (5)$$

Carbohydrate content

The difference determined the total carbohydrate content. The sum of the percentage moisture, ash, and crude protein was subtracted from 100 as described by Ihokoronye and Ngoddy (1985), summarised in Eq. 6:

% Carbohydrate = 100 − (% Moisture + % Fat + % Protein + % Ash) (6)

Determination of the viscosity of yoghurt produced from selected probiotic starters and commercial yoghurt culture

About 20 mL of the yoghurt samples were pipetted into a 50 mL Vitresol Viscometer with digital readout, calibrated in ml per second. The viscometer homogenisation speed was adjusted to 400 revolutions per minute (rpm) for 5 minutes. The homogenate was uniformly dripped through a tube dripper. Thereafter, the homogenate was read out by the Thompson Digital readout attached to the Viscometer. Values in replicates of each reading were read and recorded appropriately.

Feeding trial of prepared probiotic yoghurt

Animal experiment

The animal experiment was carried out using nine male albino Wistar rats of 8 weeks with weights varying between 85-87 g to assess the quality of probiotic yoghurt.

The parameters carried out include weight gain, feed intake, health status, survival and the persistence of the probiotic cultures in the gastrointestinal tract using enumeration procedures.

Sample A: cow milk inoculated with *L. plantarum* N24 was used for the animal studies *Sample* E: cow milk inoculated with commercial starter culture was also used, while rats fed with only water and animal feeds served as control. The care and use of animals were by the guidelines of the Institutional

Ethical Committee, with Ethical License No. UI -ACUREC/App/03/2017/005.

Rat feeding (In vivo analysis of the product)

Nine male Wistar albino rats of adult age were procured at the Veterinary Pathology Department of the University of Ibadan, Nigeria and distributed in three groups of three rats per group. The animals were housed in cages and left to acclimatise for one week under controlled 13 hours light and 11 hours dark cycles, with unrestricted access to food and water. Thirty (30 g) gram of conventional animal feed and water was given to all the animals in Group 1, Group 2 and Group 3 respectively for 28 days. An additional supplement of 1mL of probiotic yoghurt per day was given to animals in group 2. Moreover, 1mL of commercial yoghurt per day was given to animals in Group 3.

Biological evaluation (weight gain, feed intake and feed efficiency ratio) of rat fed with probiotic yoghurt during 28 days

Average feed intake

The feed intake was calculated as the differrence between feed supplied (animal conventional feeds) and the feed not consumed. The weighed portion (30 g) of conventional animal feeds was given to each rat in various groups once daily.

Average weight gain

At the beginning of the experiment, the weight of each rat in different groups was measured in grams which served as the initial weight. The weight was subsequently checked at one-week intervals for 28 days. The weight gain was measured by calculating the difference between the starting body mass of the animals and the subsequent weight recorded for that day.

Average feed efficiency ratio

The Feed Efficiency Ratio (FER) was calculated as gain in body mass (gram) divided by feed intake (gram)

Determination of haematological parameters

Blood samples were collected from the eye of each rat with a capillary tube and dispensed into a sample bottle containing ethylene diamine tetra acetic acid (EDTA), gently mixed by repeated inversion. This was carried out at 7-day intervals for 28 days. The haematological parameters examined are packed cell volume, white blood cell count and red blood cell count.

Packed cell volume (PCV)

The blood sample tube was held at an angle and the microhaematocrit (capillary) tube was introduced. The blood was allowed to track up the tube. It was allowed to continue until the tube was about 3/4 full.

The index finger was placed over the top of the capillary tube before removing it from the sample to prevent it from leaking. While keeping the finger over the end of the tube, the outside of the capillary tube was wiped clean with a piece of tissue.

The capillary tube was sealed with plasticine placed in the haematocrit centrifuge and centrifuged at 10,000 rpm for 5 minutes. The capillary tube containing the blood samples was brought out, and placed in the grove of the reader. The PCV was then determined on the scale.

Total white blood cell (WBC) count

The blood of each experimental animal was diluted at a ratio of 1:20 to give 0.02 mL of blood sample to 0.38 mL of diluting fluid (Turks solution: 2ml glacial acetic acid, 98 ml of distilled water and a tinge of gentian violet).

The haemocytometer was placed on the flat surface of the workbench. The coverslip was placed on the counting chamber. A small drop of diluted blood allowed, hanging from the pipette, to sweep into the counting chamber by capillary action, ensuring that there is no air bubble and no overfilling beyond the ruled area. The counting chamber was left on the bench for 3 minutes to allow the cells to settle.

The cells were observed by placing the counting chamber on the mechanical stage of the microscope and viewed with an x10 objective. The corner squares of the counting chamber were focused on and the white cells were counted schematically, starting from the upper left small square of each Square. The count was repeated in all the four corners of the chamber. The margin rules were applied i.e. counting the cells lying on two adjacent margins and discarding those on the other two

margins. The total white cell count (WBC) was calculated using the formula (7).

WBC = (No. of cells x Dilution factor x Depth factor)/ Area count (7)

where: *Dilution factor* = 20, *Depth factor* = 10, *Area count* = 4

Red blood cell (RBC) count

About 0.02 mL of the rat blood sample was diluted with 4.0 mL of diluting fluid. The counting chamber was thoroughly cleaned, and the coverslip was positioned correctly.

The red blood was mixed and a Pasteur pipette was used to collect the diluted blood. Holding the pipette in the 45 position, the chamber was charged. This was allowed to stand for 5 minutes. The chamber was then placed on the microscope stage and the red cells within the separated squares were counted systematically using x 10 objective.

The number of red blood cells was calculated by Eq. 8:

RBC count = (average RBCs per square) x (dilution factor) x (1/volume of square) (8)

where N= number of cells counted; area of small square A= area of small square=1/400 mm^2; D=diameter of chamber=1/10 mm; V= volume of fluid in the small square=1/400 mm x 1/10 = 1/4000 mm^3; volume of fluid in 80 small squares= 8/4000 mm^3, mm^3 of diluted blood contained N X 4000/80 cells, since dilution was 1:200.

Therefore, 1 mm^3 of blood contained N x 4000/80x 2000 = N X 10,000 cells.

Determination of probiotic cultures in the faeces of the experimental rats during 28 days

On days 7, 14, 21 and 28, freshly voided faecal contents (1 g/rat) were gathered and pooled from each rat. This was done to verify whether the L. species could endure the stress in the gastrointestinal tract (GIT).

The excrement was serially diluted after being homogenised in physiological saline. The diluted homogenates (0.1 ml) were plated on MRS agar to count lactobacilli.

The number of colony-forming units on the plates was measured after the plates were incubated for 48 hours at 37^o C (Oyetayo, Adetuyi & Akinyo-soye, 2003).

Statistical analysis

Data was analysed using descriptive statistics and Analysis of Variance (ANOVA) with Duncan Multiple Range Test for significance at $p \leq 0.05$ according to statistical procedure SAS 2002 of Minitab 14.0 and COSAT 16.0. Means and standard deviation were also presented. Data were also presented in table

RESULTS

Proximate analysis of yoghurt produced with selected probiotic starters and commercial yoghurt culture

The proximate analysis of the yoghurts produced with individual selected probiotic starters *L. plantarum* N24, *L. plantarum* N17, *L. brevis* N10, *L. casei* N1, were compared with the commercial yoghurt culture comprising (*Bifidobacterium longum, L. rhamnosus, L. casei, L. helveticus, L. bulgaricus, L. acidophilus,* and *Streptococcus thermophilus*) (Table 1).

The moisture content ranged bet-ween 85.16% and 86.75%. The highest mois-ture content (86.75%) was observed in sample C but was not significantly different at $P \leq 0.05$ from samples A, and B, while the least was in samples E at 85.16%.

Sample E had the highest protein content (4.43%), but was not significantly different at $P \leq 0.05$ from samples A, B and D while the least was sample C (3.84%). Sample A had the highest fat content of 4.95% and the least (3.51%) carbohydrate content when compared to other probiotics.

Viscosity (mPa.s) of probiotics and commercial yoghurt produced with different starter cultures using cow

As indicated in Table 2, the viscosity of the yoghurt made with the commercial yoghurt culture (sample E) and the yoghurt made with the single strain of *L. plantarum* N24 (A), *L. plantarum* N17 (B), *L. brevis* N10 (C), *L. casei* N1 (D), were also contrasted.

The yoghurt samples had viscosities ranging from 30.30 to 46.90±0.28 mPa.s. In contrast to the lowest viscosity (30.30 mPa.s) generated from commercial yoghurt culture sample C

Table 1.
Proximate analysis of yoghurt produced from cow milk with selected probiotic starters and commercial yoghurt culture

Sample	**Proximate content** (%)				
	Moisture	**Fat**	**Protein**	**Ash**	**Carbohydrates**
A	86.52±0.04^{a}	4.95±0.02^{a}	4.23±0.90^{a}	0.87±0.01^{b}	3.51±0.31^{d}
B	86.63±0.05^{a}	4.86±0.01^{b}	4.13±0.06^{b}	0.82±0.05^{c}	3.56±0.02^{d}
C	86.75±0.04^{a}	3.61±0.02^{e}	3.84±0.07^{c}	0.90±0.21^{ab}	4.91±0.02^{c}
D	85.22±0.02^{b}	4.12±0.02^{c}	4.32±0.06^{a}	0.93±0.01^{a}	5.40±0.04^{b}
E	85.16±0.02^{b}	3.91±0.03^{d}	4.43±0.08^{a}	0.80±0.02^{d}	5.71±0.06^{a}

Data presented as means of triplicates + standard deviation; $^{a, b,...}$ Means denoted with the same letters within a column are not significantly different at P≤0.05 according to the Duncan Multiple Range Test (DMRT) for separation of statistically significant means; A-Yoghurt produced from L. plantarum N24 using cow milk; B-Yoghurt produced from L. plantarum N17 using cow milk; C-Yoghurt produced from L. brevisN10 using cow milk; D-Yoghurt produced from L. casei N1 using cow milk; E-Yoghurt produced from commercial yoghurt culture using cow milk

Table 2.
Viscosities (mPa.s) of yoghurt produced using probiotics starter and commercial stater culture

Samples	**Viscosity** (mPa.s)	**Viscosity range**
A	34.65±0.21^{b}	+
B	41.70±0.14^{a}	+
C	46.90±0.28^{a}	+
D	31.40±0.14^{c}	+
E	30.30±0.10^{c}	+

Data presented as means of triplicates + standard deviation; $^{a, b, c}$ Means denoted with the same letters within a column are not significantly different at P≤0.05 according to the Duncan Multiple Range Test (DMRT) for separation of statistically significant means; Viscosity range = 28-50 mPa.s, ≥ 28 = viscous, + = All samples are within the viscosity range; A-Yoghurt produced from L. plantarum N24 using cow milk; B-Yoghurt produced from L. plantarum N17 using cow milk; C-Yoghurt produced from L. brevisN10 using cow milk; D-Yoghurt produced from L. casei N1 using cow milk; E-Yoghurt produced from commercial yoghurt culture using cow milk

Table 3.
Biological evaluation of rat fed with regular animal feed, probiotic yoghurt produced with *L. plantarum* N24 and yoghurt made with a commercial starter culture

Treatments	**Parameters**			
	Time intervals (days)	**Average weight gain** (g)	**Average feed intake** (g)	**Average FER**
Group 1		6.67±0.58^{b}	7.67±0.58^{a}	0.87±0.01^{b}
Group 2	7	11.00±1.00^{a}	8.00±0.00^{a}	1.38±0.12^{a}
Group 3		10.67±1.50^{a}	8.00±0.00^{a}	1.33±0.14^{a}
Group 1		10.33±1.50^{c}	9.33±1.15^{c}	1.11±0.13^{a}
Group 2	14	14.33±1.53^{a}	13.33±1.15^{a}	1.08±0.10^{a}
Group 3		13.00±1.73^{b}	11.00±1.00^{b}	1.20±0.20^{a}
Group 1		15.00±1.00^{c}	12.33±0.57^{c}	1.22±0.04^{b}
Group 2	21	25.33±1.15^{a}	17.67±0.57^{a}	1.57±0.27^{a}
Group 3		19.00±1.73^{b}	14.67±1.52^{b}	1.31±0.21^{b}
Group 1		19.33±0.58^{c}	11.67±0.57^{c}	1.66±0.13^{b}
Group 2	28	40.00±1.15^{aA}	20.00±1.73$^{a\,A}$	2.02±0.25$^{a\,A}$
Group 3		28.67±2.00^{b}	17.00±3.00^{b}	1.71±0.21^{b}

FER-feed efficiency ratio; Data presented as means of triplicates + standard deviation; $^{a, b, c}$ Means denoted with the same letters within a column are not significantly different at P≤0.05 according to the Duncan Multiple Range Test (DMRT) for separation of statistically significant mean; ^{A}Very significant within all treatments at all-time intervals which was the best

had the highest value (46.90±0.28 mPa.s). Though the degree of viscosity varied, all samples were viscous.

Biological evaluation of rats fed with probiotic yoghurt, commercial yoghurt and standard feed

The average weight gained varied from 6.67 to 11.00 g on the 7th day to 19.33± 0.58 to 40.00± 1.15 g on the 28th day with group 2 showing the highest average weight gain of (40.0 ± 1.15 g), which was very significant compared to the comparable groups. Group 1 showed the lowest average weight gain (19.33 ± 0.58 g) (Table 3).

Additionally, on the seventh day, the average feed intake varied from 7.67 ± 0.58 to 8.00 ± 0.00 g. Group 2 and Group 3 had the highest average feed intake (8.00 ± 0.00 g), whereas Group 1 had the lowest (7.67 ± 0.58 g).

There was no significant difference (P<0.05) in the average weight gain between the experimental groups. The average feed intake for each experimental group increased by the 14th, 21st and 28th day with the Group 2 exhibiting the highest values of 13.33±1.15, 17.67±0.57, and 20.00±1.73, respectively (Table 3).

Moreover, the Feed Efficiency Ratio (FER) as shown in Table 3 revealed that on the seventh day of feeding, Group 2 had the highest (1.38 ± 0.12), which was not significantly different from Group 3 (1.33 ± 0.14) but was both statistically different from Group 1 with the least value (0.87 ± 0.01) at P ≤ 0.05. Nonetheless, during the 21-day feeding period, the average FER varied from 1.22 ± 0.04 to 1.57 ± 0.27. Group 2 had the highest average efficiency ratio (1.57 ± 0.27), whereas Group 1 had the lowest (1.22 ± 0.04). According to the statistical study, average FERs of Group 1 and Group 3 differed from that of Group 2 but were not substantially different at P < 0.05.

Haematological parameters

The findings of the haematological study of the Wistar rats in all three groups over 28 days are displayed in Fig. 1.

The mean packed cell volume (PCV) for experimental animals in group 1 ranges from 40.33%, 40.49%, 40.75% and 40.93%, group 2 ranges from 40.33%, 44.67%, 47.33% and 49.67%. In contrast, animals in Group 3 had their PCV ranging from 37%, 44%, 45.67% and 46.33% on the 7th, 14th, 21st and 28th day, respectively. Animals belonging to Groups 1 and 2 reached the highest percentages (at 40.33% level each) on day 7. These groups were not significantly different from each other (P < 0.05), but they were significantly different (P ≥ 0.05) from Group 3 animals, which exhibited the lowest value, at 37.00 ± 0.00%).

Fig. 1 also displays the experimental rat Red Blood Cell (RBC) count after 28 days of eating. On the seventh day, the experimental rats in Group 2 had the highest RBC count (6.67 x 10^6/ML), while Group 3 was the lowest.

Additionally, the statistical analysis showed that, at P < 0.05, there was no significant difference between groups 1, 2, and 3. Nevertheless, on every sampling day, the RBC counts of the experimental groups' values did not differ significantly, with Group 2 having the highest RBC count. The experimental rat groups' white blood cell (WBC) counts are displayed in Fig. 1. On the seventh day of feeding, Group 2 had the lowest value (6.28 x 10^9/L), whereas Group 3 had the greatest value (11.30 × 10^9/L). Additionally, the statistical analysis revealed that Group 3 differed considerably from the other experimental groups. Group 2's WBC reached its maximum at day fourteen, 8.88 x 10^9/L, although it was still within the rat's haematological reference range. According to the statistical analysis, Groups 2 and 3 did not differ significantly from one another; however they differed considerably from Group 1, which had the lowest value (5.45 x 10^9/L). Although Group 3 had the greatest WBC value (8.43 x 10^9/L) and Group 2 had the lowest (7.81 x 10^9/L) at 21 days of feeding, there was no significant difference between any of the experimental groups at P < 0.05. Additionally, on the 28th day of feeding, the WBC count increased in Group 2. Group 2 had the greatest WBC count (9.13 x 10^9/L), and was substantially different from the Group 3's lowest result (7.30 x 10^9/L).

Survival or persistence of probiotic culture in the gastrointestinal tract

The amount of *Lactobacilli* in the faeces of the experimental rat groups was counted at 7-day intervals throughout 28 days to determine their capacity to survive or persist in the gastrointestinal tract.

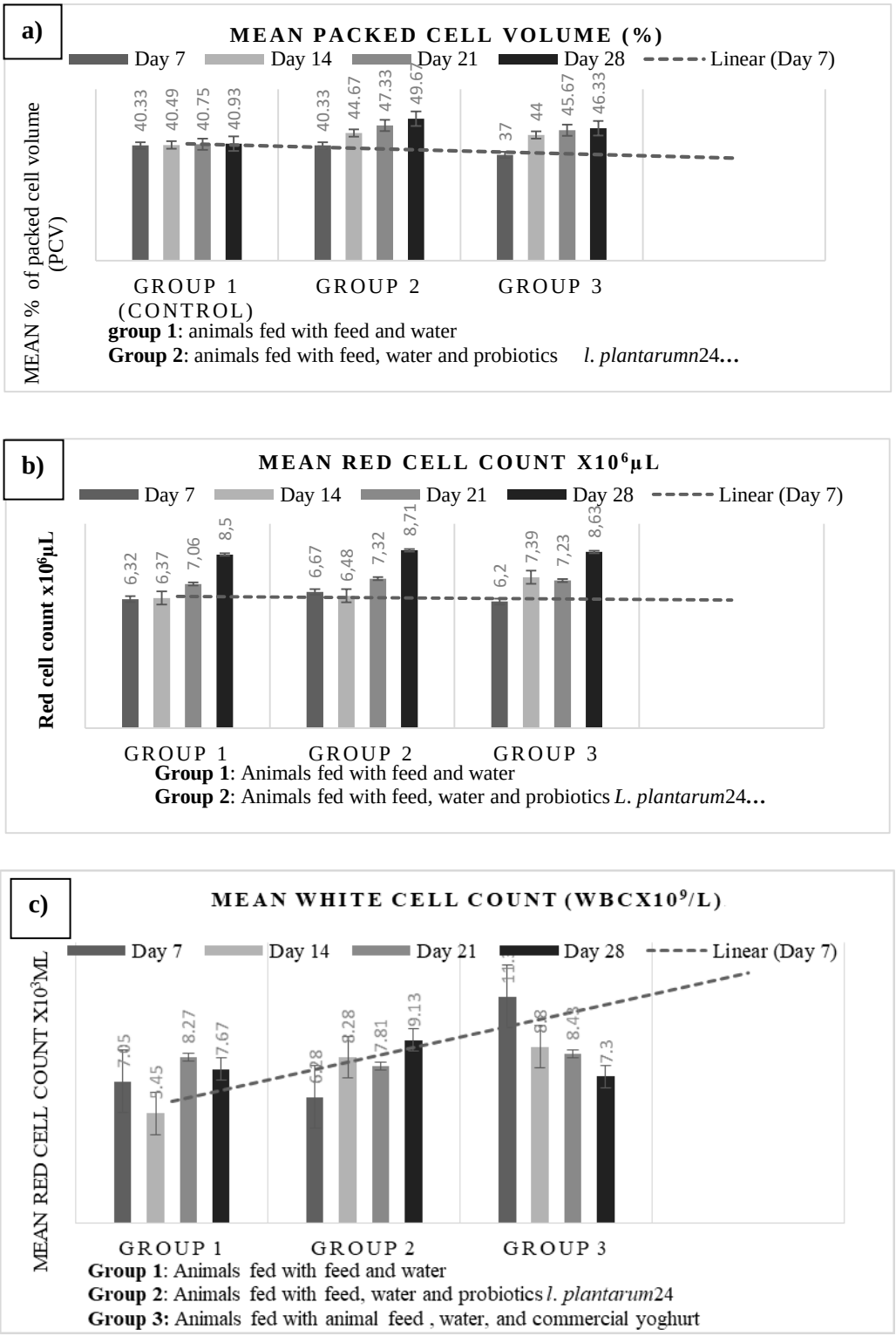

Figure 1. The mean packed cell volume (a), red cell count (b) and total white cell count (c) of the experimental animals on days 7, 14, 21 and 28. Readings of each parameter were recorded in triplicates

Although group 3 showed the greatest number of lactic acid bacteria at day 0 ($0.40x10^6$ CFU/mL), statistical analysis revealed that there was no significant difference between them ($P \leq 0.05$) Table 4. Additionally, Table 4 demonstrates that on the seventh day of feeding, the group provided animal feeds, water, and probiotic yoghurt containing *L. plantarum* N24 (Group 2) had the highest count ($1.8x10^6$ CFU/mL).

Table 4
Determination of faecal *Lactobacilli* count among the different groups of experimental animals ($x10^6$ CFU/mL)

Treatments	Time (days)	Lactobacilli count
Group1	0	0.38 ± 0.06^{a}
Group2		0.39 ± 0.06^{a}
Group3		0.40 ± 0.07^{a}
Group 1	7	0.58 ± 0.05^{b}
Group 2		1.80 ± 1.06^{a}
Group 3		0.38 ± 0.03^{b}
Group 1	14	0.41 ± 0.10^{b}
Group 2		3.23 ± 1.60^{a}
Group 3		1.76 ± 1.00^{a}
Group 1	21	0.39 ± 0.09^{c}
Group 2		11.6 ± 0.86^{a}
Group 3		4.14 ± 3.2^{b}
Group 1	28	0.46 ± 0.04^{c}
Group 2		$22.0^{*}\pm 16.0^{aA}$
Group 3		3.57 ± 0.70^{b}

*Data presented as means of triplicates + standard deviation; $^{a, b, c}$Means denoted with the same letters within a column are not significantly different at P≤0.05 according to the Duncan Multiple Range Test (DMRT) for separation of statistically significant means; ^{A}Very significant within all treatments at all-time intervals which was the best; * 22.0 indicates 2.2 x 107 CFU/mL; Group 1(control): Rats fed with animal feeds and water. Group 2: Rats fed with animal feeds, water and probiotic yoghurt produced with L. plantarum N24. Group 3: Rats fed with animal feeds, water and yoghurt produced with commercial starter culture*

Statistical analysis showed that Group 2 differed significantly from the other matching control groups ($P \leq 0.05$). Additionally, on the 21st day of feeding, comparing Group 2 to Groups 1 and 3, the faecal lactic acid bacteria count rose dramatically to $11.6x10^7$ CFU/mL. On day 28, Group 2 also showed a significant rise compared to the other groups ($P \leq 0.05$).

DISCUSSION

Animal models, *in vitro* tests, and human clinical studies have all shown the efficacy of probiotics and their potential health advantages (James & Wang, 2019). Probiotic bacteria are crucial for the development of gut flora and are responsible for producing probiotic foods. Yoghurt is one of the most widely consumed fermented dairy products due to its nutritional value, health advantages, and sensory qualities; its usage is rising globally. In this study, we produced probiotic yoghurt using *L. plantarum* N24, *L. plantarum* N17, *L. brevis*i N10, and *L. casei* N1 isolated from raw cow milk. The potential benefit of the probiotic yoghurt made using *L. plantarum* N24 on some haematological parameters, physical attributes, health status and its persistence and survival within the gastrointestinal tract was evaluated against yoghurt prepared with a commercially available starter culture in experimental animals. The choice of *L. plantarum* N24 was based on its good proximate values, viability during cold storage, survival in acidic pH, and bile salts and also had above 40% cell surface hydrophobicity, suppressed the growth of some selected food-borne pathogens inoculated in yoghurt within 48 h, and also proved to be safe, being a non-producer of gelatinase and DNAse.

In this study, the proximate content of the probiotic yoghurt produced with *L. plantarum* N24 revealed an increase in the moisture, ash, fat and protein content when compared to the yoghurt with the commercial starter culture. The moisture and protein content recorded in this study was also higher when compared to those reported by Hossain, Hoque, Ahmed and Ahmed (2024), but lower when compared to

the fat, ash and carbohydrate contents reported by the same author. The observed increase in the moisture content may be attributed to the fact that *L. plantarum* N24 does not produce significant amounts of exopolysaccharides resulting in syneresis which may lead to an increase in moisture content. The low carbohydrate content seen in this study can also be linked to a number of factors. During fermentation, *L. plantarum* N24 convert lactose into lactic acid, which lowers the pH of the yoghurt. As lactose is consumed during fermentation, the overall carbohydrate content of the yoghurt decreases (Hoxha, Evstatieva & Nikolova, 2023). In some cases, lactic acid bacteria possess enzymes capable of breaking down complex carbohydrates, such as starches or dietary fibres. During storage, these enzymes may hydrolyse complex carbohydrates into simpler sugars, which can then be utilized by the bacteria or further metabolized. This breakdown of complex carbohydrates can contribute to a decrease in the overall carbohydrate content of probiotic yoghurt (Wang, et al., 2021).

In this present study, indigenous selected probiotic starter (*L. plantarum* N24) was able to produce higher average viscosities of 34.65±0.21 mpa.s when compared to the commercial starter culture 'yoghourmet' containing (*Bifidobacterium longum, L. rhamnosus, L. casei, L. helveticus, L. bulgaricus, L. acidophilus*, and *Streptococcus thermophilus*) with viscosity of 30.30±0.10 mpa.s which occurs at pH just above the dairy milk isoelectric point (pH 5.2) and the observed viscosities are indicative of gelation.

The viscosity recorded in this study for both the probiotic and commercial yoghurt were however, below the 1.77 Pa.s average viscosity of the commercial yoghurt as reported by Ares et al. (2007). Although, the results, based on the reference value of the viscometer indicate the viscosity of both the probiotics and the commercial starter culture are within the reference range of 28-50 mpa.s.

The result of the viscosity in this study was at variance and considered lower when compared to the 2.307 Pa.s to 1.058 reported by Dahlan and Sani, (2017) and 560 mpa.s and 615 mpa.s reported by Dan et al. (2023). Stirring, heat treatment, pH, duration of fermentation, exopolysaccharides production, and formulation of starter culture and dry matter content may have been responsible for the difference in value of the viscosity.

In this study, the viscosity was read within 2 hours of production and the probiotic yoghurt still outperformed the commercial yoghurt with combined starter culture which is an indication of the higher viscosity capabilities of *L. plantarun N24* above the commercial combined formulation of *Bifidobacterium longum, L. rhamnosus, L. casei, L. helveticus, L. bulgaricus, L. acidophilus,* and *Streptococcus thermophile*. This shows that there is no correlation between single-strain cultures, combined formulation and viscosities (Aktas, Ürkek, Aktaş, Çetin & Şengül 2023).

The proximate contents and quality of the probiotic yoghurt (cow milk inoculated with *L. plantarum* N24) with the viability of the probiotic cultures showed that it supported the growth and development of the rats in terms of weight gain, increased feed intake with a good efficiency ratio. The statistical analysis showed that Group 2 weight growth differed considerably ($P < 0.05$) from the other experimental animals and comparable groups. Over 28 days, the average weight gain of the animals in Group 2 rose from 11.00±1.00 g on day 7 to 40.00±1.15 g on day 28, an average feed intake of 8.00±0.00g on day 7 increasing to 20.00±1.73 g on day 28, with an average feed efficiency ratio which also rose from 1.38±0.12 g on day 7 to 2.02±0.25 g on day 28. These results were significantly higher when compared to animals in control group 1 with an average weight of 6.67±0.58 g on day 7 to 19.33±0.58 g on day 28, an average feed intake of 7.67±0.58 g on day 7 to 11.67±0.57 g on day 28 with increased feed efficiency ratio of 1.66±0.13 g from 0.87±0.01 g and the animals in Group 3 given commercial yoghurt as supplement having an average weight gain of 28.67±2.00 g, feed intake of 17.00±3.00 g and feed efficiency ratio of 1.71±0.21 g on day 28 from weight of 10.67±1.50 g, feed intake of 8.00±0.00 g and efficiency ratio of 1.33±0.14 g on day 7.

The results of the present study showed that both the probiotic yoghurt inoculated with *L. plantarum N24* and the commercial yoghurt with combined starter improved the feed utilisation and growth performance parameters in the experimental animals. This might be in part

due to an attribution of the increased digestibility coefficient of the diet and modulating the digestive physiology of the experimental animals by enhancing intestinal enzyme activity which can improve the growth of the rats fed with probiotic yoghurt. There is also the possibility of *L. plantarum* N24 suppressing the growth and activities of depressing microflora enhancing their feeding capabilities. The ability of probiotic food to improve body weight, feed intake and feed efficiency ratio has also been reported by other workers (Bibi, Ashraf, Shehzadi, Rehman & Bukhari 2023; Soltani et al., 2019; Aboderin & Oyetayo, 2006).

Haematological parameters are important indicators of the general health condition of experimental animals. This study showed that the experimental animals in control Group 1, animals supplemented with probiotic yoghurt in group 2 and the animals supplemented with commercial yoghurt with formulated starter culture in group 3 had improved health status. The packed cell volume (PCV) of control group 1 had no significant increase in their PCV of 40.44% on day 7 to 40.93 by the end of the 4th week. Although, there was little improvement and consistency in the increase of the red cell count (6.32±0.03, 6.37±0.09, 7.06±0.19, and 8.50±0.50) at weekly intervals throughout 4 weeks. The marginal improvement can be attributed to the fact that the results of the PCV are directly proportional to the availability of the red blood cells.

In this study, the results of the haematological studies revealed that the experimental animal dose with yoghurt containing *L. plantarun* N24 showed signs of improved health based on their haematological status with a substantial increase in the packed cell volume (PCV) and the red cell count (RCC) and performance in term of weight gain. The result revealed an increase in PCV from 40.33% to 49.67% with a concomitant increase in the red cell count from 6.67±0.36 to 8.71±0.20 from the first week to the fourth week. This result is in line with the report of other studies. In a similar study, de Carla Dias et al. (2020) reported that the concentration of haemoglobin, PCV, and RBC rose in Wistar albino rats who received oral doses of *L. plantarum*. The activation of haematopoietic organs and certain lactic acid bacteria, such as *Lactobacilli*, was also documented by Korčok, Tršić-Milanović, Ivanović and Đorđević, (2018) to indirectly raise the availability of dietary iron through several mechanisms, including lowering intestinal pH, resulting in increased red cell count and packed cell volume.

According to Obazelu, Aruomaren and Nwangwu (2021), probiotics enhanced health outcomes by increasing red blood cell count, packed cell volume, and haemoglobin levels without negatively altering blood haematological parameters which is consistent with the report of this study. The total white blood cell (WBC) count showed inconsistency in the value of the results within the 4 weeks of treatment. However, the counts were within the reference value. For instance, the WBCs for group 1 was 7.05±0.61on on day 7, dropping to 5.45±0.43 which was below the reference value by the second week. The WBCs rose to 8.27±0.03 (10^9/L) by the 3rd week and finally dropped to 7.67±0.19 by day 28. Similarly, Wistar albino rats who received oral doses of *L. plantarum* N24 had a similar trend in their white blood cell count from 6.28±1.27 on day 7, 8.28±0.44 on day 14, dropped to 7.81±0.53 on day 21, and rose to 9.13±0.07 there was the slight difference in the value of the WBCs. However, the differences were not too significant. However, the observable improvement in WBCs of Wistar albino rats who received oral doses of *L. plantarum* N24 is evidence of the fact that probiotics have immunostimulatory effects.

Probiotics are becoming a more appealing option for avoiding infectious diseases. *Lactobacilli* are advantageous to the host and are a component of the commensal microflora in both humans and rats. The effects of various *Lactobacilli* strains most likely vary from one another. Rats fed *L. plantarum* N24 throughout the inoculation days in this investigation showed a considerable increase in the number of *Lactobacilli* count in their faeces from 0.39±0.06 on day zero to 22.0± 16.0 on day 28 when compared to the control group with *Lactobacilli* count of 0.38± 0.06 on day zero to 0.46 ±0.04 on day 28 and Group 3 feed with commercial starter culture having a *Lactobacilli* count of 0.40±0.07 on day zero to 3.57 ± 0.70 on day 28. The remarkable improvement in the *L.* count of the Wistar rats in Group 2 fed with the probiotic may be attributed to the improvement and interaction of *L. plantarum* N24 with the gut immune sys-

tem, enhancing intestine health and preventing systemic chronic low-grade inflammation, with a possible elevation of intestinal level of polyamines which is generated by the colonic microbiota which is activated by the probiotics *L. plantarum* N24 which on the long run may lengthen life expectancy (Kibe et al., 2014).

CONCLUSIONS

This study has once again demonstrated the potential of probiotics in improving the Health status of experimental animals and, by extension, human health. This study revealed that the Wistar rats fed with probiotic yoghurt (cow milk inoculated with *L. plantarum* N24) resulted in progressive weight gain, increased feed consumption, a good feed efficiency ratio, and improved haematological parameters. The study also revealed an excellent improvement and interaction of *L. plantarum* N24 with the gastrointestinal tract by maintaining stability and supporting intestinal health.

AUTHOR CONTRIBUTIONS

Conceptualisation, S. A.; Methodology, S.A., C.O.F., I.O. and A.A.; Investigation, formal analysis, validation, writing-original draft preparation, S.A.; Writing-review and editing, C.O.F.; Supervision, S.A. and I.O.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. No funding was received in the course of this study.

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EFEKAT IZOLOVANOG PROBIOTSKOG SOJA *LACTOBACILLUS PLANTARUM* N24 NA RAST I HEMATOLOŠKE PERFORMANSE MUŽJAKA ALBINO PACOVA

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Sažetak: Bakterije mlečne kiseline (LAB) su poznati probiotski mikroorganizmi čije prisustvo u hrani, poput jogurta, pruža ogromne koristi. Istraživali smo potencijal *Lactobacillus plantarum* N24 na fizičke i hematološke parametre albino Wistar pacova. Za izolaciju LAB iz sirovog kravljeg mleka korišćene su standardne mikrobiološke tehnike, dok je identifikacija na nivou sojeva sprovedena sekvenciranjem 16S rRNA gena. Probiotski jogurt je pripremljen korišćenjem *L. plantarum* N24. U *in vivo* proceni, tri grupe (G1–G3) albino Wistar pacova (n = 9) posmatrane su na sledeći način: G1: pacovi hranjeni životinjskom hranom; G2: pacovi hranjeni životinjskom hranom i pripremljenim probiotskim jogurtom; G3: pacovi hranjeni životinjskom hranom i komercijalnim jogurtom sa živim probioticima. Ogledni pacovi su praćeni u pogledu porasta težine, određenih hematoloških parametara i broja laktobacila. Podaci su analizirani korišćenjem deskriptivne statistike i ANOVA pri p-vrednosti od 0,05. Studija je pokazala značajan dobitak težine, sa 11,00 ± 1,00 g posle 7 dana; do 40,00 ± 1.15 g posle 28 dana. Kod životinja u grupi G2 primećeno je značajno poboljšanje u unosu hrane i odnosu efikasnosti ishrane u poređenju sa ostalim grupama. Parametri kao što su PCV (%), WBC ($x10^9$/L), RBC ($x10^6$/ML) i broj laktobacila ($x10^6$ CFU/mL) bili su značajno bolji u G2 (49,7 ± 2,4, 9,1 ± 0,07, 8,71 ± 0,20, 22,0 ± 16,0) nego u G1 (39,3 ± 1,5, 7,67 ± 0,19, 8,50 ± 0,50, 0,46 ± 0,04) i G3 (46,3 ± 1,5, 7,30 ± 0,33, 8,63 ± 0,20, i 3,57 ± 0,70, redom), što ukazuje na potencijalne koristi *L. plantarum* N24 kao probiotika. Zaključno, *L. plantarum* N24 predstavlja prikladnog kandidata za probiotik i mogao bi se koristiti kao dodatak za podsticanje rasta i održavanje ili povećanje korisne crevne mikroflore.

Ključne reči: *Lactobacillus plantarum N24, probiotici, jogurt, mikroflora*

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