



SYNERGISTIC BIOGENIC SYNTHESIS OF ZINC NANOPARTICLES BY PROBIOTICS *SACCHAROMYCES CEREVISIAE* AND *LACTOBACILLUS FERMENTUM* FOR FARM ANIMAL HEALTH AND NUTRITION

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Abstract

Probiotics, such as *Lactobacillus fermentum* and *Saccharomyces cerevisiae*, play a crucial role in catalyzing the biogenesis of nano-minerals, offering a transformative solution to enhance nutrient absorption in farm animals and promote agricultural sustainability. This research aims to synthesize ZnO nanoparticles using Probiotic *Lactobacillus fermentum* and *Saccharomyces cerevisiae*, assessing their antibacterial efficacy against MDR pathogens and investigating the impact of Nano zinc supplementation on growth and blood parameters in goats. Utilizing analytical methods such as Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), X-ray diffraction analysis (XRD), and ultraviolet–visible spectroscopy (UV–Vis), the produced ZnO NPs were characterized, revealing distinct bonds and structural features. ZnO nanoparticles, highlighted through FTIR (emphasizing N-H stretching at 1450-1500 cm⁻¹ and ZnO nanoparticle stretches at 400-800 cm⁻¹), SEM (revealing micro-sized bioconjugates with dimensions of 100–120 nm), and XRD (confirming a hexagonal crystal structure at approximately 36.50 degrees), demonstrated significant antibacterial activity. Goats treated with ZnO nanoparticles exhibited substantial weight

gain over 12 weeks (Initial BW: 29.2 ± 0.48 kg; Final Body Weight - Treatment C: 40.99 ± 0.58 kg, ZnO-1: 43.39 ± 0.45 kg, ZnO-2: 44.05 ± 0.43 kg), with limited physiological effects, except for notable variations in immunoglobulins (IgA, IgG) among treatment groups ($p = 0.006$ and $p = 0.003$, respectively). Notably, ZnO nanoparticles displayed antibacterial activity (ZnO-1 against *E. cloacae*: 13mm, *A. baumannii*: 12mm; ZnO-2 against *S. aureus*: 12mm, *E. cloacae*: 13mm, *P. aeruginosa*: 12mm), indicating their remarkable antimicrobial potency. This environmentally friendly and cost-effective approach to synthesizing ZnO NPs holds promise for diverse biomedical applications.

Keywords: Probiotic, nanoparticle, ZnO, antibacterial activity *Lactobacillus fermentum*, and *Saccharomyces cerevisiae*.

1. INTERODUCION

In Pakistan, the livestock industry plays a crucial role in meeting the protein demand and improving the socio-economic status of numerous families [1]. With over 11.5% contribution to the national GDP, it has become an increasingly profitable enterprise, especially after the COVID-19 pandemic [2]. As a result, there is growing interest in sustainable farming practices such as feed additives and feeding strategies. Nutrition is essential for the growth, immune function, and reproductive health of animals [3]. Progress in animal nutrition studies has resulted in customized diets and feed compositions that fulfill specific nutritional needs, promote optimal growth, and address reproductive health under different management and stress conditions [4]. Furthermore, incorporating feed supplements like probiotics can improve nutrient absorption, gut well-being, and overall animal performance [5]. Nanotechnology has become a prominent technology over the past ten years, revolutionizing various fields of applied science [6]. Nanoparticles are a key aspect of nanotechnology, consisting of nanoscale materials with particle sizes ranging from 1 to 100 nm. Their small size and high surface area to volume ratio result in unique properties different from their larger counterparts [7]. Zinc oxide nanoparticles have shown potential in biological applications, particularly as antimicrobial agents. Furthermore, there is evidence suggesting that integrating NPs into feed could improve zinc absorption and use in the body, leading to enhanced health and productivity [8]. Additionally, ZnO NPs have shown promise as dietary supplements for poultry and livestock industries by improving growth performance and immune response while also enhancing antioxidative properties. The conventional physical and chemical processes used for synthesizing ZnO NPs pose environmental concerns due to toxic chemicals involved in addition to being unfavourable economically [9]. This limitation prompts the exploration of cleaner alternatives such as biological synthesis using microbes like bacteria, yeast or algae which offer non-toxic methods at an affordable cost [10]. However these questions need further research especially concerning finding more potential microbes optimized for this specific process before implementation on large scale. Moreover, zinc oxide is one among most examined metals oxides owning semiconductor abilities characterized by deep borderline ultraviolet absorption therefore always been subject matter under detailed investigations [11]. Zinc oxide nanoparticles have shown great potential for various industrial applications, such as in pharmaceuticals, cosmetics, photocatalysts, UV light emitting devices, and agriculture [12]. Zinc plays a crucial role in physiological processes in humans, animals, and plants and is present in body tissues including muscles, bones, and skin [13]. It also functions as an integral component of numerous enzyme structures and contributes to hormone secretion, growth, reproduction the immune system, and antioxidant defense [14]. ZnO NPs are considered eco-friendly materials with biocompatibility and biodegradability; they have been recognized for their bio-safety traits by the US Food and Drug Administration [15]. Studies have demonstrated the non-toxic properties of ZnO NPs at certain concentration levels. Biologically mediated synthesis methods do not involve hazardous chemicals or materials, giving assurance of their safe application in living organisms [16]. ZnO NPs have greater efficiency due to their high surface-to-volume ratios, replacing bulk zinc oxide widely within various applications [17]. As

biological synthesis techniques advance, ZnONP usage is expanding into biomedical applications, such as nutrition improvement in human health and in animal husbandry [18]. For example, its significant antimicrobial properties make it a promising disease prevention agent, and it is also investigated as a feed supplement to enhance trace element utilization efficiency in animals' bodies [19]. The probiotic microorganism *Saccharomyces cerevisiae* is classified in the Ascomycota division and Saccharomycetes class. It is commonly used in brewing and baking, earning it the nicknames "beer's yeast" and "baker's yeast." Recognized as safe by the FDA, *S. cerevisiae* serves as a bio-therapeutic agent with beneficial enzymatic activities for use in food and pharmaceutical industries [20]. This non-pathogenic yeast contains bioactive compounds such as proteins and enzymes that support green synthesis of nanoparticles. Sriramulu and Sumathi have demonstrated its role as a capping agent for palladium nanoparticle biogenesis through a simple, cost-effective process. Considering its industrial significance, physiology, genetics, *S. cerevisiae* stands out as one of the most suitable candidates for nanoparticle synthesis [21]. The synthesis of ZnO nanoparticles by microorganisms has been documented in only a few studies. Furthermore, using probiotic strains to produce ZnO NPs could potentially enhance the effectiveness of bacteriostatic compounds against medication-blocking and therapeutically relevant infections. In previous studies, the efficacy of ZnO NPs was tested against various microbial pathogens including *Aspergillus niger*, *Fusarium oxyspora*, *Penicillium expansum*, and others [22]. The present research focuses on producing ZnO nanoparticles by employing the probiotic microorganisms *Lactobacillus fermentum* and *Saccharomyces cerevisiae* as a reducing and capping agent. The bacterium and yeast were then subjected to characterization using various techniques including UV–visible spectroscopy, scanning electron microscopy, Fourier transform infrared analysis, and XRD. Furthermore, the antibacterial effectiveness of the biogenic ZnO nanoparticles was evaluated against several MDR pathogens including *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *E. cloacae*, *A. baumannii*, and *S. enterica*. The study also aims to investigate growth performance and blood parameters in goats following Nano zinc supplementation.

2. Materials and Methodology

2.1. Isolation and identification of Microbes

Lactobacillus Fermentum and *Saccharomyces cerevisiae* were sourced from the gastrointestinal tract of animals. To culture *L. fermentum*, it was initially inoculated in Mueller Hinton broth and then incubated at 37 °C for twenty-four hours. Subsequently, the broth was subcultured on a solid medium and placed in an incubator at 37 °C for another twenty-four hours to preserve its vigor and integrity. *Saccharomyces cerevisiae* was isolated using Sabourouds Dextrose Agar as well as Potato Dextrose Agar.

2.2. Biosynthesis of Nanoparticles

ZnO nanoparticles were produced using *L. fermentum* bacterial culture, along with 1 ml of culture, and zinc acetate and sodium hydroxide at concentrations of 20 milliMolar. After incubating for 10 minutes to promote aerobic activity at room temperature, the culture was centrifuged at 10,000 rpm to collect the synthesized nanoparticles. The pellet underwent multiple washes with a 0.9% NaCl solution before being resuspended in a Tris/HCl buffer with a pH of 8.2. The cells were then subjected to ultrasonication at 100 W for eight minutes before undergoing another round of centrifugation for nanoparticle separation. Following washing, the ZnO nanoparticles were dispersed in double-deionized water after undergoing further centrifugation. In *Saccharomyces cerevisiae*'s case, the synthesis involved dissolving 2 mM zinc acetate in 100 ml of double-distilled water and adding baker's yeast extract also at a concentration of 2 mM. This mixture was continuously stirred at a temperature of 50 °C for twelve hours until it exhibited an opaque appearance, indicative of ZnO NP formation. Centrifugation within an ethanol and water solution separated out the ZnO NPs which went through additional drying overnight followed by annealing in a furnace set to 200 °C for approximately six hours afterward.

2.3. Characterization of Nanoparticles

Various methods were employed to characterize nanoparticles, such as X-ray diffraction using a PerkinElmer Spectrum One instrument in Waltham, MA, USA, Fourier transform infrared spectroscopy with a Shimadzu IRAffinity-1 model in Kyoto, Japan operating within the wave number range of approximately 4000–400 cm^{-1} and resolution of about 4–8 cm^{-1} . Scanning electron microscopy was conducted with ZEISS EVO18 in Japan and Nanosurf EasyScan 2 AFM in Hombrechtikon, Switzerland. UV-visible spectroscopic analysis was carried out using a Shimadzu UV-2450 spectrophotometer over the range of 300–800 nm to determine the optical properties of the samples.

2.4. Efficacy testing of supplementing ZnO nanoparticles in small ruminants

Thirty goats were chosen at random in a fully randomized design and divided into three similar groups (ten goats per group) for a 98-day growth trial. The average body weight of the goats was 28.2 ± 0.72 kg, and their age was 7 months. Body weights were recorded at the beginning of the experiment and every two weeks thereafter. The animals were fed with a basal diet (Table 1; control, C) consisting of Berseem hay at 40% and concentrate feed mixture at 60% according to NRC's guidelines. One group received only the control ration without any additives, while for the other two groups, Nano zinc oxide supplements were added to the control ration either at a level of 12 mg/kg DM (ZnO-1) or 12 mg/kg DM (ZnO-2). The concentrate feed mixture included yellow corn (52%), cotton seed meal (17.0%), soybean meal (10.0%), wheat bran (18%), limestone (1.7%), sodium chloride (1%) and mineral-vitamin mixtures (0.3%). Feeds were provided twice daily at 9:00 am and 4:00 pm. The animals had unrestricted access to drinking water at all times. Blood serum evaluations including total protein, albumin, globulin, urea, creatinine, blood glucose, and AST & ALT were performed using standard kits from Spectrum Germany. Immunoglobulin A, G, and Interleukin-2 concentrations in serum were determined through single radio-immuno-diffusion methods. These methods accurately quantify individual protein groups in biological fluids.

2.5. Antibacterial Activity Evaluation

The experimental procedure described in Ref. was adhered to with minor adjustments. The antibacterial efficacy was assessed using the agar well diffusion method. The synthesized NPs' antimicrobial effectiveness against various bacterial strains such as *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *E. cloacae*, *A. baumannii* and *S. enterica* was evaluated. In brief, Mueller Hinton Agar-Hi Media (250 mL) were prepared and poured into sterilized Petri plates. To evenly distribute the bacterial inoculum on a sterile Petri dish containing MH agar, a sterile cotton swab was utilized. Four wells of 6 mm diameter were then created in the agar using a cork borer for different concentrations - 30, 60, and 90 μL - and one for the control group. Following incubation under aerobic conditions at $36 \pm 1^\circ\text{C}$ for 24 h, the plates exhibited substantial bacterial growth. The inhibition of bacterial growth was measured in millimeters. A time-kill kinetics assay was employed to assess the level of ZnO nanoparticle exposure on cultures. New cultures were grown into medium-containing NPs as a test, while others were eradicated without the presence of nanoparticles acting as the control.

2.6. Statistical analysis

The information from each treatment was assessed using a one-way analysis of variance, and Tukey's test was employed to evaluate the impact of treatments, with significant differences noted at $p < 0.05$. The outcomes presented in tables represented averages with a combined standard error of the mean. The data analysis and visual representations were produced using GraphPad Prism version 7.0 by GraphPad Software in La Jolla, CA, USA.

3. Results and Discussion

3.1. UV-visible spectrophotometer

A change in the initial whitish color was detected using UV analysis after 3 hours of the reaction, regardless of the concentration of the precursor used during the 24-hour incubation period [23]. UV spectra were measured in absorption mode across wavelengths ranging from 375 to 400 (see Fig. 1). The shift in color, from light white to dark white observed in the UV-Vis spectrophotometer, is attributed to the Plasmon resonance phenomenon involving oscillations of electrons. Monitoring with UV-Vis spectrum confirms the reduction of zinc acetate to zinc oxide. A band was observed around 386–450 nm identified as a "surface Plasmon resonance band," reflecting excitation of valence electrons within ZnO nanoparticles (nanocrystals/nanospheres). The symmetrical shape of this band suggests uniform scattering associated with spherical-shaped nanoparticles. Similar findings were reported for cumin seeds [24], *Moringa oleifera* [25], *Nyctanthes flower*, *Eclipta alba* [26], and *Ulva lactuca* seaweed [27] with maximum wavelengths ranging from 320–400 nm.

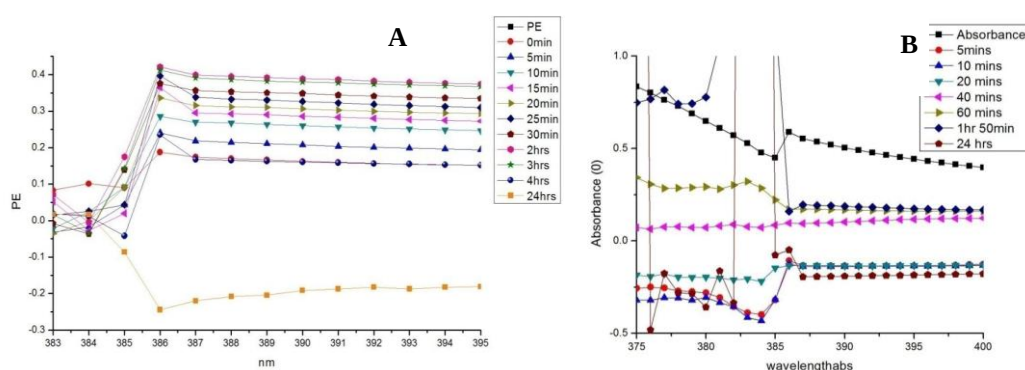


Figure 1. UV–Visible spectra of ZnO nanoparticles (A) ZnO-1(*Lactobacillus fermentum*) and (B) ZnO-2 (*Saccharomyces cerevisiae*).

3.2. Fourier Transform infrared

FTIR analysis was performed to confirm the existence of functional groups on the surface of the synthetic materials [28]. The spectrum of ZnO NPs is displayed in Fig. 2 and contains peaks corresponding to distinctive functional groups present in the synthesized zinc oxide nanoparticles. Biomolecules responsible for reducing ZnO and capping bioreduced ZnO NPs were identified based on specific bond vibration peaks at defined wavenumbers. For example, a peak around 1450-1500 cm^{-1} indicated bond stretching for N-H, while stretches for ZnO nanoparticles were found around 400-800 cm^{-1} . This suggests that proteins and metabolites such as terpenoids with functional groups surrounded the synthesized nanoparticles. The FTIR analysis also confirmed that carbonyl groups from amino acid residues and proteins have a strong ability to bind metal, indicating their potential role in capping metal nanoparticles (i.e., silver) to prevent agglomeration and stabilize the medium. Thus, biological molecules may serve dual functions by forming and stabilizing ZnO nanoparticles in aqueous medium through interactions with flavanones or terpenoids adsorbed on their surface via carbonyl groups or π -electrons. Furthermore, it is suggested that reducing sugars in solution could be responsible for reducing metal ions and forming corresponding metal nanoparticles; additionally, terpenoids may contribute to this reduction process by oxidizing aldehydic groups into carboxylic acids. Extracellular extracts of *S.cerevisiae* contain various functional categories like protein, alcohol, phenolic group carbohydrates, fatty acids which might help keep nanoparticles from aggregating by producing a covering.

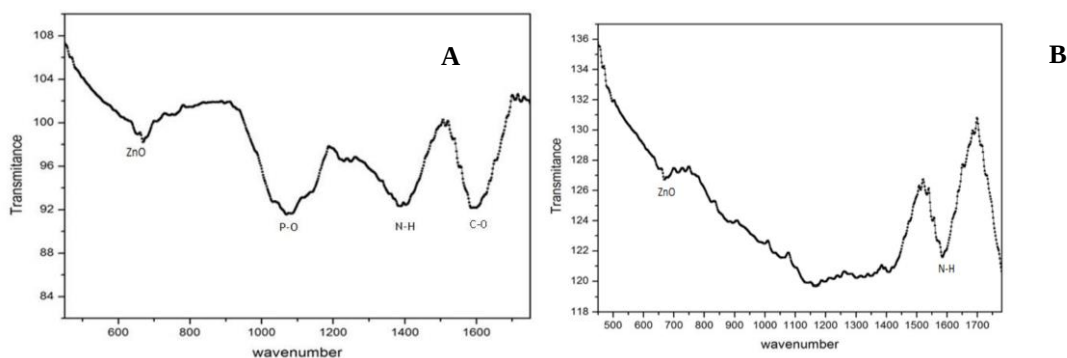


Figure 2. FTIR analysis of ZnO NPs (A) ZnO-1(*Lactobacillus fermentum*) and (B) ZnO-2 (*Saccharomyces cerevisiae*).

3.3. X-ray Diffraction Analysis

X-ray Diffraction Analysis is a method that utilizes scattered X-rays to identify nanoparticles by their crystal structure and crystallite size. This non-destructive technique is effective for analyzing powders, thin films, single crystals, and various types of nanoparticles. Understanding the crystal structure and crystallite size is crucial as they can impact the properties and potential applications of nanoparticles [29, 30]. The hexagonal phase X-ray diffraction peaks from ZnO (JCPDS data card 89-0510) were obtained to assess the purity and crystal structures of probiotic-bacteria-mediated ZnO nanoparticles with different concentrations. Both diffractograms show similar high peaks at an angle of approximately 36.50 degrees for both ZnO-1 and ZnO-2 samples, indicating similarity between them. The nanoparticle size was determined using Scherrer's formula based on parameters such as crystal size, X-ray wavelength, Bragg's angle, and full width at half maximum of the peak. The calculated size was found to be around 110 nm which aligns with sizes observed in other studies involving *Aloe barbadensis* leaf extract [31], hydrothermal growth methods [32], *Ulva fasciata* [33], and *Parkia roxburghii* pods [34].

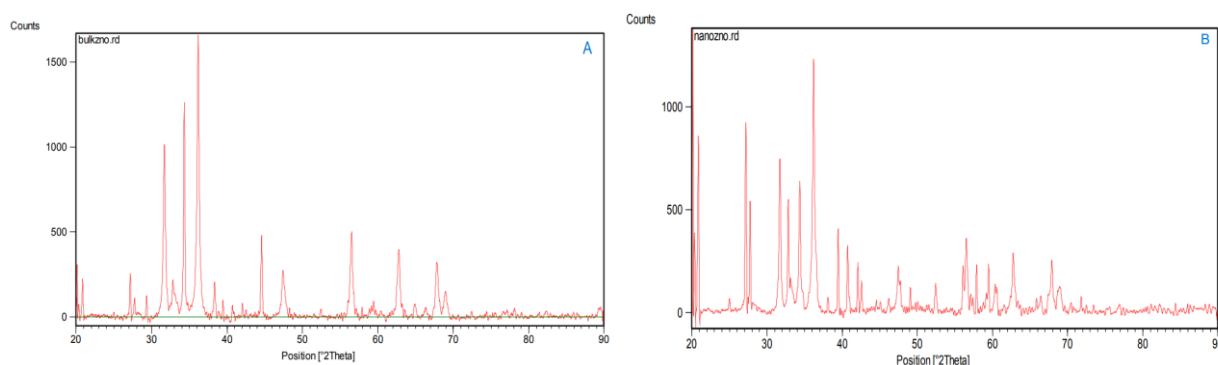


Figure 3. XRD analysis of ZnO NPs (A) ZnO-1(*Lactobacillus fermentum*) and (B) ZnO-2 (*Saccharomyces cerevisiae*).

3.4. Scanning Electron Microscope

Scanning electron microscopy employs a concentrated beam of electrons to examine a sample and generate high-resolution images of its surface through interactions with atoms, resulting in the emission of secondary electrons, backscattered electrons, and X-rays [35, 36]. This technique is valuable for investigating various materials, including nanoparticles, and can offer detailed insights into their structure, dimensions, and distribution [37]. The research on ZnO nanoparticle surface morphology involved the use of a scanning electron microscope. The examination included measurements to assess the growth and morphological characteristics of the synthesized nanostructure. Observations from SEM revealed that the synthesized zinc oxide nanoparticles appeared as micro-sized ZnO-1 and ZnO-2 ranging in size from 100–120 nm (see Figure 4). Synthesizing ZnO NPs in an aqueous medium led to increased surface energy and reduced particle

spacing due to densification [38]. Similar structures were observed in ZnO nanoparticles produced via the sol-gel method using *Elaeagnus angustifolia* [39] flower twigs, *Tabernaemontana divaricata* leaf extract[38], and *Eucalyptus globulus* leaves [40].

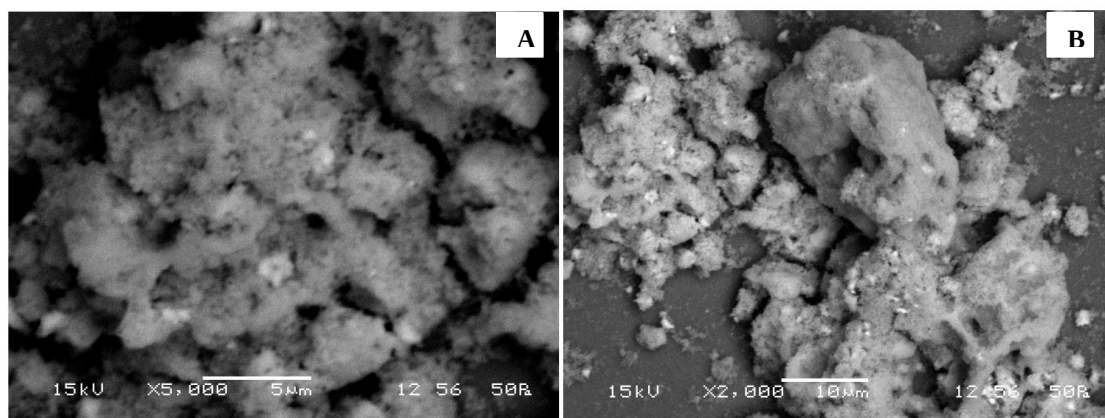


Figure 4. SEM analysis of ZnO nanoparticles (A) ZnO-1(*Lactobacillus fermentum*) and (B) ZnO-2 (*Saccharomyces cerevisiae*).

3.5. Effect of ZnO nanoparticles supplementation on Growth performance

The study explored the impact of three distinct treatments, namely C, ZnO-1, and ZnO-2, over a 12-week period. Initial body weights (Initial BW) did not differ significantly among the treatment groups (Treatment C: 29.2 ± 0.48 kg, ZnO-1: 29.94 ± 0.54 kg, ZnO-2: 29.93 ± 0.55 kg; $p = 0.574$). However, significant differences in body weights became apparent over time. At 10 and 12 weeks, Final Body Weight exhibited substantial variations between treatments (Treatment C: 40.99 ± 0.58 kg, ZnO-1: 43.39 ± 0.45 kg, ZnO-2: 44.05 ± 0.43 kg; $p < 0.001$). While Total Gain did not reach statistical significance (Treatment C: 18.21 ± 0.53 kg, ZnO-1: 20.88 ± 0.74 kg, ZnO-2: 20.09 ± 0.73 kg; $p = 0.064$), a similar trend was observed in Average Daily Gain (ADG) with p -values at 0.063 Table 1. These findings suggest potential treatment-related variations in daily growth rates. In conclusion, the study highlights the differential effects of the investigated treatments on body weight outcomes, particularly notable in the later stages of the study. Consideration of any confounding factors and further research, including a larger sample size and detailed nutritional analysis, is recommended for a comprehensive understanding of these treatment effects. Working with Iranian Angora goat kids [38] and with Jalauni male lambs [41] did not find any effect on average daily gain due to supplementation of nZnO ; the zinc level in the basal ration may have been adequate for normal growth of Jalauni lambs which could be the reason for non-significant effect on growth performance. According to the [42] rabbits fed diet enriched with various amounts of Nano-Zn or Nano-Se had higher ($P > 0.05$) live body weight at 12 weeks than the control. Additionally, rabbits fed diet supplemented with 60 mg Nano-Zn or 0.3 mg Nano- Se/kg recorded the higher ($P > 0.05$) value of body weight gain and feed conversion ratio comparing with the control group at the whole experimental period (6-12 weeks of age), while protein efficiency ratio were insignificantly improved.

Table 1: Growth performance as affected by Nano zinc supplementation

	Treatments			
Weeks	C	ZnO-1	ZnO-2	P. value
Initial BW (kg)	29.2 ± 0.48	29.94 ± 0.54	29.93 ± 0.55	0.574
2 weeks	30.97 ± 0.52	32.79 ± 0.57	32.81 ± 0.59	0.546
4 weeks	33.89 ± 0.51	34.82 ± 0.54	35.07 ± 0.52	0.285

6 weeks	35.05± 0.44	37.26 ± 0.57	37.24 ± 0.54	0.2 35
8 weeks	38.50 ± 0.56	41.09 ± 0.71	41.99± 0.69	0.0 73
10 weeks	40.99± 0.58	43.39 ± 0.45	44.05 ± 0.43	0.0 04
12 weeks	45.68 ± 0.60	49.61 ± 0.34	49.19 ± 0.33	< 0.001
Final BW (kg)	49.41 ± 0.54	53.83 ± 0.53	53.03 ± 0.49	< 0.001
Total gain (kg)	18.21 ± 0.53	20.88 ± 0.74	20 .09 ± 0.73	0.0 64
ADG (g)	170.40 ± 0.54	195.7 0± 0.11	187.60 ± 0.08	0.0 63

C: basal diet without additive (control) . ZnO-1(*Lactobacillus fermentum*): control diet supplemented with 12 mg Nano zinc oxide /kg DM. ZnO-2 (*Saccharomyces cerevisiae*): control diet supplemented with 12 mg Nano zinc oxide /kg DM. ADG: average daily gain. SEM: standard error of means.

3.6. Effect of ZnO nanoparticles supplementation on Blood biochemistry and Immunological parameters

The effects of experimental diets (Control, ZnO-1, ZnO-2) on various physiological parameters were examined. Total Protein showed no significant differences among the groups (Control: 7.18±0.02 g/dl, ZnO-1: 7.05±0.03 g/dl, ZnO-2: 7.12±0.02 g/dl; $p = 0.073$). Albumin levels remained consistent ($p = 0.517$), and while a trend towards a difference in globulin levels was noted ($p = 0.07$), it was marginally non-significant. Glucose levels did not differ significantly among the groups ($p = 0.857$). Urea and Creatinine levels showed no significant differences, indicating comparable kidney function. AST and ALT levels exhibited no significant variations ($p = 0.986$ and $p = 0.971$, respectively), suggesting similar liver function. Immunoglobulins (IgA, IgG) displayed significant differences between groups ($p = 0.006$ and $p = 0.003$, respectively). Interleukin-2 levels did not significantly differ among the groups ($p = 0.639$). In summary, while the experimental diets had limited effects on general biochemical parameters, they influenced specific immune markers, warranting further exploration of their biological significance. [43] reported that lambs supplemented with nano zinc at the rate of 20 mg/kg BW showed a higher level of creatinine in the serum. Lambs may have elevated serum creatinine levels because they were exposed to large dosages of nZnO (360–400 mg/d). Thus, groups fed with both levels of Zn supplementation showed equivalent nutritional utilization and growth performance; however, Zn bioavailability was greatly enhanced by nZnO, though. In light of this, supplementing with 20 ppm of nZnO may be preferable Table 2. [44] illustrated that there were no significant differences in the mean serum glucose values between the treated groups, while the addition of nano zinc oxide led to significant differences in total protein and blood urea nitrogen levels when compared to other treatments. There was no significant difference in the alanine aminotransferase activities between the treatments, but there was a significant difference ($P<0.05$) in the aspartate aminotransferase activities, with lower values being obtained in the group that received higher levels of treatment than the other treatments. [42] reported that there were no significant differences between the experimental rabbit groups in studied plasma criteria by dietary supplementation of Nano-Zn or Nano-Se as compared with the control group. Immunoglobulin increased significantly ($P<0.003$) due to the Nano zinc supplementation; values of IgA were 28.47, 29.99 and 29.57 mg/dl, for groups C, T1 and T3. The respective values for IgG were 127.15, 130.64 and 129.22mg/dl. Zinc deficiency reduces immune responses and disease resistance [45].

Table 2: Blood criteria of goats as affected by Nano zinc supplementation

Parameters	Experimental diets			
	Control	ZnO-1	ZnO-2	P. value
Total protein (g/dl)	7.18± 0.02	7.05±0.03	7.12±0.02	0.073
Albumin (g/dl)	4.67± 0.34	4.68± 0.02	4.65±0.02	0.517
Globulin (g/dl)	3.51± 0.44	3.37± 0.05	3.47±0.04	0.07
Glucose (mg/dl)	62.77± 0.32	63.2±0.43	64.19±0.59	0.857
Urea(mg/dl)	34.04± 0.27	32.86± 0.33	31.67±1.26	0.486
Creatinine (mg/dl)	2.27± 0.31	2.26± 0.31	2.27±0.01	0.195
AST U/L	108.06± 0.15	107.81± 0.45	107.87±1.07	0.986
ALT U/L	118.65± 0.15	118.61± 0.55	118.25±1.27	0.971
IgA, mg/dl	29.47± 0.13	30.99± 0.43	30.57±0.19	0.006
IgG, mg/dl	128.15± 0.15	131.64± 0.45	130.22± 0.37	0.003
Interleukin2 2 (Pg/ml)	77.32± 0.21	77.26± 0.22	78.23± 0.75	0.639

C: basal diet without additive (control) . ZnO-1(*Lactobacillus fermentum*): control diet supplemented with 12 mg Nano zinc oxide /kg DM. ZnO-2 (*Saccharomyces cerevisiae*): control diet supplemented with 12 mg Nano zinc oxide /kg DM. AST: Aspartate transaminase. ALT: Alanine transaminase. IgA: Immunoglobulin A. IgG: immune globulin G. SEM: standard error of means.

3.7. Antibacterial Activity of Zinc Oxide Nanoparticles

The antibacterial activity of the ZnO nanoparticles was determined via their exposure to MDR pathogens and gauging the extent of inhibited bacterial growth [46]. ZnO nanoparticles can manifest their antibacterial activity through various mechanisms. One of these mechanisms includes the dismemberment of the bacterial cell membrane, which eventually results in the death of the bacterial cell. The production of reactive oxygen species (ROS), such as hydrogen peroxide, is yet another process that contributes to the death of bacterial cells. ROS, such as hydrogen peroxide, may damage DNA and proteins, ultimately resulting in cell death. Furthermore, the nanoparticles can interact with the bacterial cell surface, thereby hindering bacterial metabolism and reproduction [47, 48]. Neomycin exhibited a uniform inhibitory effect, generating 10mm zones of inhibition against all tested bacteria, including *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *E. cloacae*, *A. baumannii*, and *S. enterica*. ZnO-1 demonstrated varied effectiveness against the bacterial strains, with varying zone sizes. Notably, it displayed a substantial inhibitory effect against *E. cloacae* (13mm) and *A. baumannii* (12mm). However, its impact on other strains ranged from 6mm to 9mm. ZnO-2 also exhibited diverse antibacterial activity. It showed a significant inhibitory effect against *S. aureus* (12mm), *E. cloacae* (13mm), and *P. aeruginosa* (12mm). However, its effectiveness against other strains ranged from 6mm to 11mm Figure 5. The supernatant was used as negative control. In a prior study, durian-rind-synthesized ZnO NPs showed significant antibacterial efficacy against *Escherichia coli* and *Staphylococcus aureus*. Zinc ions released by ZnO NPs harm the bacterial cell membrane and undermine its integrity, thereby killing a bacterium [49]. The biosynthesis of nanoparticles through green approaches, as conducted in this research, has been receiving an increasing amount of attention due to its eco-friendliness, low cost, and biocompatibility [50].

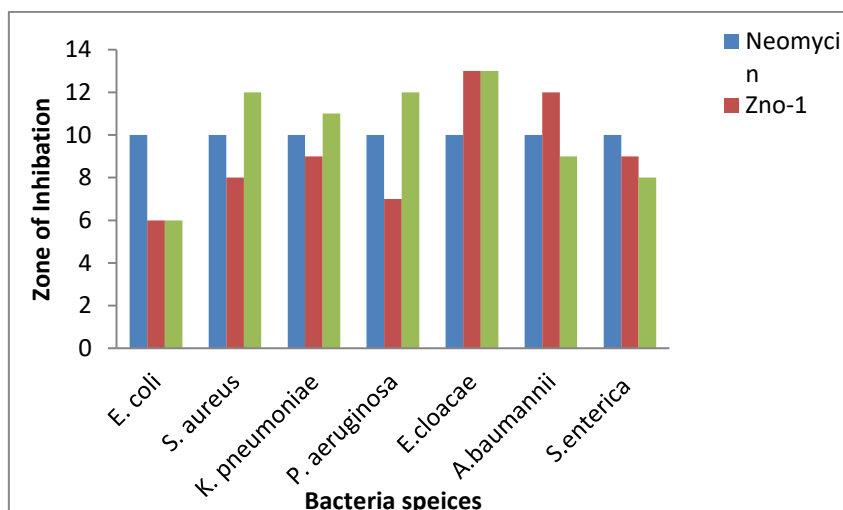


Figure 5. Antibacterial activity of ZnO-1 (*Lactobacillus fermentum*) and ZnO-2 (*Saccharomyces cerevisiae*) nanoparticles determined via their zone of inhibition against various MDR pathogens.

4. Conclusion

ZnO nanoparticles were successfully synthesized characterized using FTIR identified specific bonds (N-H stretching: 1450-1500 cm^{-1} , ZnO nanoparticle stretches: 400-800 cm^{-1}); SEM showcased ZnO NPs morphology (micro-sized bioconjugates: 100–120 nm); XRD confirmed hexagonal crystal structure (peaks at ~ 36.50 degrees). Treatment-related variations in body weights were observed over a 12-week period: Initial BW (29.2 ± 0.48 kg), Final Body Weight (Treatment C: 40.99 ± 0.58 kg, ZnO-1: 43.39 ± 0.45 kg, ZnO-2: 44.05 ± 0.43 kg), and Total Gain (Treatment C: 18.21 ± 0.53 kg, ZnO-1: 20.88 ± 0.74 kg, ZnO-2: 20.09 ± 0.73 kg). Physiological parameters showed limited effects, except for significant differences in immunoglobulins (IgA, IgG) among treatment groups ($p = 0.006$ and $p = 0.003$, respectively). ZnO nanoparticles demonstrated notable antibacterial activity: ZnO-1 against *E. cloacae* (13mm) and *A. baumannii* (12mm), ZnO-2 against *S. aureus* (12mm), *E. cloacae* (13mm), and *P. aeruginosa* (12mm). Future research should focus on optimizing ZnO nanoparticle synthesis, assessing their long-term effects in vivo, and exploring potential applications in drug delivery. Additionally, environmental impact studies and scalable production methods are crucial for responsible development.

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