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Dietary Supplementation of Zinc Oxide Nanoparticles and Yeast Culture: Effect on Nutrient Digestibility, Growth Performance, and Blood Metabolites in Ghanduzi Male Lambs

Research Article

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ABSTRACT

The worldwide prohibition of antibiotics has heightened interest in alternative feed additives, like paraprobiotics and nanominerals, to improve livestock performance. This study investigates the effects of zinc oxide nanoparticles (ZnO-Nps) and yeast culture (YC) on nutrient digestibility, growth performance, and blood metabolites in Ghanduzi male lambs. Sixteen Ghanduzi male lambs, with an average body weight of 32 ± 4.2 kg, were randomly assigned to one of four dietary treatments: a control diet without supplements; a diet supplemented with 50 mg/kg ZnO-Nps; a diet containing 5 g/kg YC; and a diet combining both ZnO-Nps and YC. The trial lasted 28 days, during which daily dry matter intake (DMI), nutrient digestibility, and growth performance metrics were recorded. Blood samples were collected for biochemical analysis. Lambs fed with the diet combining ZnO-Nps and YC showed the highest daily dry matter intake (DMI). This diet significantly enhanced the digestibility of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and organic matter (OM) compared to other groups ($P<0.001$). The final body weight (BW) and average daily gain (ADG) were also significantly higher in the ZnO-Nps + YC group (43.42 kg and 0.417 kg/day, respectively) compared to the control and YC groups ($P<0.001$). Blood metabolites showed no significant differences among treatment groups, although trends indicated improved nitrogen utilization in the supplemented groups. Combining ZnO-Nps and yeast culture substantially enhances growth performance and nutrient digestibility in Ghanduzi male lambs without adversely affecting blood metabolites. These results advocate for the utilization of ZnO-Nps and YC as potent alternatives to conventional growth promoters in sheep farming.

KEY WORDS feed additives, nanominerals, paraprobiotics, sheep farming.

INTRODUCTION

Since antibiotics were prohibited worldwide, farmers have been increasingly using other substances in sheep feed to help their animals grow faster (Valenzuela-Grijalva *et al.* 2017; He *et al.* 2020). Paraprobiotics represent an alternative feed additive employed in sheep farming systems, serving as replacements for traditional growth-promoting agents (Liu *et al.* 2019; Plaza-Díaz *et al.* 2019; Direkvandi *et al.* 2020). Paraprobiotics, which are characterized by non-viable microbial cells that offer health benefits, can potentially enhance the host's well-being. Yeast culture, a type of paraprobiotic, is a natural product obtained through yeast fermentation (Liu *et al.* 2019). Yeast culture comprises dead yeast cells, nutrients they used to grow, and substances they produced while alive. These substances include proteins, sugars, fats, vitamins, small proteins, amino acids, nucleotides, organic acids, and substances that protect cells from damage. Supplementing ruminants' diets with paraprobiotics, such as YC, offers multiple benefits: it has antimicrobial effects, inhibits the growth of enteric pathogens, regulates rumen pH, boosts feed intake and digestion, enhances the production of volatile fatty acids (VFA), and optimizes rumen function. These benefits collectively lead to improved animal performance (Tripathi and Karim, 2011; Zhang *et al.* 2016; Dias *et al.* 2018; Nawab *et al.* 2019; Mitchell and Heinrichs, 2020).

A new approach in livestock farming involves using nanominerals, particularly zinc (Zn), to integrate these essential elements into the animals' bodies. This technique enables targeted delivery of active compounds to specific organs, minimizing their rapid breakdown and enhancing health benefits (Fesseha *et al.* 2020; Reddy *et al.* 2020). Research suggests that the use of nanominerals in animal production could significantly enhance both immunity and reproduction. Zinc oxide nanoparticles provide a superior surface-to-volume ratio compared to larger ZnO particles or zinc methionine supplements and can be used in small quantities, yielding better outcomes than conventional zinc sources (Geetha *et al.* 2020; Hussein *et al.* 2020). Furthermore, ZnO-Nps not only enhance productivity but also serve as antibacterial agents, positively impacting animal immunity and breeding (Hussein *et al.* 2020).

Feed additives can significantly enhance livestock health and performance. Antioxidants like vitamin E, C, proanthocyanidins, and curcumin, along with trace elements like selenium and zinc, can reduce oxidative stress and improve overall well-being (Sun *et al.* 2023). Additionally, probiotics and prebiotics have been shown to promote growth and strengthen the gastrointestinal immune system (Garcia *et al.* 2019). Studies suggest that combining different feed additives often yields better results than using them individually (Sun *et al.* 2023). For instance, Zn-enriched yeast has been shown to benefit lambs by reducing intestinal damage and improving overall performance (NRC, 2007). However, the effects of combining zinc oxide nanoparticles (ZnO-Nps) with yeast culture on fattening lambs are less well-understood. The study focused on examining how adding zinc oxide nanoparticles, yeast culture, or a combination of both to the diet of male Ghanduzi lambs affected their ability to digest nutrients, their growth, and their blood chemistry.

MATERIALS AND METHODS

Experimental feed additives

The ZnO-Nps were purchased from Nano-Scale Borhan, a knowledge-based company in Mashhad, Iran. The YC was obtained from Kavoshbiotech company in Tehran, Iran. Figure 1 shows a scanning electron microscopy (SEM) image and the corresponding energy dispersive X-ray (EDX) spectrum of the ZnO-Nps used in this study.

Animals, diet, and treatments

The research was carried out at the Torbat-e Jam livestock farm in Iran. The lambs were housed, according to the Iranian Council on Animal Care (1995). Sixteen Ghanduzi male lambs (32±4.2 kg body weight) were randomly divided into four groups and fed different experimental diets. The diets included a control diet without supplements, a diet with ZnO-Nps (50 mg/kg), a diet with YC (5 g/kg), and a diet with both ZnO-Nps and YC (50 mg/kg and 5 g/kg, respectively).

The diets were formulated based on the NRC (2007) recommendations for a forage-to-concentrate ratio of 15:85. Before the main trial, the lambs were allowed to adjust to their new diets for 20 days. The trial then lasted for 28 days. The lambs were housed individually (2 m×2 m) (Iranian Council on Animal Care, 1995) and provided with unlimited feed (2% refusals, twice daily: 7 a.m. and 7 p.m.) and fresh water.

Animal sampling

Daily DMI and orts were recorded daily. Feed samples were dried in a forced-air oven (Behdad Co., Iran) at 65 °C for 48 hours and stored in plastic bags for analysis. Lambs were weighed daily before morning feeding and weekly thereafter. Fresh fecal samples were collected one hour after feeding for seven days (days 41-48) using leather bags. Nutrient digestibility was assessed by measuring DMI and refusals. Lambs were given a two-day adjustment period to the fecal collection bags. Bags were emptied completely twice daily. Collected feces were thoroughly mixed, weighed, and a portion was stored at -20 °C for later analysis. Fecal samples were dried in a forced-air oven at 65 °C for 48 hours and then ground to a particle size of 1 mm using a Wiley mill before analysis. Nutrient digestibility was calculated based on nutrient intake and excretion data, using the analytical procedures described in the analytical procedures section. Blood samples were collected over five consecutive days during week four, three hours after the morning feeding, using ethylenediaminetetraacetic acid (EDTA) tubes through the jugular vein (5-10 mL). The samples were centrifuged at 3000 × g for 10 minutes, and the plasma supernatant was transferred into sterile 1.5 mL micro-tubes, stored at -80 °C for subsequent analysis.

Analytical procedures

The AOAC protocol (AOAC, 2005) was used to analyze the following: of dry matter (method 930.15), ether extract (EE, method 991.36), ash (method 942.05), and crude protein (CP), (Kjeldahl method, N × 6.25, method 954.01).

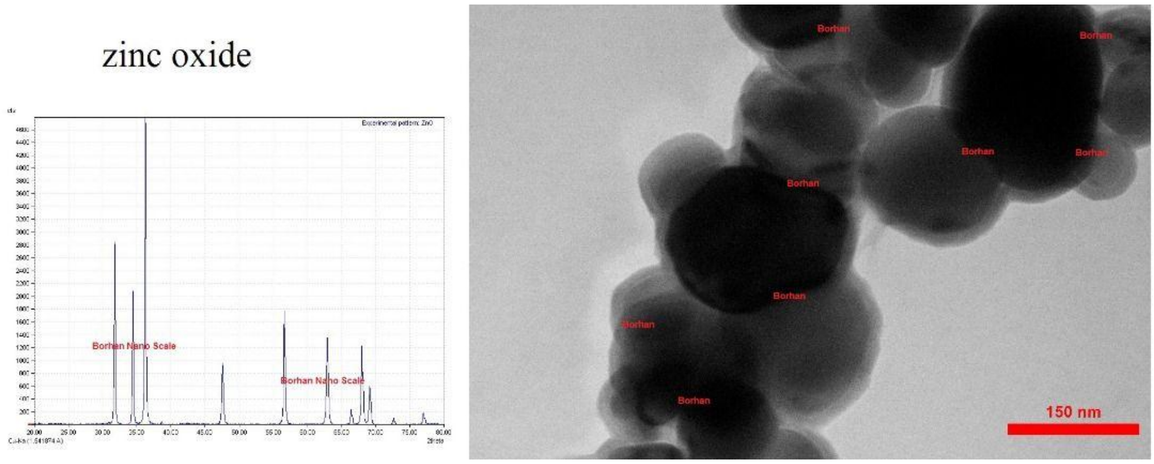

Figure 1 The company's SEM image and EDX spectrum about the purchased ZnO-Nps

For the determination of neutral detergent fiber (NDF), the procedure described by Van Soest *et al.* (1991) and the protocol of Ankom Technology (2006) was utilized, with the analysis conducted using the Ankom fiber analyzer (ANKOM, model A2001, New York, USA). The levels of total protein (T.P), albumin (Alb), globulin (Glo), blood urea nitrogen (BUN), creatinine (Cre), glucose (Glu), triglyceride (TG), cholesterol (Chol), alanine transaminase (ALT), aspartate transferase (AST), creatine kinase (CPK), gamma-glutamyltransferase (GGT), and alkaline phosphatase (ALP) were measured using an auto-analyzer (Mindray BS-200 Chemistry Analyzer, China).

Table 1 Major ingredients and chemical composition of the experimental basal diet (%)

Alfalfa hay	10.00
Wheat straw	5.00
Barley grain	40.00
Corn grain	29.80
Wheat bran	12.00
Soybean meal	10.00
Vitamins and minerals mixture [1]	5.00
Urea	2.50
Fat powder	0.70
Chemical composition	
Dry matter (% of fresh weight)	90.00
Metabolizable energy (Mcal/kg DM)	2.83
Crude protein (%)	14.85
Neutral detergent fiber (%)	26.12
Non-fiber carbohydrates (%)	48.06
Ether extract (%)	3.80
Ash (%)	7.17

Mineral and vitamin mixture (per kg): vitamin E: 1000 IU; vitamin A: 100,000 IU; vitamin D_3: 40,000 IU; Ca: 147 g; P: 5 g; Mg: 20 g; Na: 40 g; S: 4 g; Cu: 100 mg; I: 20 mg; Co: 20 mg; Mn: 800 mg; Zn: 800 mg; Fe: 400 mg; Se: 20 mg and Antioxidant: 1000 mg.

Statistical analysis

All data were analyzed using the PROC GLM procedure in SAS (2013) with the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij}: value of each observation.
μ: denotes the overall mean.
T_i: signifies the treatment effect.
e_{ij}: experimental error.

The statistical significance of the treatment differences was assessed using the Tukey test (P<0.05).

RESULTS AND DISCUSSION

Dry matter intake and nutrient digestibility

Table 2 presents the effects of individually or combined dietary supplementation of ZnO-Nps with YC on DMI and nutrient digestibility in Ghanduzi male lambs.

The findings demonstrate that the group receiving ZnO-Nps and YC had the highest DMI compared to the control and individual supplementation groups (P<0.001). Additionally, lambs fed a diet containing ZnO-Nps exhibited significantly higher nutrient digestibility for DM, CP, NDF, and organic matter (OM) compared to the control and YC groups (P<0.001). Including ZnO-Nps at levels of 100 and 200 mg/kg has been shown to enhance VFAs production, microbial CP, and OM breakdown during the 6^{th} and 12^{th} hours of an *in vitro* fermentation study (Chen *et al.* 2011). Similarly, in an *in vitro* fermentation study, an increase in microbial biomass production and a reduction in methane emissions were observed when 20 mg of zinc in the form of

ZnNps was administered, compared to higher zinc levels (40 and 60 mg/kg dry matter) (Riyazi *et al.* 2019). Incorporating ZnNps into lamb diets improved diet digestibility and feeding value, as evidenced by increased feed utilization efficiency in the experimental dietary treatments (Mohamed *et al.* 2015). Positive results have been observed in Holstein calves. Supplementation of 50 mg Zn/kg DM, either as ZnO or ZnO-Nps, has led to an increase in DM digestibility (Rajendran *et al.* 2013). The beneficial impacts of ZnO-Nps on DMI and nutrient digestibility can be attributed to their unique properties. These include a large surface area-to-volume ratio, nanoscale size, rapid and targeted movement, and catalytic efficiency. These properties enhance the bioavailability of zinc for absorption in the gastrointestinal tract (GIT) as noted by (Zhan *et al.* 2007; Seven *et al.* 2018). The growth-promoting effects of nanominerals are likely due to their ability to positively infeleunce the gut's structure and function. They can modify the gut microarchitecture in animals (Adegbeye *et al.* 2019) and improve rumen fermentation, particularly in terms of fiber digestion and redox balance (Murthy, 2013; Zhai *et al.* 2017).

Yeast culture, a safe and environmentally friendly dietary supplement, has been shown to enhance nutrient digestibility in sheep (Wang *et al.* 2022). Studies by Malekkhahi *et al.* (2015) and Song *et al.* (2021) found that adding YC to high-concentrate diets significantly improved the digestibility of both CP and NDF. Research on beef cattle and dairy cows by Lei *et al.* (2013) and Jiang *et al.* (2017) also revealed that YC can increase fiber digestibility. This consistent improvement is likely due to an increase in cellulose-degrading bacteria or the presence of metabolites in YC that stimulate these bacteria. The positive impact of YC on fiber digestibility is particularly beneficial for ruminants fed high-concentrate diets, as shown by Dias *et al.* (2018). This may explain the higher dry matter intake (DMI) and nutrient digestibility observed in the ZnO-Nps + YC group compared to the other groups in the study.

Growth performance

Initial body weights were comparable across all treatments (P=0.080) (Table 3). However, significant differences emerged in final body weights (P=0.006) (Table 3). The ZnO-Nps + YC treatment group demonstrated the highest final body weight, followed by the ZnO-Nps group. Moreover, the ZnO-Nps + YC group exhibited notably higher average daily gains (ADG) on days 14 (P=0.050) and 28 (P=0.003) compared to the control and YC groups (Table 3). The cumulative ADG and total body weight gains (BWG) were significantly superior in the ZnO-Nps + YC group (P<0.001) (Table 3). Additionally, the feed conversion ratio (FCR) was markedly improved in the ZnO-Nps + YC group relative to the control and YC groups (P=0.007) (Table 3).

Studies have shown that supplementing livestock with ZnO-Nps can lead to significant improvements in growth and gut health. Anil *et al.* (2019) found that calves given 10 mg/kg of ZnO-Nps experienced substantial increases in total and live weight. Similarly, Zaboli *et al.* (2013) observed that kids supplemented with 20-40 ppm of ZnO-Nps had significantly higher average daily gains, compared to the control group. Additionally, ZnO supplementation at lower doses (below 50-100 mg/kg of dry matter) has been shown to increase the overall number of bacteria in the gut, including beneficial intestinal bacteria. This, in turn, leads to a significant enhancement in VFAs, which play a crucial role in gut health and nutrient absorption (Pieper *et al.* 2012). Garg *et al.* (2008) further demonstrated that zinc supplements can improve food conversion efficiency by making zinc more readily available in the digestive system. This increased zinc availability contributes to a higher bacterial count and better utilization of ingested feed.

Studies on yeast products have shown benefits for livestock, including: increased feed efficiency, improved daily weight gain, enhanced overall animal health and well-being (Kim *et al.* 2000; Haddad and Goussous, 2005; Rozeboom *et al.* 2005; Ding *et al.* 2008).

Research has also demonstrated that supplementing ruminants with yeast products can positively influence DMI, rumen pH, and overall nutrient digestibility (Callaway and Martin, 1997; Dann *et al.* 2000). Yeast and yeast products may enhance animal performance by supporting animal health through positive effects on bacterial colonization in the GIT, and potentially increasing nutrient digestibility (Harris and Webb, 1990). The impact of dietary treatments on blood metabolites in Ghanduzi male lambs is summarized in Table 4. While no significant differences were found among the various treatment groups, certain trends emerged, particularly in BUN levels. Lambs fed the ZnO-Nps + YC diet exhibited the highest BUN levels, followed by those receiving ZnO-Nps alone.

Table 5 outlines the effects of individual or combined dietary supplementation of ZnO-Nps with YC on liver enzymes and creatine phosphokinase in Ghanduzi male lambs. Although ALT, AST, CPK, GGT, and ALP were unaffected by the inclusion of ZnO-Nps and YC in the basal diet, some trends were observed, especially in GGT and ALP levels. Blood biochemistry values are crucial to the body's physiological state (El-Kholy *et al.* 2017). Previous research has indicated that adding ZnO-Nps to ruminant diets doesn't significantly impact specific biochemical parameters (Zaboli *et al.* 2013; Swain *et al.* 2019; Abd El Rahim *et al.* 2023).

Table 2 Effects of individual or combined dietary supplementation of zinc oxide nanoparticles (ZnO-Nps) with yeast culture (YC) on dry matter intake and nutrient digestibility

Items	Treatments				SEM	P-value
	Control	ZnO-Nps	YC	ZnO-Nps + YC		
DMI (kg/day)	1.25[a]	1.51[b]	1.36[c]	1.56[d]	0.024	< 0.001
Nutrient digestibility						
DM	0.71[a]	0.75[bc]	0.72[ab]	0.76[c]	0.005	< 0.001
CP	0.73[a]	0.75[bc]	0.74[ab]	0.78[c]	0.005	< 0.001
NDF	0.55[a]	0.62[bc]	0.58[ab]	0.64[c]	0.008	< 0.001
OM	0.73[a]	0.77[bc]	0.75[ab]	0.78[c]	0.005	< 0.001

DMI: dry matter intake; CP: crude protein; NDF: neutral detergent fiber and OM: organic matter.
Control: basal diet without supplements; ZnO-Nps: 50 mg/kg DM basal diet; YC: 5 g/kg DM basal diet and ZnO-Nps + YC: 50 mg and 5g/kg DM basal diet, respectively.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effects of individual or combined dietary supplementation of zinc oxide nanoparticles (ZnO-Nps) with yeast culture (YC) on growth performance

Items	Treatments				SEM	P-value
	Control	ZnO-Nps	YC	ZnO-Nps + YC		
Initial BW (kg)	27.9	35.2	32.1	32.5	1.05	0.080
Final BW (kg)	33.9[a]	44.1[ab]	39.4[a]	43.4[b]	1.31	0.006
Average daily gain (ADG) (kg/d)						
Day 07	0.2	0.3	0.3	0.2	0.02	0.200
Day 14	0.2[a]	0.3[bc]	0.3[ab]	0.4[b]	0.02	0.050
Day 21	0.1	0.2	0.1	0.2	0.28	0.670
Day 28	0.1[a]	0.3[bc]	0.2[ab]	0.4[c]	0.41	0.003
Total ADG	0.2[a]	0.3[b]	0.2[ab]	0.3[c]	0.01	< 0.001
Total BWG (kg)	6.0[a]	8.8[b]	7.3[ab]	10.8[c]	0.49	< 0.001
FCR	7.6[a]	5.2[b]	7.1[a]	4.6[b]	0.40	0.007

BW: body weight; BWG: body weight gains and FCR: feed conversion ratio.
Control: basal diet without supplements; ZnO-Nps: 50 mg/kg DM basal diet; YC: 5 g/kg DM basal diet and ZnO-Nps + YC: 50 mg and 5g/kg DM basal diet, respectively.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effects of individual or combined dietary supplementation of zinc oxide nanoparticles (ZnO-Nps) with yeast culture (YC) on blood metabolites

Items	Treatments				SEM	P-value
	Control	ZnO-Nps	YC	ZnO-Nps + YC		
T.P (g/dL)	8.0	8.5	7.6	9.1	0.37	0.57
Alb (g/dL)	2.4	2.3	2.4	2.1	0.05	0.30
Glo (g/dL)	5.5	7.1	5.3	7.8	0.62	0.48
Alb/Glo	0.4	0.3	0.4	0.3	0.02	0.15
Cre (mg/dL)	0.7	0.6	0.7	0.6	0.03	0.84
Glu (mg/dL)	99.0	88.3	102.	107.6	3.52	0.27
TG (mg/dL)	28.6	31.3	19.3	28.3	2.20	0.24
Chol (mg/dL)	53.0	56.3	64.3	69.6	3.84	0.45
BUN (mg/dL)	6.3	11.0	7.0	13.6	1.16	0.06

T.P: total protein; Alb: albumin; Glo: globulin; Cre: creatinine; Glu: glucose; TG: triglyceride; Chol: cholesterol and BUN: blood urea nitrogen.
Control: basal diet without supplements; ZnO-Nps: 50 mg/kg DM basal diet; YC: 5 g/kg DM basal diet and ZnO-Nps + YC: 50 mg and 5g/kg DM basal diet, respectively.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effects of individual or combined dietary supplementation of zinc oxide nanoparticles (ZnO-Nps) with yeast culture (YC) on alanine transaminase, aspartate transaminase, creatine phosphokinase, gamma glutamyltransferase, and alkaline phosphatase

Items	Treatments				SEM	P-value
	Control	ZnO-Nps	YC	ZnO-Nps + YC		
ALT (U/L)	55.0	51.6	51.6	55.3	1.99	0.89
AST (U/L)	132.3	121.0	130.3	114.0	6.59	0.79
CPK (U/L)	488.6	307.6	413.0	303.0	42.4	0.38
GGT (U/L)	84.3	40.0	62.3	41.3	7.33	0.08
ALP (U/L)	95.6	22.0	69.1	28.9	11.8	0.06

ALT: alanine transaminase; AST: aspartate transaminase; CPK: creatine phosphokinase; GGT: gamma glutamyltransferase and ALP: alkaline phosphatase.
Control: basal diet without supplements; ZnO-Nps: 50 mg/kg DM basal diet; YC: 5 g/kg DM basal diet and ZnO-Nps + YC: 50 mg and 5g/kg DM basal diet, respectively.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

However, the results in Table 4 align with a study by Belewu and Adewumi (2021), which found that West African Dwarf (WAD) goats fed diets with 40 and 80 mg/kg of ZnO-Nps experienced a notable decrease in BUN levels compared to the control group. Additionally, Novoselec *et al.* (2017) observed that incorporating ZnO-Nps, ZnO, and Zn-Met at 28 mg/kg DM in sheep diets led to lower serum BUN levels. The higher plasma BUN concentrations in the ZnO-Nps + YC group might be due to improved diet digestibility and increased N intake.

Fluctuations in AST and ALT blood levels can signal ZnO-Nps toxicity (Wang *et al.* 2017), potentially leading to liver damage (Kurtz and Travlos, 2017). However, the similar AST and ALT levels in the lambs suggest that including ZnO-Nps in their diet did not cause any liver cell injury. This finding aligns with previous research on sheep fed ZnO-Nps (Mohamed *et al.* 2017; Swain *et al.* 2019), or alternative zinc sources like Zn-methionine, Zn glycine, and Zn-proteinate (Alimohamady *et al.* 2019).

The addition of YC did not affect serum metabolite level. These findings are consistent with previous research on lambs (Malekkhahi *et al.* 2015; Mahmoud *et al.* 2020) and dairy cows (Dias *et al.* 2018). Blood urea nitrogen is a byproduct of protein metabolism and indicates the amount of dietary protein and its utilization by the body. Increased DMI typically leads to higher CP intake. Excess protein is metabolized, producing urea, which is excreted in urine, raising BUN levels (Song *et al.* 2021). Liver function parameters ALT, AST, and CPK were unaffected by YC in this study. Previous research by Abou Elenin *et al.* (2016) and Song *et al.* (2021) suggest that YC has no adverse or beneficial effects on liver metabolism.

In the present study, ZnO-Nps treatment was found to lower GGT and ALP levels in livestock compared to other groups. Livestock can experience various stressors throughout their lives, including disease exposure, environmental challenges, handling stress, nutritional problems, relocation, and new surroundings. These stressors can activate the hypothalamic-pituitary-adrenal (HPA) axis and potentially the immune system, leading to decreased performance (Burdick *et al.* 2011). Probiotics, like yeast and yeast-derived products, can boost immunity and health by modifying the gut microbiome (White *et al.* 2002; Mullins *et al.* 2013; AlZahal *et al.* 2014). A healthy gut flora improves nutrient absorption and immune function (Bauer *et al.* 2006; Czarnecki-Maulden, 2008). Additionally, yeast cell wall polysaccharides may prevent harmful bacteria from attaching to the gut, reducing the risk of infections (Bäumler *et al.* 1997).

CONCLUSION

Combining zinc oxide nanoparticles and yeast culture significantly boosted growth performance and nutrient digestibility in Ghanduzi male lambs without negatively impacting blood metabolites. These results suggest that ZnO-NPs and YC could be effective alternatives to traditional growth promoters in sheep farming. Future research should focus on long-term effects of ZnO-NPs and YC on lamb health and performance, determining the optimal dosages of these supplements, understanding the underlying mechanisms that contribute to the observed benefits, and assessing the implications for animal health and welfare within livestock production systems.

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