



EFFECTIVENESS OF YEAST PROBIOTICS IN IMPLEMENTING THE "NO ANTIBIOTICS EVER" STRATEGY IN BROILER FARMING

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Summary

Matiukha, I., O. Krasovska, O. Vishchur, N. Broda, D. Mudrak, M. Romanko & V. Ushkalov, 2025. Effectiveness of yeast probiotics in implementing the "No Antibiotics Ever" strategy in broiler farming. *Bulg. J. Vet. Med.* (online first).

NAE stands for "No Antibiotics Ever" and refers to a type of poultry diet and management practice in which chickens are raised without the use of any antibiotics from hatch to harvest. This practice is part of a broader trend in the poultry industry to meet consumer demand for antibiotic-free meat and to address concerns about antibiotic resistance. Instead of antibiotics, NAE rely on enhanced biosecurity measures, vaccination programmes, and the use of probiotics, prebiotics, and other natural health-promoting additives to maintain bird health. The study on the effectiveness and safety of probiotic yeast strains was conducted on Ross 308 broiler chickens, divided into five groups. Each group received a specific diet, with or without probiotic supplementation. Blood, tissue, and droppings samples were collected for the analysis of biochemical, immunological, and productive indicators. The research demonstrates that with the complete absence of antibiotics during the growing period, the inclusion of probiotics in broiler diets contributed to an increase in the levels of short-chain fatty acids in the faeces, which play a crucial role in ensuring optimal digestion, immune response, pathogen protection, nutrient absorption, and high productivity levels. The application of *Kluyveromyces marxianus*, isolated, identified, and characterised in the Research and Development laboratory as a probiotic feed additive for broiler chickens, shows promising potential for improving productivity, enhancing gut microbiota balance, and supporting digestive function through increased short-chain fatty acid production.

Key words: biochemical parameters, broiler, immune response, probiotic, yeast

INTRODUCTION

The "No Antibiotics Ever" (NAE) initiative in poultry feeding is a crucial issue today due to the growing concern about antibiotic resistance and the increased consumer demand for antibiotic-free meat. Antibiotic resistance, resulting from the overuse of antibiotics in animal agriculture, poses significant public health risks as it can lead to the development of resistant bacterial strains that affect humans (Van Boeckel *et al.*, 2017). The NAE programme aims to eliminate the use of antibiotics at any stage of poultry production, necessitating alternative methods to ensure bird health and growth.

Probiotics, including yeast probiotics such as *Saccharomyces cerevisiae*, have emerged as viable alternative for antibiotics. These probiotics enhance gut health, boost the immune system, and inhibit pathogenic bacteria, thereby maintaining bird health and performance without antibiotics (Gaggia *et al.*, 2010; Allen *et al.*, 2013). The implementation of yeast probiotics in poultry diets complies with consumer expectations and helps mitigate the risks associated with antibiotic use in agriculture.

Yeast probiotics, particularly those from the *Saccharomyces* genus, offer several advantages over bacterial probiotics in animal nutrition. Yeast probiotics are more robust and can survive the harsh conditions of the gastrointestinal tract better than many bacterial probiotics, ensuring they reach the intestines alive where they exert their beneficial effects (Klemenčič *et al.*, 2012). Additionally, yeast probiotics such as *Saccharomyces cerevisiae* have shown superior abilities in enhancing gut health and boosting immune responses due to their complex cell wall structure, which includes β -glucans, mannan-oligosaccharides, and other bio-

active components that promote the growth of beneficial bacteria and inhibit pathogens (Chee *et al.*, 2010). Thus, Lytvynenko *et al.* (2024) reported that the use of a probiotic feed additive (*Bacillus subtilis* 12P-130, *Bacillus licheniformis* 12P-896 and *Saccharomyces cerevisiae* AF 338) at all stages of raising calves contributed to increased economic efficiency of the livestock industry, reducing the level of use of antibacterial agents and obtaining ecological products.

Recent research has identified several new yeast strains within the *Saccharomycetaceae* family such as *Kluyveromyces marxianus*, that hold significant potential as probiotics. These strains exhibit strong probiotic properties, including the ability to survive gastrointestinal conditions, adhere to intestinal epithelial cells, and modulate the host's immune response (Fonseca *et al.*, 2008). *Kluyveromyces* strains have shown promising results in improving gut health and enhancing nutrient absorption due to their unique metabolic activities and bioactive compounds. Studies have demonstrated that these yeast strains can inhibit pathogenic bacteria and promote the growth of beneficial microbiota, thereby contributing to a healthier gut environment (Loureiro & Malfeito-Ferreira, 2003). The utilisation of these new strains in animal nutrition could lead to more effective and sustainable probiotic formulations, reducing the reliance on antibiotics and improving overall animal health and performance.

MATERIALS AND METHODS

Experimental design

A total of 75 14-day old Ross 308 chicks with similar weight were divided into 5 re-

plicates (experimental units) of 15 birds each and assigned to one of the 5 experimental diets. Animals were raised for 49 days according to the Experimental design and probiotic usage recommendation.

The experiment was carried out under the supervision of certified veterinarians. Chickens were vaccinated at hatchery before entering the groups according standards, then placed in floor pens with wood shavings as litter. They received supplemental heat in the first period, water was provided through a plastic waterer, and feed was offered *ad libitum* according to the experimental group. Animals were fed the starter formula from days 1 to 14, grower from days 21 to 28 and finisher formula from day 28 to 49. The dietary treatments after the beginning of the experimental period as well as ration formulations are reported in Table 1.

The health and vitality status of the subjects were evaluated twice daily and at the end of the trial, a post-mortem exam was performed on all animals. On days 15, 21, 28 and 40, all the birds were weighed, and average daily gain (ADG) was calculated. On days 28 and 49, five broilers from each replication were sacrificed and samples of blood and tissues

were collected for further analyses.

All procedures on animals were carried out in compliance with European Union regulations (EU Council. Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purposes, 1986. EU Commission).

Yeast strains origin

Saccharomyces cerevisiae yeasts were grown at the facilities of Enzym Group, on a molasses medium in accordance with the developed technology regulations to produce this strain. After receiving the yeast cream, it was dried by a special technology to obtain a final product encapsulated in the outlier of inactive yeast cells. As a result, the product EnzActive – live *Saccharomyces cerevisiae* with a yeast cell count $\geq 1.5 \times 10^{10}$ CFU/g was produced.

The yeasts *Kluyveromyces marxianus* (KM S1 – 1.4×10^{10} cfu/g and KM S2 – 4.25×10^{10} cfu/g), were isolated, selected, identified and grown in the Enzym Company's R & D Centre. *Kluyveromyces marxianus* KM CS (B0399), a commercial strain from the yeast market, was used for comparative purposes.

Table 1. Groups differentiation by diets

Groups		All diets without antibiotics or other growth promoters	
		14–28 days of age	28–49 days of age
Control		Basic balanced grower diet (GBd)	Basic balanced finisher diet (FBd)
Experimental	<i>S. cerevisiae</i> - EnzActive		
	<i>Kl. marxianus</i> - KM S1	Basic balanced grower diet (GBd) + 0.1% probiotic	Basic balanced finisher diet (FBd) + 0.1% probiotic
	<i>Kl. marxianus</i> - KM S2		
	Commercial <i>K. marxianus</i> - KM CS (B0399)		

Blood sampling

At days 14 and 21 of trials, blood samples were collected in EDTA anticoagulated tubes. Aliquots of each blood sample were immediately used for determining biochemical and immunological parameters. The rest was centrifuged, and plasma was collected and stored at $-80\text{ }^{\circ}\text{C}$ for further enzymatic and chemical analyses.

Blood biochemical parameters

Malondialdehyde (MDA) was determined in blood plasma stored at $-80\text{ }^{\circ}\text{C}$ with a commercial kit for measuring thiobarbituric acid reactive substances (TBARS). The colour reaction between malondialdehyde and thiobarbituric acid was measured at 532 nm using a UV/VIS spectrophotometer (Gildford Instrument Laboratories, Inc., Oberlin, OH) and the results were compared against a standard curve made with 100, 50, 25, 12.5 and 0 nmol/mL dimethylacetyl malondialdehyde.

Lipid hydroperoxides (HLP) were determined according to the standard method by precipitation of blood plasma proteins with trichloroacetic acid, extraction of lipids with ethanol with subsequent interaction of the studied extracts with ammonium thiocyanate (Vlizlo, 2012).

Superoxide dismutase (SOD) spectrophotometric assay based on the inhibition of nitroblue tetrazolium (NBT) reduction by superoxide radicals was used to measure the decrease in absorbance at 560 nm, reflecting SOD activity (Beauchamp & Fridovich, 1971).

The alanine transaminase (ALT) and aspartate aminotransferase (AST) were measured using commercial kits (AS101 and AL100, Randox, United Kingdom) at 546 nm.

In vitro trials

Sensitivity to aggressive conditions of the gastrointestinal microbiota was determined in simulated gastric conditions (final pH ~ 2.5) and in intestinal conditions (final pH ~ 6.5) by adding dry *K. marxianus* yeasts to a container with appropriate conditions in a ratio of 1:9. The flask (pH ~ 2.5 , simulated gastric environment) was placed in an orbital shaker incubator at $37\text{ }^{\circ}\text{C}$ and 110 rpm for two hours; every 30 minutes, 1 mL of the suspension was taken from the flask, serial dilutions were made and inoculated onto OGYE selective nutrient medium. Then the contents of flask 1 were transferred to flask 2, which simulates the condition of the small intestine (pH ~ 6.5), and incubated for an additional four hours with sampling at every hour. Serial dilutions were made from all sampling points and plated on OGYE selective nutrient medium.

The thermal stability of *K. marxianus* isolates was determined by culturing and heating yeast cultures dissolved in nutrient medium and dry cultures at $80\text{ }^{\circ}\text{C}$ for 20 min, followed by seeding tenfold dilutions (10^{-1} to 10^{-7}) into Petri dishes with Yeast and Mold Agar Composition and incubation at $25\text{ }^{\circ}\text{C}$ under anaerobic conditions for 48–72 h and subsequent counting of the colonies formed.

Study of poultry faeces

Faecal samples for short-chain fatty acid (SCFA) determination were collected and stored in sealed containers. The samples were homogenised in distilled water 1:10, centrifuged (10,000 rpm, 10 min), the supernatant was filtered ($0.45\text{ }\mu\text{m}$) and used for SCFA determination by gas chromatography using a capillary column with a polyethylene glycol (PEG) stationary phase. SCFA content was quantified by comparing retention times and peak

areas with those of standards; the concentration of each SCFA was calculated using a calibration curve, and the results were expressed in $\mu\text{mol/g}$ faeces (Zhao *et al.*, 2019; Wang *et al.*, 2020).

Statistical analysis

All data were first analysed using one-way ANOVA. When the overall P value was below the significance threshold ($P < 0.05$), pairwise comparisons were performed using the t-test to assess differences between individual groups.

RESULTS

In vitro trials

Fig. 1 presents the growth of the three different yeast strains (*K. marxianus* KM S2, commercial *K. marxianus*, and commercial probiotic strain of *S. cerevisiae*) cultivated in a Solaris M30 laboratory fermenter. Two key parameters were evaluated: the amount of active dry biomass (ADB) and the yield percentage. The *K. marxianus* KM S2 strain exhibited the highest productivity, generating approximately 50 g/L of active dry biomass. This indicates its high capability for growth and biomass production under the conditions provided by the Solaris M30 fer-

menter. Additionally, this strain had the highest yield, around 53% indicating efficient substrate conversion into biomass, making KM S2 a promising candidate for further research and potential commercial applications. In contrast, the commercial *K. marxianus* strain demonstrated significantly lower productivity of 10 g/L of active dry biomass. Its yield was also considerably lower, approximately 45%. The EnzActive strain showed intermediate results, producing approximately 30 g/L of active dry biomass with yield of around 51%, slightly lower than KM S2 but significantly higher than the commercial strain.

In terms of thermostability, the *K. marxianus* KM S2 strain demonstrated the highest activity in dry yeast (4.25×10^{10} cfu/g) and the highest level of thermostability (3.1×10^{10} cfu/g) (Table 2). EnzActive (*S. cerevisiae*) also exhibited high activity (2.7×10^{10} cfu/g) and thermostability (1.3×10^{10} cfu/g), confirming its suitability for high-temperature conditions, although it was surpassed by *K. marxianus* KM S2 in these parameters. The KM S1 strain showed high activity (1.4×10^{10} cfu/g) but significantly lower thermostability (3.0×10^5 cfu/g), limiting its applicability in high-temperature environments. The commercial *K. marxianus* strain ex-

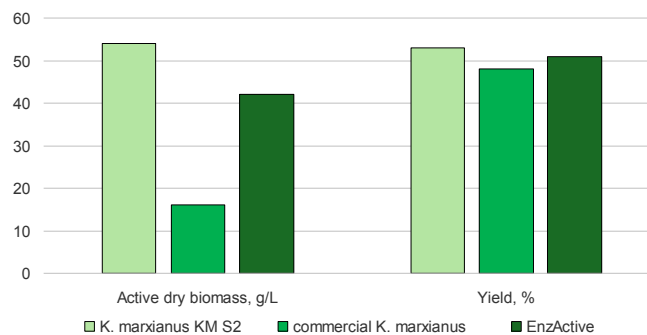


Fig. 1. Growth of probiotic strains – average active dry biomass and average yield.

Table 2. Thermostability of *K. marxianus* KM S2 compared to other strains.

Strains	Activity of dry yeast, cfu/g	Thermostability (85 °C/20 min), cfu/g
EnzActive (<i>S. cerevisiae</i>)	2.70×10^{10}	1.30×10^{10}
<i>K. marxianus</i> KM S1	1.40×10^{10}	3.00×10^5
<i>K. marxianus</i> KM S2	4.25×10^{10}	3.10×10^{10}
Commercial <i>K. marxianus</i> KM CS	1.20×10^{10}	3.40×10^{10}

Table 3. *In vitro* viability of probiotic strains under the conditions of the gastrointestinal tract

Time points	Strains			GIT sections
	<i>S. cerevisiae</i> EnzActive	<i>K. marxianus</i> KM S1	<i>K. marxianus</i> KM S2	
Beginning	2.05×10^{10}	4.25×10^{10}	1.40×10^{10}	Stomach
30 min	2.00×10^{10}	5.50×10^9	5.80×10^9	
1 h	1.90×10^{10}	5.30×10^9	5.80×10^9	
1.5 h	1.80×10^{10}	5.00×10^9	5.75×10^9	
2 h	1.70×10^{10}	5.00×10^9	5.70×10^9	
3 h	1.60×10^{10}	4.50×10^9	4.50×10^9	
4 h	1.55×10^{10}	4.00×10^9	4.50×10^9	Intestine
5 h	1.45×10^{10}	3.40×10^9	4.00×10^9	
6 h	1.40×10^{10}	3.00×10^9	3.00×10^9	

hibited the lowest activity (1.2×10^9 cfu/g) and thermostability (3.4×10^6 cfu/g), indicating its lower efficiency compared to the other strains under conditions requiring high productivity and heat resistance.

The strain *K. marxianus* KM S2 had positive characteristics under conditions simulating the passage through the gastrointestinal tract. From the start of the experiment to the end of the six-hour period, KM S2 maintained stable viability, with several key aspects highlighted. Under stomach conditions, an aggressive environment for many microorganisms characterised by low pH, KM S2 was able to retain a substantial portion of its viability during the first two hours in the acidic stomach environment. The initial count was 1.4×10^{10} cfu/g, and even after two hours of exposure to the acidic medium, KM S2 retained 5.7×10^9 cfu/g (Table 3).

After passing through the stomach, KM S2 exhibited stable viability in the less acidic environment of the intestines. The counts decreased only slightly, from 5.7×10^9 cfu/g at the two-hour mark to 3.0×10^9 cfu/g after six hours.

In vivo trials

The blood alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity in broilers supplemented with probiotics is crucial for evaluating liver health and overall metabolic status. ALT and AST are markers of liver function, and their activity can reveal potential liver damage or stress. In our studies, blood ALT activity on the 14th day of the experiment was within the normal range. However, on the 28th day, the ALT activity in the control group and the group fed a commercial probiotic exceeded the up-

per limit. In contrast, the ALT levels in broilers that received experimental probiotics in their diet, including EnzActive, remained within the normal range (Fig. 2). Blood AST activities were normalised on the 28th day of the experiment in birds fed EnzActive and KM S1. The activity was slightly enhanced in broilers that received the KM S2 probiotic, but the changes remained within physiological norms. The commercial probiotic showed excessive enzyme activity on day 28th compared to day 14 day, which may indicate metabolic disturbances.

It is crucial to investigate changes in oxidative stress and antioxidant defense in the blood of broilers when studying the addition of probiotic yeast to their diets. Our research results showed that by the

end of the study, the level of lipid peroxidation products in the blood of broilers in the experimental groups was lower than in the control group (Fig. 3). This effect was especially pronounced with the addition of the probiotic KM SC2 to the diet. Consequently, the activity of superoxide dismutase (SOD) was higher in the blood of broilers with probiotics in their diet, as was the content of reduced glutathione compared to the control group. The highest SOD activity was recorded in the group supplemented with KM SC2.

Short-chain fatty acids (SCFAs) in the faeces of broilers are critical parameters in evaluating the efficacy of probiotics in poultry trials. The addition of yeast-based probiotics significantly influenced the concentrations of short-chain fatty acids

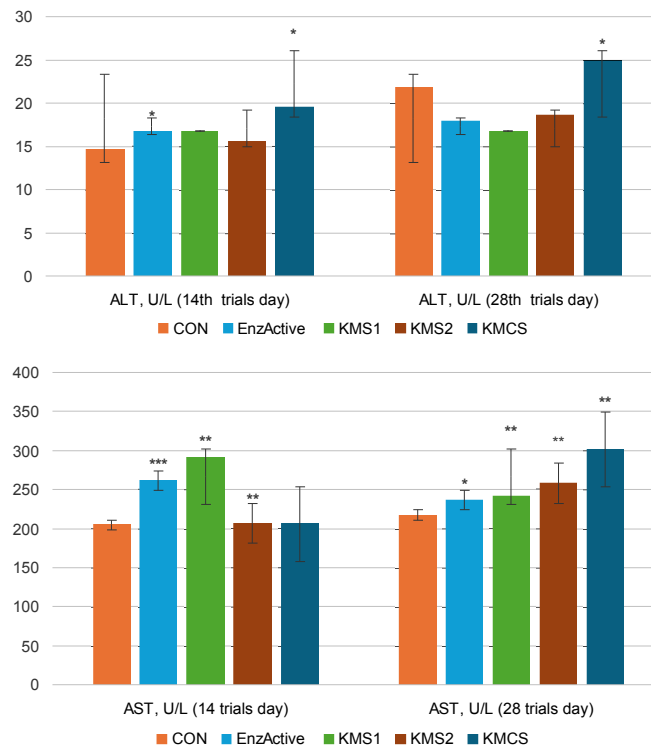


Fig. 2. Activity of serum aminotransferases (mean \pm SD) in broilers from the different groups during the experiment. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ compared to the CON group.

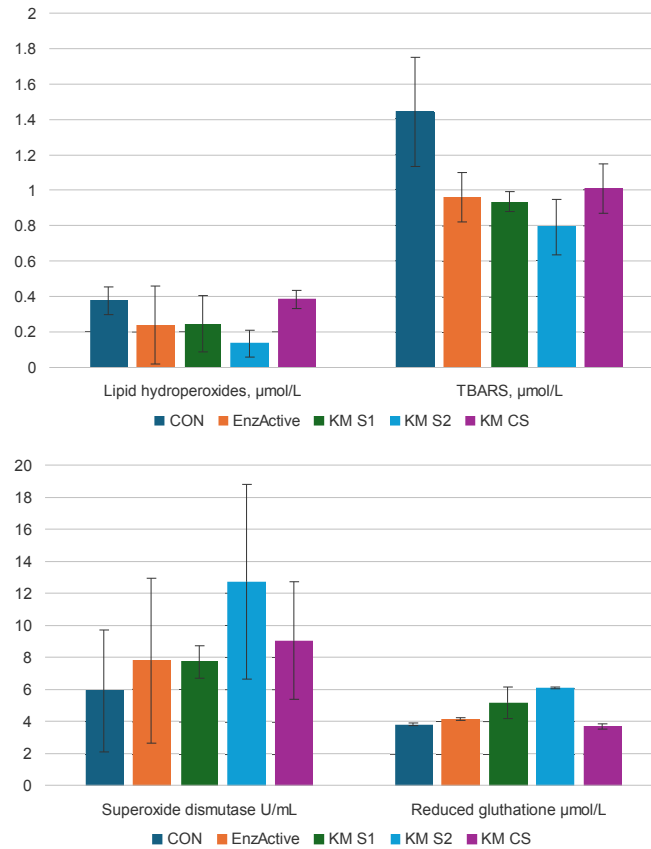


Fig. 3. Lipid peroxidation products, activity of superoxide dismutase and reduced glutathione content (mean \pm SD) in the blood of broilers from the different groups.

(SCFAs) in the faeces of broilers compared to the control group (CON), which received basal diet without probiotics.

The acetate level in the control group was the lowest, approximately 7 mmol/L. All probiotic-supplemented groups showed a significant increase in acetate concentration, with the highest values observed in the KM S2 (*K. marxianus* strain 2) and EnzActive (*S. cerevisiae*) groups, reaching levels of 28–38 mmol/L and 22–28 mmol/L, respectively. The KM S1 and KM SC groups also demonstrated elevated acetate levels but with greater variability (Fig. 4). Propionate, an impor-

tant energy source for intestinal epithelial cells, had the lowest concentrations in the control group, around 1–3 mmol/L. The addition of probiotics enhanced propionate production, with the most notable effect in the KM S2 group, where levels reached 10 mmol/L (Fig. 4).

EnzActive, KM S1, and KM SC also stimulated propionate production, though within a lower range of 3–8 mmol/L. Regarding butyrate, a key SCFA for maintaining intestinal mucosal integrity and immune response, its concentration was lowest in the control group (0.3–0.7 mmol/L). The highest increase in butyrate

was observed in the commercial probiotic KM SC group, reaching levels of 5.2 mmol/L, indicating a strong positive effect on the gut microbiota. The other probiotic groups showed moderate butyrate increases in the range of 1.5–2.5 mmol/L. Thus, among the tested probiotics, the *K. marxianus* S2 strain was the most effective in stimulating the production of

major SCFAs (acetate, propionate, and butyrate), achieving the greatest increase in acetate and propionate levels. The *S. cerevisiae*-based probiotic also significantly raised SCFA levels, especially acetate. The commercial *K. marxianus* probiotic (KM SC) stood out with the highest butyrate levels, suggesting a specific beneficial effect on the intestinal

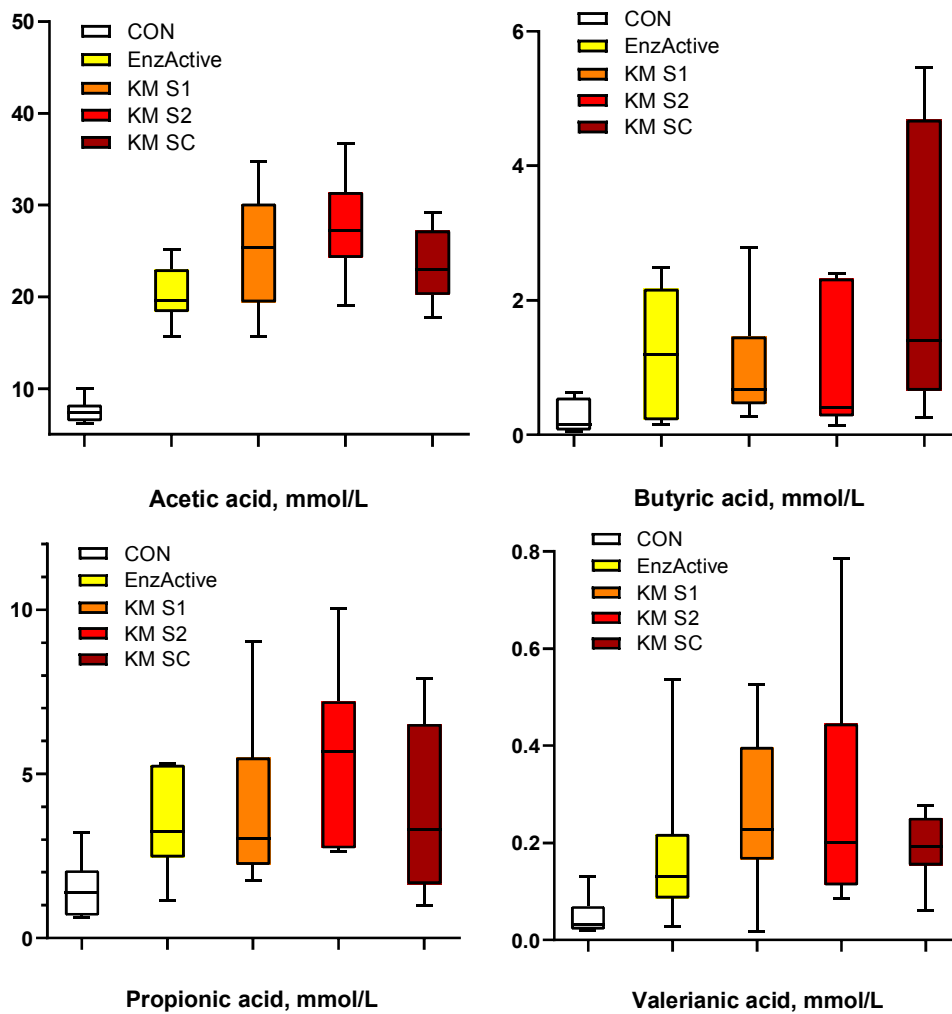


Fig. 4. Short-chain fatty acids in the faeces of broilers from the different groups.

mucosa. Higher levels of butyric and valeric acids was detected in the faeces of birds fed the probiotic *K. marxianus* in relation to the birds fed the standard diet (Fig. 4, 5).

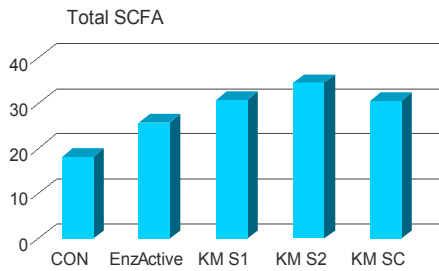


Fig. 5. Total short-chain fatty acids content in the faeces of broilers from the different groups.

DISCUSSION

In the context of using probiotic strains of the yeast *K. marxianus* in agricultural animals, it is important to highlight several key aspects that define their potential as probiotics. Research demonstrates that *K. marxianus* can significantly improve animal health and productivity by influencing gut microbiota, enhancing immune response, and combating pathogens. For example, *K. marxianus* yeasts exhibit significant probiotic potential for improving the health and productivity of broilers. The addition of *K. marxianus* to the diet of chicks led to improvements in growth, nutrient digestion, and immune response modulation. Specifically, birds receiving a diet supplemented with *K. marxianus* showed increased body weight, improved feed conversion ratio, and strengthened immune system due to increased relative mass of immune organs and elevated activity of specific immunoglobulins (Lane & Morrissey, 2010). Other studies have also shown that the addition of probiotic

yeasts *K. marxianus* can significantly enhance the performance of broiler chicks. Bolla *et al.* (2013) revealed that including *K. marxianus* in the diet positively affected weight gain and improved feed conversion ratio in chicks. In this experiment, the use of different doses of *K. marxianus* (0.25–2.5 g/kg) contributed to increased average daily weight gain and reduced feed conversion ratio, indicating more efficient nutrient utilisation.

In our studies, probiotics based on the KM S2 strain isolated in R&D laboratory demonstrated high technological growth and stability characteristics compared to other probiotic products. This suggests that while EnzActive may be beneficial under certain conditions, *K. marxianus* KM S2 remains the most efficient option for achieving maximum productivity. The cultivation of strains in the Solaris M30 laboratory fermenter revealed that KM S2 was the most effective among the strains studied. Its high productivity and efficiency in laboratory conditions highlighted its potential as a probiotic for further research and commercial use.

The initial count was 1.4×10^{10} cfu/g, and even after two hours of exposure to the acidic medium, KM S2 maintained 5.7×10^9 cfu/g. This indicates a high level of resistance, which is a crucial quality for probiotic strains, as the ability to survive stomach conditions is critical for reaching the intestines. This ability to maintain viability in the intestines is a vital aspect, as it is in the intestines that probiotics exert their beneficial effects, including enhancing the microbiota and strengthening the immune system. Additional research has shown that the introduction of *K. marxianus* may also impact immune response improvement and intestinal tract structure. For instance, experiments demonstrated that the addition of *K. marx-*

ianus leads to increased weight of immune organs such as the spleen and thymus, as well as improved intestinal villi structure, which may contribute to better nutrient absorption (Wang *et al.*, 2018). A study conducted in Korea showed that *K. marxianus* yeast isolated from kefir, can survive in the gastrointestinal tract of animals better than other probiotic yeasts, such as *Saccharomyces boulardii* (Youn *et al.*, 2022). The goal was to compare the survival and adaptation properties of different yeasts under the complex conditions of the gastrointestinal tract, including high acidity and the presence of bile acids. Among all the samples, *K. marxianus* yeasts demonstrated high cell surface hydrophobicity, allowing them to interact better with intestinal epithelial cells, which improves their adhesion and survival in the gastrointestinal tract. As in our studies, when comparing several strains, we established better viability and stability of *K. marxianus*, other authors also successfully prove this ability of the strains. Based on the presented data, the *K. marxianus* KM S2 strain possessed the best characteristics for use as a probiotic. Its high productivity, dry yeast activity, and excellent thermostability combined with good survival in the simulated gastrointestinal tract make it a promising candidate for further research and potential commercial application. These properties ensure its ability to effectively colonize the gut and maintain viability under conditions typical for the gastrointestinal tract and during the processing and storage of probiotic products.

There is enough evidence that broilers show inflammatory changes in the liver through their life. A study showed that the increased ALT and AST rate in the serum of the COBB 500 broiler could be the cause for general liver lesions while the

increased level of AST might be lowered due to lessened growth rate caused by reduced food intake with increased temperature (Tang *et al.*, 2022). Probiotic strains were found to be effective in reducing liver injury and normalisation of AST and ALT activity in blood (Hong *et al.*, 2024). They influence gut health and immune response, which can, in turn, impact liver function. Monitoring these enzymes helps in understanding the safety and efficacy of probiotics in poultry diets, ensuring they promote overall health without causing adverse effects. Research has shown that probiotics can modulate enzyme activity, highlighting their role in maintaining liver health in broilers (Mountzouris *et al.*, 2010; Swiatkiewicz *et al.*, 2014). The changes in the blood activity of both enzymes in birds from the groups that received probiotics were within physiological norms and did not indicate any protein metabolism disorders or liver and myocardium dysfunctions. In contrast, the enzyme response upon the addition of the commercial product used for comparative evaluation prompts consideration of the underlying cause of these changes, as the alterations in activity were significant. The impact of yeast probiotics on ALT and AST activity in broilers was significant. Studies have shown that incorporating yeast-based probiotics into broiler diets can positively influence liver enzyme levels, potentially reduce liver stress and enhancing metabolic efficiency. For instance, Zhang *et al.* (2020) demonstrated that broilers fed yeast probiotics exhibited lower ALT and AST levels compared to control groups, indicating improved liver function and reduced tissue damage. The results of our experiment proved that the addition of target probiotics to the diet had caused no destructive or other pathological changes in the liver due

to the normalisation of the blood activity of transferases.

Oxidative changes, provoked by the influence of various stress factors (including thermal), can cause significant cellular damage, leading to impaired growth, health issues, and reduced overall performance in broilers. Probiotic yeasts in broiler diets can significantly influence oxidative stress parameters, including lipid peroxidation and superoxide dismutase (SOD) activity. By enhancing antioxidant defenses, probiotic yeasts help mitigate oxidative damage and support overall health. Research has shown that dietary supplementation with probiotic yeasts reduced blood lipid peroxidation products and increased SOD activity in broilers. This effect is attributed to the probiotic's ability to improve the antioxidant status of the birds, promoting better growth performance and health outcomes (Mokhtar *et al.*, 2011; Gao *et al.*, 2015; Borges & Borges, 2020). Aluwong *et al.* (2013) proved that yeast probiotic supplementation increased body weight and enhanced serum antioxidant enzyme activities of broiler chickens. Antioxidant enzymes are most effective when acting synergistically with one another or with other components of the antioxidant barrier of the organism. It has been shown that nutrition plays a vital role in maintaining the prooxidant-antioxidant balance (Cowey, 1986). In the present study, both blood GSH content and SOD activity were increased. The most important growth processes early in life are characterised with the generation of reactive oxygen species (ROS) through cellular division and apoptosis (Buetler, 2004). A similar study (Chee *et al.*, 2010) in turkeys reported that mannanoligosaccharides a component of *S. cerevisiae* used as dietary additive stimulate the mechanisms

of oxidative defense and improve the growth performance of the birds. Our research has corroborated literature data (Borges, 2020) on the positive effect of probiotic yeast on reducing the effects of oxidative stress in poultry. Moreover, the tested *K. marxianus* strain caused a significant increase in antioxidant protection and a decrease in the accumulation of the final metabolites of peroxidation.

Short-chain fatty acids (SCFAs) in the faeces of broilers are important indicators of gut health and microbial activity, particularly when yeast-based probiotics are added to their diet. Acetate, propionate and butyrate are key metabolic products of gut microbial fermentation and serve as indicators of a healthy and balanced gut microbiome. These SCFAs play a crucial role in maintaining gut integrity, promoting beneficial microbial populations, and inhibiting the growth of pathogens. The presence and concentration of SCFAs reflect the probiotic's ability to enhance beneficial microbial activity, improve nutrient absorption, and support gut health. Higher SCFA levels often correlate with better gut integrity, reduced pathogen load, and overall improved growth performance in broilers. Thus, SCFAs in faeces is a valuable metric for assessing the functional impact of probiotics on poultry health and productivity (Hassan *et al.*, 2020).

Studies have consistently demonstrated that the inclusion of yeast probiotics in poultry diets results in higher SCFA concentrations in faeces, reflecting a more efficient and healthier gut environment (Awad *et al.*, 2009; Ding *et al.*, 2019; Kim *et al.*, 2020). The addition of yeast probiotics, such as *Saccharomyces cerevisiae*, enhanced the production of SCFAs, including acetate, propionate, and butyrate (Wang, 2020; Zhang *et al.*,

2020). Our research data demonstrated that the inclusion of probiotics in broiler diets, with complete absence of antibiotics during the growing period, contributed to increased levels of short-chain fatty acids in the faeces in all experimental groups. Higher levels of SCFAs like propionic and acetic acid can lower the pH in the intestines, creating an environment that is not favorable for pathogenic bacteria and more favorable for beneficial microbes. This helps maintain a healthy balance of gut microbiota. Butyric acid is an important energy source for the cells lining the gut, supporting their growth and function. This can lead to better nutrient absorption and overall improved growth performance in broilers. SCFAs are absorbed by intestinal cells and used as an energy source, which can enhance the health and function of the gut lining. A healthier gut lining improves the absorption of nutrients, leading to better feed efficiency and growth performance in broilers. In summary, the increase in SCFA levels when broilers are fed probiotics suggests that the probiotics are effectively enhancing gut fermentation, promoting a healthier gut environment, and contributing to better overall health and performance of the birds. The most interesting result was shown by the *K. marxianus* S2 strain.

CONCLUSIONS

The results of this study confirm the effectiveness of *Kluyveromyces marxianus*, particularly the KM S2 strain as a probiotic yeast for broiler chickens raised without antibiotics. The *in vitro* tests showed its strong resistance to gastric conditions, high thermostability, and biomass productivity, while *in vivo* trials demonstrated its positive influence on blood antioxidant status, liver enzyme

activity, and short-chain fatty acid levels in broiler faeces. These findings point to improved gut health, immune function, and overall bird performance.

Importantly, the probiotic effect was most pronounced in the KM S2 group, suggesting that this strain has great potential for practical use in poultry feeding strategies aimed at meeting the “No Antibiotics Ever” (NAE) standards. By supporting physiological function and productivity without the need for antibiotics, *K. marxianus* could reduce the reliance on conventional antimicrobial growth promoters in line with both public health goals and consumer expectations.

Although these results are promising, further studies under commercial conditions and with larger sample sizes would be valuable to confirm the long-term benefits and determine optimal inclusion rates for field application.

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Paper received 04.02.2025; accepted for publication 17.09.2025

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