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Influence of dietary probiotic formulations onrumen characteristics, digestibility, and blood biochemistryin Saidisheep

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Abstract

Although in vitro data with mixed ruminal fluid has shown positive effects of a blend of probiotics on measures of fermentation, digestibility, and microbial profiles, there is a lack of in vivo data with mature small ruminants. Therefore, the current study evaluates the potential of a blend of probiotics (ABLB) alone or combined with *Saccharomyces cerevisiae* (SC) on rumen characteristics, digestibility, and blood biochemistry in sheep. Five males Saidi sheep (54.14 ± 1.67 kg body weight) were randomly allocated in a 5 × 5 Latin square design. Animals were fed basal diet or supplemented alone with 1 g/day/animal of a bacterial formula containing *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Bacillus licheniformis*, and *Bifidobacterium bifidum* (1:1:1:1) at two levels: 2×10^9 (ABLB2) and 4×10^9cfu/g (ABLB4) as the 2nd and 3rd alone or combined with SC (1 gram/ animal/day) as 4th (ABLB2+SC) and 5th (ABLB4+SC) tested diets. The results show that all probiotic supplements significantly reduced ($P<0.05$) NH3-N concentrations and pH values at 0 and 3 hours after feeding compared to the control. In contrast, ruminal TVFA, MCP, and protozoa count increased ($P<0.05$) with ABLB2+SC at both sampling times. Compared to the control, significant improvements ($P<0.05$) were observed with ABLB2+SC in DM, CF, CP, and EE digestibilities, as well as TDN, SV, and DCP (%) values of the tested diets. Additionally, ABLB2+SC supplements led to significant increases in serum protein and glucose concentrations ($P<0.05$). AST activities showed a slight statistical increase ($P=0.031$) with the inclusion of SC in the probiotic formula. Conversely, ABLB2+SC resulted in the lowest levels of serum urea, creatinine, triglycerides, and cholesterol ($P<0.05$). This study highlights the potential benefits of using probiotics in ruminant nutrition, particularly in improving rumen physiology, blood health, and nutrient utilization.

Keywords: probiotic formulations, sheep, rumen characteristics,digestibility,blood indices.

1. INTRODUCTION

At the beginning of the third millennium, animal health and production sectors faced many challenges to reach maximal animal productivity without adverse impacts on the safety of their products and the environment. The global movement to prohibit the use of antibiotics in animals has prompted the exploration of safe alternatives such as direct-fed microbial (DFM), which includes prebiotics, probiotics, post-biotics, enzymes, and antimicrobial growth promoters[1-3].Khan et al. reviewed that several genera of bacteria (*Bifidobacterium* spp., *Bacillus* spp., and *Lactobacillus* spp.) and yeast (*Saccharomyces cerevisiae*) are successfully used in different combinations as DFM for domestic ruminants[4].

Previous studies in sheep found that dietary supplementation with probiotics formulations significantly decreased the ruminal NH_3-N [5,6], induced a significant growth in the volatile fatty acids production[7], the total protozoa numbers [8], and the microbial protein mass[6,9]. Furthermore, several researchers have shown that dietary supplementation with probioticformulations showed positive results in nutrient digestibility in sheep [5,10] and improved the blood biochemistry[9,7]

When lactic acid bacteria (LAB) are combined with yeast and other bacterial strains, they can exhibit synergistic activities[11]. Therefore, this work aimed to evaluate the impacts of using different probiotic formulations with or without *Sacharomycescervisiae*in the diets of Saidisheepwhich are fed a 50:50 concentrate/roughage ratio on rumen characteristics, digestibility, and blood biochemistry.

2. MATERIALS AND METHODS

The experimental workwas accomplished at the Animal Nutrition Research Unit, Animal Production Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.Animals, procedures, and protocols in this experiment were inspected and accepted by the Institutional Animal Care and Use Committee of the Faculty of Agriculture, Sohag University, Sohag, Egypt (Sohag-IACUC/6/12/1/2024/01).

2.1.Experimental design, animals,and diets

This study was conducted at the farm of the Agriculture College, Zagazig University. Five healthy mature male Saidi sheep (54.14 ± 1.67 kg body weight) were enrolled in this study. The animals were kept in metabolic cages (120 cm height ×150 cm length × 70 cm width) during the study period. Animals were fed basal diets, consisting of a mixed diet (50:50 forage to concentrate ratio), as presented in Table 1. The basal diet consisted of yellow corn (35%), soybean meal (7.5%), wheat bran (6.5%), berseem hay (50%) enriched with common salt (2.5kg/ton), Limestone (6 kg/ton), and mineral and vitamin mixture (1.5 kg/ton). Free access to water was available throughout the day. Diets were offered twice a day (8 a.m. and 4 p.m.). All animals housed in the same building and were reared following conventional farm management with natural photoperiod. The probiotic blend used in this experiment comprised of *Lactobacillus acidophilus, Lactobacillus bulgaricus, Bacillus licheniformis,* and *Bifidobacterium bifidum (*ABLB) at ratios of 1:1:1:1. The experimental groups were as follows:

i. Group 1 (CON): animals were fed basal diets without additives and served as the control group.
ii. Group 2 (ABLB2): animals were fed basal diets supplemented with 1 g/day/animal of a probiotic blend at a level of 2×10^9cfu (ABLB2).
iii. Group 3 (ABLB4): animals were fed basal diets supplemented with 1 g/day/animal of a probiotic blend at a level of 4×10^9cfu (ABLB4).

iv. Group 4 (ABLB2+SC): animals were fed basal diets supplemented with 1 g/day/animal of a probiotic blend at a level of 2×10^9cfu (ABLB2) + Saccharomyces cerevisiae (SC, 1g /day/animal).
v. Group 5 (ABLB4+SC): animals were fed basal diets supplemented with 1 g/day/animal of a probiotic blend at a level of 4×10^9cfu (ABLB2) + Saccharomyces cerevisiae (SC, 1g /day/animal).

These animals were allotted at random in a 5 × 5 Latin square design. The daily dose of tested additives was mixed with 50 g of offered feed and delivered to each animal once a day in the morning feed. In the 105-day experimental period (21 days each), each period entailed 14 days as a preliminary phase and 7 days for the collection of samples (4 days experimental digestion samples and 3 days for rumen and blood samples).

2.2.Digestibility trail

During days 15 to 18representative samples of diets and feces from each metabolic cage were collected and oven-dried, ground, screened and preserved for chemical analysis. Dry matter (DM), ash, crude protein (CP), ether extract (EE), and crude fiber (CF) in feed and fecal samples were determined according to the AOAC[12]. The percentages of organic matter (OM) and nitrogen-free extract (NFE) were estimated. Thereafter, the digestibility of nutrients and the nutritive value of each experimental diet were calculated.

2.3. Rumencharacteristics

At days 19 to 21, samples of rumen fluid were drawn by a stomach tube (150 mL/ lamb) at 0 and 3 hours of feeding. The collected sample was split into two parts. The first one was filtrated through 4layers of cheesecloth to measure the pH values immediately by a pH meter and then stored at -18 °C until chemical analysis. Later, the ammonia nitrogenconcentrations as illustrated by Conway [13], total TVFA as described by Warner [14], and microbial protein mass as demonstrated by Shultz and Shultz [15]were evaluated. The second part of the rumen liquid sample was strained over a layer of cheesecloth for the protozoa count.

2.4. Blood sampling

Blood samples were withdrawn from the jugular vein by using a sterile syringe from all tested animals at 8 a.m. in the morning and then transferred into one sterile tube. The tube was supplemented without heparin to obtain serum by centrifugation for 10 minutes at 1006 g. The serum stored in Eppendorf at -18 °C until the biochemical assays for glucose, albumin, globulin, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, cholesterol, and triglycerides.

2.5. Statistical analysis

The SAS software V.9.1.3 SP4 (SAS Institute Inc., Cary, North Carolina, USA)was used to analyze the collected data using the General Linear Model (GLM) procedure. Rumen fermentation nutrient digestibility and blood parameters data were statistically analyzed using the analysis of variance (ANOVA) model for Latin square design. Duncan's multiple range tests were used to find the differences among the treatments[16]. The model is composed as follows:

$$Y_{ijk} = \mu + R_i + C_j + T_k + e_{ijk}$$

Where:

Y_{ijk}:An observed value of each trait.

μ: The overall mean.

R_i: The i^{th} row effect.

C_j: The j^{th} column effect.

T_k: The k^{th} (1,2,3,4,5) treatment effect.
e_{ijk}: The random effect of error.

3. RESULTS

3.1. Rumen characteristics

The ruminal NH_3-N and pH values in the control were statistically higher than in all supplemented diets before and after 3 hrs of feeding (P<0.001; P=0.024 and P=0.001, respectively). The lowest levels of NH_3-N concentrations were observed withthe low level (2×10^9cfu/g) of the bacterial probiotic formula plus SC (ABLB2+SC). However, before and after sampling times, there were no notable disparities in the ruminal pH values and NH_3-N and TVFAconcentrations among the four diets (Table 2).While before feeding, the TVFA concentrations were significantly higher (P<0.001) with all supplements than in the control. Yet, both of ABLB2 and ABLB2+SC led to considerable (P=0.040) elevations in TVFA levels compared to the control at 3 hrs of feeding.No remarkable changes were detected in ruminal microbial crude protein (MCP) among all supplements beforefeeding. However, only the ABLB2+SC diet exhibited significant elevation in MCP values at 0 and 3 hrs of feeding (P=0.039 and P=0.033, respectively) relative to the control. The protozoa count without supplements was significantly lower (P=0.035) than those in the ABLB2+SC before the morning meal, but 3 hrs later, both values of protozoa count with ABLB2 and ABLB2+SC were superior (P=0.015) the control (Table 2).

3.2. Nutrientdigestibility

It was evident that the combinations of probiotic ABLB2+SC generated the highest (P<0.001) DM digestibility value and the best (P>0.05) amelioration in OM and NFE digestibilities related to the other treatments (Table 3). Besides, all the tested probiotic blends achieved crucial (P<0.001) enhancements in CP and EE digestibilities, especially those augmented with the SC, where the ABLB2+SC recorded the utmost values. Also, ABLB2+SC had the highest (P=0.005) CF digestibility value among all treatments (Table 3). The maximum averages of total digestible nutrients (TDN) and starch value (SV) were noticed with diet ABLB2+SC. Furthermore, all supplements exhibited better (P<0.001) digestible crude protein (DCP) values than the control diet, and ABLB2+SC showed a superior value.

3.3. Blood parameters

In comparison with the control, only the ABLB2+SC supplements promoted a significant rise (P=0.040) of serum glucose concentrations. The mean albumin values were significantly (P=0.037) higher with all the supplemented diets than in control, except with the high dose of the multi-strain bacteria formula (ABLB4). Moreover, all probiotic supplements significantly (P<0.001) increased the levels of globulin and total protein in the serum compared to the control lambs. Using the ABLB2 + SC was accompanied by the highest level of serum proteins. Concerning the values of ALT activity, the examined supplements did not induce any significant changes. While the AST activities were statistically (P=0.031) increased with including the SC in the tested supplements in comparison to the control. Except for ABLB2, all probiotic supplements significantly lessened serum urea concentrations in parallel to the control (P<0.001). The combination of ABLB2 and SC resulted in the lowest level of serum urea concentration. As well, a remarkable (P=0.030) reduction in the concentration of serum creatinine was achieved only with ABLB2+SC supplements. Likewise, all the tested probiotic combinations resulted in

significant declines (P<0.001) in the mean values of triglycerides, excluding that with the low dose of the multi-strain bacteria formula (ABLB2) compared to the control.Furthermore, all tested supplements afforded significant reductions (P=0.001) in cholesterol levels compared to the control. The lowest value of cholesterol concentration was in ABLB2+SC. All the measured values of serum metabolites in this study were within the normal ranges for sheep, according to Kaneko et al. [17] and Varanis et al. [18].

4. DISCUSSION

4.1. Rumen fermentation parameters

Probiotics have valuable impacts on ruminant's well-being and productivity by stabilizing the ruminal environment [19, 20].

The obtained results showed that, all probiotic supplements drastically lowered the NH_3-N concentrations, especially ABLB2+SC, than the control by 31.88 and 26.84 % at 0 and 3 hrs of feeding, respectively. Comparable results by Jia et al. revealed that concentrations of NH_3-N significantly (P=0.007) declined by 18.29 and 21.18%, respectively, with dietary *S. cerevisiae* alone and a blend of *B. licheniformis* and *S. cerevisiae* in fattening lambs[6]. Also, Mousa et al. [21] noticed a reduction (P<0.05) in ruminal NH_3-N with *S. cerevisiae* in Rahmani sheep. However, a minor decrease (1.03%) was detected when Farafra rams fed a diet containing *L. acidophilus*[7]. In a meta-analysis, Ogbuewu&Mbajiorgu, concluded an advantageous influence of the dietary SC on ruminal NH_3-N level in growing goats[22]. In another investigation on Holstein cows, NH_3-N concentration significantly (P<0.05) lessened after 3 hrs of feeding by 22.16% with *B. licheniformis* supplementation [23]. This may indicate that more NH_3-N is used to synthesize microbial protein.

The values of the TVFA have an opposite trend, where all probiotic formulas increased the TVFA production in the rumen at both sampling times. The ABLB2+SC additives elevated the levels of TVFA by 47.35 and 17.29%, at 0 and 3 hrs of feeding, respectively, compared to control. The significant raise of ruminal TVFA by supplementation of the probiotic is consistent with earlier studies by Mousa et al. [21] with *S. cerevisiae*in Rahmani sheep, Sheikh et al. [8] with *L. acidophilus* plus *S.cerevisiae* in Corriedale sheep, Ogbuewu&Mbajiorgu, [22]with *S.cerevisiae* in young goats, and Qiao et al. [23] with *B. licheniformis* in Holstein cows. In Farafra sheep, the significant (P=0.021) high ruminal TVFA levels with the probiotics were possibly yielded from enhancements in the activities of ruminal microorganisms, fermentation rate, and the availability of fermentable carbohydrates [7].

The obtained results of NH3-N and TVFA reflected on the values of rumen pH.Relative to the control, the pH values with all tested probiotic combinations were statistically decreased at 0 and 3 hrs post-feeding. The changes at the lowest pH values in ABLB4+SC were small and nearly identical [-0.31 (4.10%) and -0.32 (4.53%) units] at 0 or 3 hrs of feeding, respectively, comparedto the control. These findings are matched with a preceding study by Chen et al. [9] when incorporating *B. licheniformis* and *L. plantarum* into lambs' diet, a slight significant (P=0.002) reduction (-0.22 unit; 3.21%) in the ruminal pH value was induced. They suggested that probiotics encouraged more lactic acid production, which reduced the ruminalpH.Additionally, *B. licheniformis* supplementation caused a lower pH (P<0.05) value ofthe ruminalliquor of Chinese Holstein cows [23]. In contrast, yeast competes with lactate-producing and promoting lactate-utilizing bacteria, decreasing lactate accumulation. Accordingly, *S. cerevisiae* can sustain the normal ruminal pH ([24,22]). Also, growing the protozoal count in rumen fluid under all the tested probiotic supplements was declared in our trial. This finding supports that protozoa stabilize the ruminal

pH since the protozoa are faster than bacteria in consuming the lactate [25]. Formulating yeast and other bacterial strains with lactic acid bacteria (LAB), synergistic activities could appear [11].

In this work, the protozoa count was higher in ABLB2+SC than in control by 21.98 and 35.08% at 0 and 3 hrs of feeding, respectively. These findings were in concurrence with Sheikh et al. [8] who found that the rumen protozoa count with the dietary probiotics (*S. cerevisiae* plus *L. acidophilus*) was significantly higher (P<0.01) than the control group in the Corriedale sheep. Likewise, Kumar et al. [26] illustrated that *S. cerevisiae* raised protozoa count significantly (*P*<0.01) by 31.35% in Murrah buffalo bulls at 4 hrs post-feeding. Nevertheless, no significant variations were noticed in the protozoal count when supplementing Farafra sheep with *L. acidophilus*, *B. subtilis*, or *Ruminococcusalbus*[7]. In the rumen, protozoa (10^5 to 10^6 /mL) comprise about half of the microbial biomass. Most ruminal protozoa are ciliated, while some species are flagellated [27]. Protozoa are active and may be responsible for 30-40% of the total ruminal activity. It has a role in degrading plant cell walls (ciliate protozoa), starch digestion, lipid hydrolysis, and the microbial protein massin the rumen [28].

It is widely accepted that MCP is a reliable indicator of feed utilization. Incorporating ABLB2+SC in Saidi sheep diets was clearly connected with a substantial enhancement of MCP mass (29.64 and 32.49%, respectively) at 0 and 3 hrs of feeding compared to the control. Soliman et al. [29] recorded that the MCP significantly improved (*P*<0.05) when supplemented diets of Barki rams with a multi-strain probiotic involving *L. acidophilus* and *B. licheniformis*. Likewise, Jia et al. [6] revealed that production of MCP significantly (*P*=0.043) advanced by 31.22% with dietary probiotics (*B. licheniformis* plus *S. cerevisiae*) in lambs. Also, Chen et al. [9] supplied lambs with a probiotics blend (including *B. licheniformis*, *L. plantarum*, and *B.subtilis*) and detected a considerable (*P*<0.001) improvement in the MCP by 157.14%. To synthesize the MCP, nitrogen, energy, and other nutrients such as sulfur are required [30].

In the present investigation, the rumen fermentation parameters were enhanced by probiotic supplements. Improved ruminalTVFAconcentrations, reduced NH3-N concentration and stability of pH values which reflected on the synthesis of more MCP.

4.2.Nutrient digestibility

The probiotic blends with different mechanisms of action could offer better results than adding them separately. Supplementing ruminants with these combinations may improve their health status and nutrient availability [20]. It was noticeable that the advancement in nutrient digestibility was attributed to the mixture of ABLB and SC at the low dose of tested diets (ABLB2+SC). The improvements in DM, OM, CF, CP, EE, and NFE digestibilitiesin ABLB2+SCwere estimated by11.78,10.65,11.61, 19.81,15.45, and 7.04%, respectively, relative to the control. Numerous authors discussed the positive impacts of probiotic strains on total tract digestibility. Mousa et al. [21] documented that the dietary *S. cerevisiae* of Rahmani yearling ameliorated the DM, CF, and CP digestibilities (P<0.05). Also, Soliman et al. [29] supplemented Barki sheep diets with a multi-strain probiotic involving *L. acidophilus and B. licheniformis*. They obtained that all nutrient digestibilities, except NFE, were higher than the control (*P*<0.05).Incorporating *B. licheniformis* in Dorper crossbred sheep diets increased (*P*<0.001)the DM, OM, neutral detergent fiber, and CP digestibilities[5]. Feeding Farafra lambs a diet containing *L. acidophilus* improved the digestibility (*P*≤0.01) of OM and non-structural carbohydrates relative to the control [7]. Supplementing growing Barki lambs with probiotics containing *L. acidophilus* and *B. licheniformis*, *S. cerevisiae* and exogenous enzymes significantly (*P<0.05*) enhanced fiber digestion.[10]

The improvement results of ruminal TVFA, NH_3-N, and MCP (Table 2) can reflect the efficacy of dietary nutrient utilization with the probiotic supplements. Seo et al. [31] found that a combination of *S. cerevisiae* and *B. licheniformis* boosted the nitrogen utilization by the rumen microorganisms and altered the fermentation in sheep. Various researchers revealed that S. cerevisiae can promote lactate-utilizing bacteria, thus adjusting ruminal pH for fiber breakdown[6]. Consequently,improving fiber digestion in the diet enhances nutrient availability for rumen metabolic processes. Additionally, protozoa in the rumen actively contribute to lipid hydrolysis [28]. Therefore, the noteworthy increase in protozoa count in ABLB2+SC may account for the higher digestibility of EE.

The improvement of most nutrient digestibility's reflected on the nutritive values of the tested diets as a percentage of TDN, SV, and DCP. The bestvalues were obtained with the ABLB2+SC. Several researchers obtained similar results with the dietary probiotics in different sheep breeds: Saleem et al. [32] in Saidi, Mousa et al. [21] in Rahmani, Deng et al. [5] in Dorper crossbred, and Soliman et al. [29] in Barki.

4.3.Blood metabolites

The health and performance of animals are indications of their metabolic state. Probiotics can regulate the metabolism in the body [33]. The significant increase in serum glucose concentrations (11.22%) with ABLB2+SC supplements which may be related to the improvement of TVFA production in the rumen[6] the obtained results areharmonious with earlier studies. Hussein, [34] illustrated that incorporating *L. sporogenes* and *Saccharomyces cerevisia*in Najdi lambs' diets improved (P<0.05) plasma glucose concentration.In the same line, Chen et al. [9]noticed that the probiotics (*B. subtilis*, *B. licheniformis*, and *L. plantarum*) treatment significantly (P=0.001) increased (14.25%) the concentrations of lambs' serum glucose.

Albumin, globulin, and total protein concentrations were significantly increased with probiotics additiveswhich may be related to the improvement of digestible dietary protein with probiotic additives. These results are in line with Sallam et al. [10]who supplemented growing Barki lambs with probiotics containing *L. acidophilus* and *B. licheniformis*, *S. cerevisiae* and exogenous enzymes, and Analog sheep with B. subtilis and B. licheniformis [35]. These findings may suggest that the improvement in the measured plasma proteins was related to including the dietary bacterial and yeast formula at the low level (ABLB2+SC) in our study on dietary protein's utilization and bioavailability[36,10].

Remarkable drops in serum urea concentration with most probiotic supplements were detected in this study, with ABLB2+SC (by 9.86%). Comparable findings were noticed by Ding et al. [37] in crossbred lambs (with yeast), Saleem et al. [32] in weaned Saidi lambs (with *Pediococcus* strains) and Hamdon et al. [7]in growing Farafra lambs (with *B. subtilis*, *L. acidophilus* or*Ruminococcusalbus*). Results in Table 2 exposed that use of the tested probiotics ameliorated the dietary protein utilization, as evidenced by the decreased NH_3-N and the upgraded MCP levels in parallel with the lower serum urea levels. Regarding serum creatinine, just the ABLB2+SC caused a significant decrease by 35.79% in its concentration relative to the control.Hamdon et al. [7] demonstrated that dietary bacterial probiotic declined serum creatinine by about 21% in Farafra lambs. Plasma urea and creatinine concentrations serve as effective markers of glomerular filtration in mammals, even in cases unrelated to kidney function. By monitoring these levels in animals, the health of their renal system can be assessed. Considering this, the probiotic formulas used in the current trial have been shown to safeguard kidney functions and maintain overall physiological well-being.The obtained results showed no considerable impact on ALT activity. In contrast, adding S. cerevisiae to the investigated

probiotics resulted in slight but statistically significant increases in AST levels. Only 3.48% was the difference between the highest value of AST in ABLB2+SC and the control. Similar observations were detected by incorporating *S. cerevisiae*[21] or by including *Lactobacillus* and *Bifidobacterium* strains in the ewe's diet[36]. The ALT and AST are enzyme-related metabolites that play a role in amino acid catabolism and interorgan nitrogen transport. Their activities can indicate the condition of hepatocytes or myocytes. Thereby, the tested probiotic combinations have no adverse effect on the functions of the liver or muscles in lambs.

Regarding lipid-related metabolites, concentrations of triglycerides and cholesterol were decreased by 4.62 and 11.64%, respectively, in ABLB2+SC relative to the control. The findings in this study are consistent with those stated by Hamdon et al. [7] who noticed that bacterial probiotics induced significant decreases ranging from 9.2 to 16.9% in serum cholesterol concentration of growing Farafra lambs. Additionally, probiotics led to reductions of 8.10% [35] and 6.83% [9] in the serum cholesterol of sheep. Further, Shetawy et al. content of serum cholesterol was significantly lowered (P=0.001) by 7.04% with probiotics supplements (*Bacillus subtillis* + *Bacillus licheniformis*; 1:1) in Holstein's calves[38]. On the other side, serum triglycerides findings in the present experiment are in the same trend as Shetawy et al. [38] who declared that levels of triglyceride were significantly reduced (P=0.001) by 37.98 in Holstein's calves under probiotics treatment. In the lambs subject to probiotic treatments, serum triglyceride declined by 15.63% [9] and 11.22% [10].However, Kafilzadeh et al. [36]described that the probiotic supplementation of lactating Sanjabi ewes have noinfluences (P>0.05) on the average plasma contents of triglyceride and cholesterol.Song et al. registered that the liver synthesizes bile acids from cholesterol, and probiotics can lower cholesterol by accelerating the circulation of bile acids[33]. They added that short-chain fatty acids are metabolites of probiotics and could inhibit cholesterol synthesis. Probiotics such as *L. acidophilus* can generate cholesterol ester acyl-CoA inhibitors that obstruct the enzyme 3-hydroxy-3-methyl-glutaryl coenzyme A reductase, which in turn is a vital enzyme for cholesterol synthesis. Cholesterol coprecipitates with deconjugated bile acids, reducing lipid solubilization and absorption from the diet [39]. Moreover, certain strains of *Lactobacillus* that hydrolyze bile salts can lower the hydrolysis of triglycerides by inhibiting the responsible enzyme, lipoprotein lipase [40]. Furthermore, bacterial probiotics may eliminate cholesterol by incorporating it into their cell membrane during their growth, as reviewed by Pandey et al. [41]. Consequently, the assessed dietary probiotics could be a beneficial approach to regulate lipid metabolism.

CONCLUSION

The combination of probiotics has multiple mechanisms of action that can yield better results. Using a multi-strain probiotic is more effective than individual components. This study demonstrated that the enhancement in rumen parameters, nutrient digestibility, blood parameters, antioxidants, and immunity was influenced by the level of combined bacterial strain supplements and SC supplementation. We recommend incorporating a formulation of *L. acidophilus, L. bulgaricus, B. licheniformis*, and *Bifidobacterium bifidum* at a daily dose of 2×10^9cfu along with SC at 2×10^7cfu in the regular diets of sheep to improve their performance and physiological responses.

Data availability: All data generated or analyzed during this study are included in this published article.

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Conflict of conflicts of interest statement

The authors declare no conflict of interest.

Animal welfare statement

Work procedures were approved by Sohag Institutional Animal Care and Use Committee (Approval protocol No: Sohag-IACUC/6/12/1/2024/01).This study was approved by the Scientific Research Ethics Committee of Mansoura University in accordance with Animal Research Reporting of *in Vivo* Experiments (ARRIVE) guidelines.

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Table 1. Chemical composition of the basal diet (% on DM basis)

Items			
Ingredients		Kg/Ton	
Yellow corn		350	
Soybean meal		75	
Wheat barn		65	
Common salt		2.5	
Limestone		6	
Mineral and vitamin mixture*		1.5	
Berseem hay		500	
Proximate chemical composition (on DM basis)			
Items	Concentrate mixture	Berseem hay	Total mixed diets (calculated)
%			
Dry matter	88.93	91.28	90.1
Organic matter	87.84	85.8	86.82
Crude protein	14.2	15.1	14.65
Ether extract	4.98	1.66	3.32
Crude fiber	9.58	35.9	22.74
Nitrogen free extract	59.08	33.14	46.11
Ash	12.16	14.2	13.18

*: minerals and vitamins mixture contained: Copper 30000 mg, Iodine 800 mg, Selenium 300 mg, Iron 10000 mg, MgO 80000 mg, Zinc 100000 mg, Cobalt 400 mg, Vit. A 10000000 IU, Vit. D_3 2500000 IU, Vit. E 35000 IU, and $CaCO_3$ to 3 Kg.

Table 2. Influence of dietary probiotic formulations on rumen parameters of Saidi sheep.

Items		Treatments					*P*-value
		Control	**ABLB2**	**ABLB4**	**ABLB2+SC**	**ABLB4+SC**	
Before feeding							
pH		7.56 ^{a}±0.04	7.29 ^{b}±0.01	7.33 ^{b}±0.08	7.29 ^{b}±0.07	7.25 ^{b}±0.09	0.024
NH_3-N	mg/dL	46.67 ^{a}±0.66	32.38 ^{b}±1.07	33.25 ^{b}±1.41	31.79 ^{b}±0.68	32.08 ^{b}±0.58	<0.001
TVFA	mEq/L	10.20 ^{b}± 0.52	14.38 ^{a}± 0.67	14.48 ^{a}±1.11	15.03 ^{a}±0.23	14.93 ^{a}±0.40	<0.001
MCP	mg/dL	15.18 ^{b}± 0.11	16.32 ^{ab}± 0.61	17.06 ^{ab}±1.91	19.68 ^{a}±1.56	17.16 ^{ab}±0.49	0.039
Protozoa	10^5/mL	9.10 ^{b}±0.41.23	10.18 ^{ab}± 0.28	9.24 ^{b}± 0.56	11.10 ^{a}± 1.35	9.57 ^{ab}±0.83	0.035
3 hrs post-feeding							
pH		7.06 ^{a}±0.06	6.79 ^{b}± 0.04	6.77 ^{b}± 0.04	6.76 ^{b}±0.06	6.74 ^{b}±0.05	0.001
NH_3-N	mg/dL	49.44 ^{a}±0.43	37.33 ^{b}±1.28	38.21 ^{b} ± 1.30	36.17 ^{b} ±0.37	37.04 ^{b} ±1.13	<0.001
TVFA	mEq/L	18.28 ^{b}±1.20	20.66 ^{a}± 0.57	20.01 ^{ab}±0.77	21.44 ^{a}±0.34	20.43 ^{ab}±0.71	0.040
MCP	mg/dL	11.79 ^{b}± 0.66	13.08 ^{b}± 0.42	13.32 ^{b}± 1.03	15.62 ^{a}±0.52	13.68 ^{ab}±0.19	0.033
Protozoa	10^5/mL	12.63 ^{c}± 0.66	16.19 ^{ab}± 0.58	13.19 ^{c}±0.75	17.06 ^{a}±1.95	14.21 ^{bc}±0.89	0.015

ABLB2 and ABLB4: 2×10^9cfu/g and 4×10^9cfu/g of the bacterial formula. SC: 2×10^7cfu/g of yeast.pH: potential of hydrogen. NH_3-N: ammonia nitrogen. TVFA: total volatile fatty acids. MCP: microbial crude protein.

$^{a, b \text{ and } c}$:means in the same row with different superscripts are significantly (P<0.05) different.

Table 3. Influence of dietary probiotic formulations on nutrient digestibilities and nutritive values of the experimented diets

Items	**Treatments**					***P*-value**
	Control	**ABLB2**	**ABLB4**	**ABLB2+SC**	**ABLB4+SC**	
Digestibility %						
Dry matter	75.40^{c}±0.78	79.34^{b}±0.71	81.74^{b}±0.22	84.28^{a}±0.87	81.42^{b}±0.42	<0.001
Organic matter	66.10±1.41	69.53±1.16	71.13±1.05	73.14±1.76	70.46±1.46	0.104
Crude protein	66.49^{d}±0.96	72.55^{c}±0.96	74.10^{bc}±0.63	79.66^{a}±0.92	76.99^{ab}±0.66	<0.001
Ether extract	65.44^{d}±0.87	69.25^{c}±0.94	71.86^{bc}±0.89	75.55^{a}±1.76	72.92^{ab}±0.45	<0.001
Crude fiber	66.23^{c}±0.42	68.19^{bc}±1.60	70.16^{b}±0.24	73.92^{a}±1.42	71.04^{ab}±0.10	0.005
Nitrogen free extract	65.95±1.97	69.01±1.26	70.40±1.56	70.59±2.19	66.95±1.76	0.451
Nutritive value %						
Total digestible nutrients	61.65^{b}±1.30	64.86^{ab}±1.06	66.35^{a}±0.95	68.29^{a}±1.61	65.25^{ab}±1.04	0.041
Starch value	49.81^{b}±1.29	52.94^{ab}±1.05	54.40^{a}±0.95	56.27^{a}±1.59	53.27^{ab}±1.03	0.043
Digestible crude protein	11.64^{d}±0.17	12.70^{c}±0.17	12.97^{bc}±0.11	13.94^{a}±0.16	13.47^{ab}±0.11	<0.001

ABLB2 and ABLB4: 2×10^{9}cfu/g and 4×10^{9}cfu/g of the bacterial formula. SC: 2×10^{7}cfu/g of yeast.$^{a, b, c \text{ and } d}$:means in the same row with different superscripts are significantly ($P<0.05$) different.

Table 4. Influence of dietary probiotic supplements on blood biochemistry of Saidi sheep

Items		**Treatments**					***P*-value**
		Control	**ABLB2**	**ABLB4**	**ABLB2+SC**	**ABLB4+SC**	
Glucose	**mg/dL**	70.13^{b}±1.08	74.88^{ab}±0.98	73.13^{ab}±1.41	78.00^{a}±2.09	76.38^{ab}±4.15	0.040
Albumin	**g/dL**	4.98^{b}±0.15	5.32^{a}±0.10	5.22^{ab}±0.16	5.40^{a}±0.14	5.40^{a}±0.08	0.037
globulin	**g/dL**	2.40^{c}±0.06	2.60^{b}±0.06	2.80^{a}±0.09	2.88^{a}±0.05	2.80^{a}±0.07	<0.001
Total protein	**g/dL**	7.36^{d}±0.08	7.92^{c}±0.16	8.02^{bc}±0.09	8.28^{a}±0.19	8.20^{ab}±0.03	<0.001
ALT	**U/L**	24.28±0.84	24.80±1.53	24.48±0.73	25.28±0.85	24.82±1.17	0.956
AST	**U/L**	59.20^{b} ±0.41	60.28^{ab}±0.72	60.38^{ab}±0.76	61.26^{a}±1.07	61.18^{a}±0.77	0.031
Urea	**mg/dL**	35.50^{a}±0.81	34.50^{ab}±0.50	34.26^{b}±0.49	32.00^{c}±0.71	32.76^{c}±0.37	<0.001
Creatinine	**mg/dL**	1.90^{a}±0.15	1.58^{a}±0.11	1.70^{a}±0.12	1.22^{b} ±0.12	1.71^{a}±0.18	0.030
Triglycerides	**mg/dL**	61.88^{a}±0.64	60.98^{ab}±0.73	60.24^{bc}±0.72	59.02^{d}±0.61	59.50^{cd}±0.50	<0.001
Cholesterol	**mg/dL**	73.00^{a}±1.28	67.13^{b}±0.89	66.88^{b}±1.29	64.50^{b}±1.46	66.25^{b}±1.52	0.001

ABLB2 and ABLB4: 2×10^{9}cfu/g and 4×10^{9}cfu/g of the bacterial formula. SC: 2×10^{7}cfu/g of yeast.ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.$^{a, b, c \text{ and } d}$ means in the same row with different superscripts are significantly (P<0.05) different.