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The Effect of Adding Multi-Strain Probiotics (MSP) on the Hematological, Immunological and Antioxidant Parameters of Male Saidi Sheep

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Abstract

THIS study was conducted to evaluate the effect of adding multi-strain probiotics (MSP) with or without dry yeast to the diet on blood, immune characteristics and antioxidant status of male Saidi sheep. Five males (54.14 ± 1.67 kg average body weight) were used and randomly distributed in a 5 × 5 Latin square design to evaluate MSP administration for a period of 105 days. The five treatments were given the basic diet (50:50% rough/concentrate), where the first treatment was control and without other additives, while 2nd and 3rd groups were added at a rate of 1 g/day/animal to the other four treatments at a level of (2 x 10^9 cfu/g) or (4 × 10^9 cfu/g), ABLB2 and ABLB4, respectively. The 4th (ABLB2+SC) and 5th (ABLB4+SC) treatments were ABLB2 and ABLB4 enriched with 1 g/day/animal of dry yeast *Saccharomyces cerevisiae* (SC) was added at a level of (2 × 10^7 cfu/g). The results showed that adding MSP to the basal diet had no effect on blood parameters. While ABLB4 and ABLB4+SC groups had better values of GSH-Px and TAC and significantly reduced the level of MDA compared to the control ($P < 0.001$). There was a slight improvement in the IgM concentration, along with a significant increase in IgA and IgG levels, as well as lysozyme activity in the blood serum in all MSP groups. In conclusion, adding multi-strain probiotics with yeast to the basic diet enhanced the antioxidant capacity and immune response in blood serum in Egyptian male sheep.

Keywords: MSP, Saidi sheep, Immunity, Antioxidant, Hematological parameters.

Introduction

Recently, there has been a growing demand for animal products and heightened public concerns about the negative impacts of livestock farming. As a result, the industry is under pressure to enhance animal welfare, reduce environmental harm, and guarantee the safety of animal-derived products. This has sparked a surge in interest in natural feed additives, such as direct-fed microbials (DFM), which include substances like enzymes, probiotics, and prebiotics [1-4]. Probiotics, which are deemed safe by the Food and Drug Administration (FDA), consist of non-pathogenic microorganisms such as bacteria, yeasts, and fungi [5]. It plays a vital role in regulating host metabolism, bolstering barrier function, modulating the immune system, and exhibiting antimicrobial properties [6]. Probiotics are mainly lactic acid creating bacilli, mostly lactobacilli (*L. acidophilus* DDS-1, *L. casei*, *L. lactis*, *L. rhamnosus*, *L. salviarius*) and *bifidobacteria* (*B. longum, B. infantis, B. bifidum*), and yeasts (*Sacharomyces boulardii* and *Sacharomyces cervisiae*) [7].

Above all, there is evidence that adding probiotics to the diets has improved blood hematological parameters [8], blood biochemistry [9], immunity response [6, 10], and antioxidant parameters [10,11]. In recent years, many commercial products have been formulated to contain a mixture of microorganisms and their fermentation products [10,11]. This approach aims to ensure the efficacy of the product and provide a multi-factorial response.

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As part of a series of research studies evaluating the effects of probiotic combinations and yeast on animal performance, physiological status, and nutritional efficiency, thus the present study aimed to assess the effects of adding two levels of a novel combination of four bacterial probiotics with or without yeast to the diet on the blood hematological parameters, antioxidant status, and immune response of Saidi sheep.

Material and Methods

The experimental work was conducted at the Animal Nutrition Research Unit, Animal Production Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. The animals, procedures, and protocols in this experiment were reviewed and approved by the Institutional Animal Care and Use Committee of the Faculty of Agriculture, Sohag University, Sohag, Egypt (Sohag-IACUC/6/12/1/2024/01).

Experimental design, animals, and diets

Five healthy male Saidi sheep (54.14 ± 1.67 kg body weight) were randomly assigned in a 5 × 5 Latin square design to evaluate the basal diet and four other tested diets supplemented with probiotic combinations. In the first treatment, sheep were fed a basal diet consisting of 50% roughage and 50% concentrate, as presented in Table 1. The second and third treatments sheep were given the basal diet supplemented with 1 g/day/animal of a bacterial probiotics combination containing *Lactobacillus acidophilus, L. bulgaricus, Bacillus licheniformis,* and *Bifidobacterium bifidum* at a ratio of 1:1:1:1 (ABLB) at two levels: 2×10^9 (ABLB2) and 4×10^9 cfu/g (ABLB4), respectively. While sheep in the fourth (ABLB2+SC) and fifth (ABLB4+SC) treatments received the second and third diets enriched with 1 g/day/animal of *Saccharomyces cerevisiae* (SC) 2×10^7 cfu/g. The dietary feed samples were analyzed for dry matter (DM), ash, crude protein (CP), ether extract (EE), and crude fiber (CF) according to the official methods of the AOAC [12]. The organic matter (OM) was calculated by subtracting ash content from 100, while the nitrogen-free extract (NFE) content was estimated as 100% - (CF + CP + EE + ash). Animals were housed and fed individually in metabolic cages (150 cm length × 70 cm width × 120 cm height). The tested diets were offered twice daily (8 a.m. and 4 p.m.), 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. Free access to water was available throughout the day.

Blood sampling and blood parameters

At the end of each experimental period, blood samples were collected from the jugular vein by using a sterile syringe from all tested animals at 8:00 a.m. in the morning and then transferred into two sterile tubes. The first tube was supplemented with heparin for the hematological assay to estimate hemoglobin (Hb), hematocrit (HCT), red blood cells (RBC), white blood cells (WBC), lymphocytes (LYM), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (PLT). The second tube was without heparin to obtain serum by centrifugation at 3000 rpm for 10 minutes. The serum was collected in Eppendorf tubes and then stored at -18 °C until biochemical analysis. Immunoglobulins were measured by a microplate reader method described in ELISA kit manufacture catalog of IgA (CSB-E13681Sh), IgG (CSB-E14400Sh) and IgM (CSB-E13682Sh) at wavelength of 450 nm. Serum lysozyme activity was measured using a spectrophotometer at a wavelength of 450 nm. Commercial kits from Biodiagnostic Egypt were utilized to determine malondialdehyde (MDA) (CAT No: MD2529), glutathione peroxidase (GSH-Px) (CAT No: GP2524), and total antioxidant capacity (TAC) (CAT No: TA2513#) following the standard protocols provided by the suppliers.

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. The data of blood parameters 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. The Statistical model used to analyze the data was $Y_{ijk} = \mu + R_i + C_j + T_k + e_{ijk}$

Where Y_{ijk} is the observed value of each trait, μ is the overall mean, R_i is the i^{th} row effect, C_j is the j^{th} column effect, T_k is the k^{th} (1,2,3,4,5) treatment effect, e_{ijk} is the random effect of error.

Results

Blood hematological parameters

Table 2 shows that dietary probiotic blends have no significant impact on the estimated hematological parameters in Saidi sheep, except for a notable increase (P=0.002) in white blood cell (WBC) count with both doses of the multi-strain probiotics combination plus SC (ABLB2+SC and ABLB4+SC) compared to the multi-strain probiotics combination without SC (ABLB2 and ABLB4) and the control group. Additionally, the lymphocyte percentage was higher in ABLB2+SC and ABLB4+SC compared to the control group by 1.76 and 3.24 units, respectively.

Antioxidant status

Fig.1 (A-C) shows how the multi-strain probiotics enhance serum antioxidant parameters in the tested sheep fed the experimental diets. The probiotic supplements led to statistically significant improvements in GSH-Px (Fig. 1A) and TAC (Fig. 1B) values (P=0.010 and P=0.025, respectively), while the mean MDA (Fig. 1C) values were significantly reduced compared to the control (P=0.001). The highest resistance to oxidative stress (OS) was observed with ABLB2+SC2 supplements.

Immune response

Fig.2 (A-D) shows a slight increase in serum IgM levels (Fig. 2C) (P=0.346) when MSP was included in the sheep diets. Additionally, there were significant increases in serum IgG (Fig. 2A), IgA (Fig. 2B) concentrations, and lysozyme activity (Fig. 2D) with the supplemented diets compared to the control (P<0.001, P=0.038, and P=0.008, respectively). Saidi sheep in the ABLB2+SC group had better results for immunological biomarkers than other groups.

Discussion

In this study, incorporating *Saccharomyces cerevisiae* with bacterial strains (*L. acidophilus, L. bulgaricus, B. licheniformis, and B. bifidum*) in the diets of Saidi sheep led to a significant increase in blood WBC count. Similar findings were reported by Hussein [13], who included *L. sporogenes* and *S. cerevisiae* in the diets of Najdi male lambs; El-Mehanna *et al.* [14], who fed probiotics containing L. bulgaricus to Noemi male lambs; El-Ashker *et al.* [8], who supplemented Ossimi male lambs with a combination of *Lactobacillus bulgaricus* and *Lactobacillus fermentum,* and Mousa *et al.* [11], who added B. subtilis to Barki lamb's diets. Additionally, a study on ewes by Milewski & Sobiech [15], showed improved immunity with an increase in WBC count due to dietary S. cerevisiae.

Song *et al.* [6] reviewed that B-lymphocytes and macrophages can be stimulated by probiotics. Our study indicated that the probiotic combination with SC significantly reinforced the white blood cell count. To our knowledge, the mechanisms responsible for *Saccharomyces cerevisiae* increasing the production of WBC remain unclear. However, Milewski *et al.* [15], discussed that beta-glucans in the cell wall of SC can stimulate the immune cells. The WBC count and lymphocyte percentage values fall within the reference range (5 to 11 × 10^9/L and 60 to 65%, respectively) for sheep [4]. This implies that the tested lambs have a normal physiological and immunological status.

Several antioxidant parameters in the blood can serve as biomarkers of oxidative stress. One such index is GSH-Px, an enzyme that contains selenium and catalyzes the reduction of hydrogen peroxide and lipid peroxides when combined with GSH [6].

The serum MDA level is a product of oxidative stress and serves as an indicator of the intensity of lipid peroxidation. Estimating TAC can help evaluate the oxidation status. Moreover, overexpression of lysozyme can protect against oxidative stress by boosting natural antioxidant reserves and providing resistance to molecules like advanced glycation end products that contribute to acute or chronic oxidative stress [1].

The results of the blood antioxidant parameters in this study are consistent with findings from other studies. For example, Mousa *et al.* [11] found that supplementing Barki lambs with *Bacillus subtilis* for 30 days resulted in significant improvements in GSH, TAC, and lysozyme activity, as well as a significant reduction in MDA values. Similarly, supplementing Holstein calves with bacterial probiotics, including *L. plantarum* and *B. subtilis,* significantly increased the activity of superoxide dismutase at 80 days of age, indicating that probiotics can enhance TAC [16]. Jia *et al.* [10] also reported a significant increase in GSH-Px values when supplementing fattening lambs with a probiotic mixture containing *B. licheniformis* plus SC, although they did not observe significant impacts on MDA and TAC values. Additionally, Izuddin *et al.* [17] found higher GSH-Px and lower MDA levels in serum when incorporating secondary metabolites of probiotic bacteria *Lactobacillus* spp. in Dorper lambs' diet. In mice, supplementation with *B. subtilis* or *L. Plantarum* increased serum TAC and GSH-Px levels while reducing MDA levels [16].

Magistrelli *et al.* [18] demonstrated that probiotics containing Lactobacillus and Bifidobacterium strains prevented reactive oxygen species production in human peripheral blood mononuclear cells. Moreover, selenium in SC activates the enzyme glutathione peroxidase, preventing oxidative damage to the cell membrane. Additionally, yeast is a source of B-complex vitamins, which act as precursors of vital co-enzymes like Nicotinamide Adenine Dinucleotide (NAD) and Flavin Adenine Dinucleotide (FAD), responsible for biological oxidation. Therefore, the probiotic supplements in the current investigation may enhance antioxidant indices and reduce oxidative stress in sheep.

Many mechanisms of how probiotic bacteria affect the immune system are attributed to an increase in innate or acquired immunity that induces both the systemic and mucosal immune responses

[19]. Enhancing the production of antibodies is one of these ways that can improve the host immunity [20]. B-lymphocytes produce serum immunoglobulins (IgA, IgG, and IgM) to fight infections. Serum antibodies are important indicators of humoral immunity in ruminants [10]. The first antibody secreted during an initial exposure to infectious organisms is IgM. The antibody IgA has a significant role in mucosal immunity by excluding pathogenic bacteria. On the other hand, IgG is the prevalent antibody in blood plasma antibody and plays a crucial function in the systemic immune response [21]. Lysozyme is a ~14 kDa protein found in animal tissues and mucosal secretions. It can stimulate the production of immunoglobulins, enhance neutrophil activities and bacterial phagocytosis, and hydrolyze the peptidoglycan of the cell walls in pathogenic bacteria.

The results presented here demonstrate that ABLB2+SC supplements led to a relative increase (105.78%) in the lymphocyte percentage (Table 2) compared to the control group. Overall, the probiotic strains used in this study have beneficial effects on animal immunity. Song *et al.* [6] also reported that probiotics can stimulate B-lymphocytes and macrophages. Furthermore, combining lactic acid bacteria (LAB) with other bacteria and yeast has shown synergistic effects in commercial products [20]. This combination can enhance the immune response against pathogenic bacteria and mitigate the negative effects of infections [18].

Several studies have highlighted the advantages of dietary supplementation with *Lactobacillus*-based probiotics in improving mucosal immunity [17]. *Bacillus*-based probiotics in the diet can increase IgG1 levels as part of an anti-spore immune response [18]. *Bifidobacteria* are known to produce essential active compounds such as vitamins and amino acids and support lymphoid tissue development. Supplementation of Comisana male lambs with L. acidophilus and a combination of *Bifidobacterium animalis* and *B.* longum improved immune-regulatory functions and humoral responses, respectively. Additionally, *Bifidobacterium* and *L. plantarum* supplements have been shown to modulate lipid metabolism and enhance the immune response in the host [22]. The inclusion of SC in the diet positively influenced humoral immunity indicators in lambs [15].

The results of the blood immunoglobulin response in our study were similar to previous studies. Milewski et al. [15] stated a significant ($P \leq 0.01$) increase in serum concentrations of gamma globulins in lambs fed diets including SC. Also, Jia *et al.* [10] noticed that lambs fed rations supplemented with a probiotics blend containing SC and *Bacillus licheniformis* resulted in a significant increase in the serum levels of IgA, IgG, and IgM. In another study by Chen *et al.* [9] it was recorded that probiotic treatment (*B. subtilis, B. licheniformis,* and *Lactobacillus plantarum*) significantly (P=0.001) increased the serum concentration of IgG in lambs but did not affect the serum concentration of IgA and IgM. Similarly, Sun *et al*. [21] demonstrated the benefits of probiotics addition (*B. subtilis natto*) on immune function showing a significant enhancement of IgG, with slight increases in IgA and IgM levels in the serum of Holstein's calves. Other authors displayed that there was a tendency to increase the blood concentration of IgG through supplementing Holstein dairy calves with *B. subtilis* and *B. licheniformis* [16], *L. acidophilus* and *L. plantarum*, a bacterial blend containing *B. subtilis* and *L. plantarum* [16], and when including *B. amyloliquefaciens* or *B. subtilis* in beef calves' diets. Concerning serum lysozyme, Mousa *et al.* [11], observed a significant (P=0.004) increase in its activity in the serum after 30 days of adding *B. subtilis* to diets of Barki lambs. In the same context, after 30 days of treatment with a combination of probiotics (*L. delbrueckii ssp. Bulgaricus* and *L. fermentum*), the lysozyme activity in the serum of Ossimi lambs was significantly improved according to the study conducted by El-Ashker *et al.* [8]. Likewise, Devyatkin *et al.* [23] determined the positive influence of *B. subtilis* and *B.licheniformis* supplements on serum lysozyme activity in sheep, which elevated by about 52% with probiotic addition relative to the control ones. Furthermore, lambs fed the SC diet presented a significant ($P \leq 0.01$) increase in serum lysozyme activity [15].

Finally, the OS is connected to the immune response, which can impact on the health status of animals [24]. The enhancement of antioxidant capacity with probiotic supplements was orchestrated with the stimulated immune function [11, 17]. The probiotic formula used in this study improved both resistance to OS and immunity.

Conclusions

A combination of probiotics with different mechanisms of action could provide better results and potentiated probiotics are more effective than their components separately. In the present study, it is noted that the improvement in blood hematological, antioxidants and immunity was not only related to the level of combined bacterial strain supplementation but was also associated with the SC supplementation. Therefore, we emphasize the inclusion of formulations of *Lactobacillus acidophilus, Lactobacillus bulgaricus, Bacillus licheniformis*, *Bifidobacterium bifidum* at a level of 2×10^9 cfu/g plus SC at a level of 2×10^7 in the

regular diets of sheep to enhance performance and physiological responses. The use of probiotics and their combinations in animal nutrition needs further studies to evaluate their effects on animal performance and nutritional efficiency.

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Conflicts of interest

The authors declared no competing interests.

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Author contributions

Ali S. A. Saleem, and Khaled M. Al-Marakby: Methodology, Formal analysis, Conceptualization, Mohamed Y. Elaref, Sabry M. Bassiony; Supervision, Writing – original draft, Validation Project administration, Sameh Abdelnour, Amera A. Helal, and Usama M. Abdel-Monem; Data curation, Investigation Writing – review & editing, Writing – original draft, Validation.

TABLE 1. Ingredients and chemical composition of the basal diet on dry matter basis %.

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		
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 combination (ABLB) with or without SC on haematological parameters of Saidi sheep

Items[2]	**Treatments**[1]					***P*-value**
	Control	**ABLB2**	**ABLB4**	**ABLB2+SC**	**ABLB4+SC**	
Hb, g/dL	10.64±0.37	10.85±0.31	10.75±0.26	10.95±0.45	10.78±0.36	0.987
HCT, %	32.74±0.54	33.28±1.03	33.18±0.70	33.63±0.83	33.35±0.84	0.937
RBC, 10^6/µL	12.78± 0.54	13.15±0.38	13.17±0.71	13.33±0.66	13.18±0.59	0.969
WBC, 10^3/µL	7.14 ^{b}± 0.11	7.77 ^{b}±0.24	7.49 ^{b}±0.54	8.90 ^{a}±0.92	8.71 ^{a}±0.80	0.002
LYM, %	62.14± 3.04	62.48±2.78	64.85±1.37	65.73±2.24	65.38±1.36	0.476
MCV, fL	25.91± 1.46	25.47±1.27	25.57±1.48	25.60±1.57	25.53±1.22	0.999
MCH, pg/cell	8.43± 0.58	8.29±0.36	8.30±0.53	8.34±0.60	8.20±0.20	0.998
MCHC, g/dL	32.47± 0.74	32.85±1.76	32.42±0.56	32.75±1.88	32.35±1.04	0.998
PLT, 10^9/L	347.0±41.52	372.5±42.50	350.3±38.10	386.3±45.04	357.8±41.94	0.969

[1]ABLB2 and ABLB4: 2×10^9 cfu/g and 4×10^9 cfu/g of the bacterial combination. SC: 2×10^7 cfu/g of yeast. [2]Hb: Hemoglobin, HCT: Haematocrit, RBC: Red blood cells, WBC: White blood cells, LYM: Lymphocytes, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: platelets. $^{a \text{ and } b}$ means in the same row with different superscripts are significantly ($P<0.05$) different.

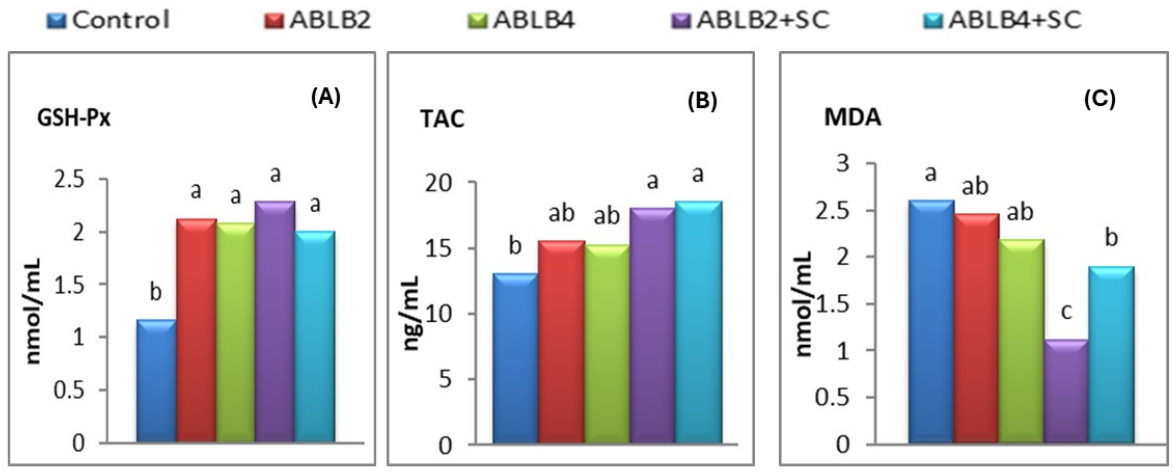

Fig. 1. Influence of dietary probiotic combination (ABLB) with or without SC on antioxidant parameters including GSH-Px (Fig. 1A), TAC (Fig. 1B) and MDA (Fig. 1C) of Saidi sheep.

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. GSH-Px: glutathione peroxidase. MDA: malondialdehyde. TAC: total antioxidant capacity. [a, b, c] means in the same row with different superscripts are significantly ($P<0.05$) different.

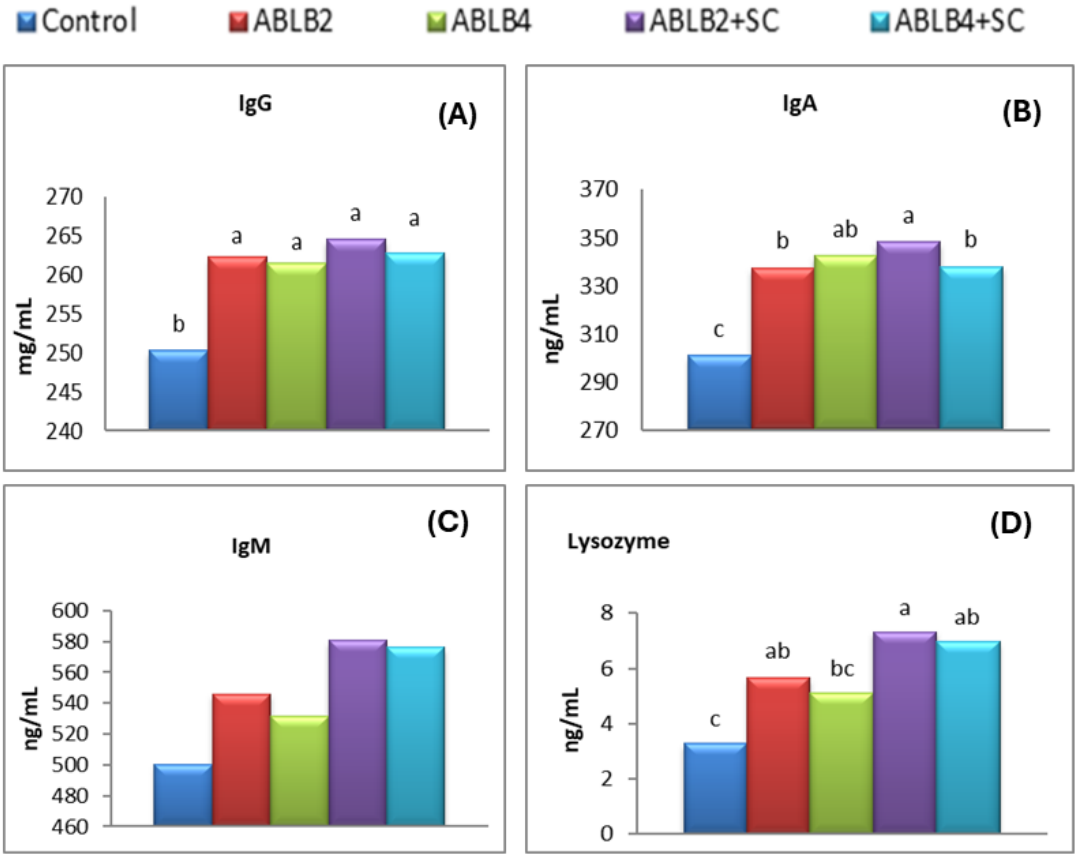

Fig. 2. Influence of probiotic formulations on blood immunoglobulin response including IgG (Fig. 2A), IgA (Fig. 2B), IgM (Fig .2C) and lysosome activity (Fig. 2D) of Saidi sheep.

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. IgA, IgM and IgG: Immunoglobulins A, M and G, respectively.

References

1. Arowolo, M. A. and He, J. Use of probiotics and botanical extracts to improve ruminant production in the tropics: A review. *Anim. Nutr.*, **4**(3), 241-249 (2018).
2. Direkvandi, E., Mohammadabadi, T. and Salem, A. Z. Oral administration of lactate producing bacteria alone or combined with Saccharomyces cerevisiae and Megasphaera elsdenii on performance of fattening lambs. *J. Appl. Anim. Res.*, **48**(1), 235-243 (2020).
3. Ban, Y. and Guan, L. L. Implication and challenges of direct-fed microbial supplementation to improve ruminant production and health. *J. Anim. Sci. Biotechnol*., **12**(1), 109(2021).
4. Castro-Pérez, B. I., Núñez-Benítez, V. H., Estrada-Angulo, A., Urías-Estrada, J. D., Gaxiola-Camacho, S. M., Rodríguez-Gaxiola, M. A. and Plascencia, A. Evaluation of standardized mixture of synbiotic-glyconutrients supplemented in lambs finished during summer season in tropical environment: growth performance, dietary energetics, and carcass characteristics. *Can. J. Anim. Sci*., **102**(1), 155-164 (2021).
5. Mahesh, M.S., Mohanta, R.K. and Patra, A.K. (2021). Probiotics in Livestock and Poultry Nutrition and Health. In: Goel, G., Kumar, A. (eds) Advances in Probiotics for Sustainable Food and Medicine. Microorganisms for Sustainability, vol **21**. Springer, Singapore. https://doi.org/10.1007/978-981-15-6795-7_7.
6. Song, X., Liu, Y., Zhang, X., Weng, P., Zhang, R. and Wu, Z. Role of intestinal probiotics in the modulation of lipid metabolism: Implications for therapeutic treatments. *Food Sci. Hum. Wellness*, **12**(5), 1439-1449 (2023).
7. Kumar, D. S., Chigurupati, D., Prasad, S. and Prasad, R. M. V. Effect of yeast culture (Saccharomyces cerevisiae) on ruminal microbial population in buffalo bulls. *Buffalo Bull.*, **32**(2), 116-119 (2013).
8. El-Ashker, M., Risha, E., Abdelhamid, F. and Ateya, A. Potential immune modulating properties and antioxidant activity of supplementing commercially available lactoferrin and/or Lactobacillus sp. in healthy Ossimi lambs. *Pol. J. Vet. Sci.*, **21**(4), 705–713(2018).
9. Chen, H., Guo, B., Yang, M., Luo, J., Hu, Y., Qu, M. and Song, X. Response of growth performance, blood biochemistry indices, and rumen bacterial diversity in lambs to diets containing supplemental probiotics and Chinese medicine polysaccharides. *Front. Vet. Sci.*, **8**, 681389 (2021).
10. Jia, P., Cui, K., Ma, T., Wan, F., Wang, W., Yang, D. and Diao, Q. Influence of dietary supplementation with Bacillus licheniformis and Saccharomyces cerevisiae as alternatives to monensin on growth performance, antioxidant, immunity, ruminal fermentation and microbial diversity of fattening lambs. *Sci. Rep.*, **8**(1), 16712 (2018).
11. Mousa, S., Elsayed, A., Marghani, B. and Ateya, A. Effects of supplementation of Bacillus spp. on blood metabolites, antioxidant status, and gene expression pattern of selective cytokines in growing Barki lambs. *J. Adv. Vet. Anim. Res.*, **6**(3), 333 (2019).
12. AOAC. Official Methods of Analysis of AOAC International. 18th ed. In: Latimer GW, editor. Washington DC: Oxford University Press Oxford. (2019). doi: 10.1093/9780197610138.001.0001.
13. Hussein, A. Effect of biological additives on growth indices and physiological responses of weaned Najdi ram lambs. *J. Exp. Biol. Agric. Sci.*, **2**(6), 597-607 (2014).
14. El-Mehanna, S. F., Abdelsalam, M. M., Hashem, N. M., El-Azrak, K. E. M., Mansour, M. M. and Zeitoun, M. M. Relevance of probiotic, prebiotic and synbiotic supplementations on hemato-biochemical parameters, metabolic hormones, biometric measurements and carcass characteristics of sub-tropical Noemi lambs. *Int. J. Anim. Res.*, **1**(10), 1-12 (2017).
15. Milewski, S., Wójcik, R., Zaleska, B., Małaczewska, J., Tański, Z. and Siwicki, A. K. Effect of Saccharomyces cerevisiae dried yeast on the meat performance traits and selected indicators of humoral immunity in lambs. *Acta Vet. Brno.*, **82**(2), 147-151 (2013).
16. Wang, H., Yu, Z., Gao, Z., Li, Q., Qiu, X., Wu, F. and Su, H. Effects of compound probiotics on growth performance, rumen fermentation, blood parameters, and health status of neonatal Holstein calves. *J. Dairy Sci.*, **105**(3), 2190-2200 (2022).
17. Izuddin, W. I., Humam, A. M., Loh, T. C., Foo, H. L. and Samsudin, A. A. Dietary postbiotic Lactobacillus plantarum improves serum and ruminal antioxidant activity and upregulates hepatic antioxidant enzymes and ruminal barrier function in post-weaning lambs. *Antioxidants*, **9**(3), 250 (2020).
18. Magistrelli, L., Amoruso, A., Mogna, L., Graziano, T., Cantello, R., Pane, M. and Comi, C. Probiotics may have beneficial effects in Parkinson's disease: in vitro evidence. *Front. Immunol*., **10**, 969 (2019).
19. Galdeano, C. M. and Perdigon, G. The probiotic bacterium Lactobacillus casei induces activation of the gut mucosal immune system through innate immunity. *Clin. Vaccine Immunol.,* **13**(2), 219-226 (2006).
20. McAllister, T. A., Beauchemin, K. A., Alazzeh, A. Y., Baah, J., Teather, R. M. and Stanford, K. The use of direct fed microbials to mitigate pathogens and enhance production in cattle. *Can. J. Anim. Sci.*, **91**(2), 193-211 (2011).

21. Sun, P., Li, J., Bu, D., Nan, X. and Du, H. Effects of Bacillus subtilis natto and different components in culture on rumen fermentation and rumen functional bacteria in vitro. *Curr. Microbiol*., **72**, 589-595 (2016).

22. Fang, Z., Lu, W., Zhao, J., Zhang, H., Qian, L., Wang, Q. and Chen, W. Probiotics modulate the gut microbiota composition and immune responses in patients with atopic dermatitis: a pilot study. *Eur. J. Nutr.*, **59**, 2119-2130 (2020).

23. Devyatkin, V., Mishurov, A. and Kolodina, E. Probiotic effect of Bacillus subtilis B-2998D, B-3057D, and Bacillus licheniformis B-2999D complex on sheep and lambs. *J. Adv. Vet. Anim. Res.*, **8**(1), 146 (2021).

24. Celi, P., Gabai, G. Oxidant/antioxidant balance in animal nutrition and health: The role of protein oxidation. *Front. Vet. Sci.*, **2**, 48 (2015).

تأثير إضافة البروبيوتيك (MSP) متعدد السلالات على الصفات الدموية والمناعية ومضادات الأكسدة في ذكور الأغنام الصعيدية.

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الملخص

أجريت هذه الدراسة لتقييم تأثير إضافة البروبيوتيك متعدد السلالات مع أو بدون الخميرة الجافة إلى العليقة على صفات الدم والمناعة وحالة مضادات الأكسدة في ذكور الأغنام الصعيدية. تم استخدام خمسة ذكور بمتوسط وزن جسم (54.14 ±1.67 كجم)، وتم توزيعهم عشوائياً في تصميم مربع لاتيني 5 ×5 لتقييم النظام الغذائي الأساسي والمكملات الأربعة الأخرى لمدة 105 أيام. أعطيت المعاملات الخمس العليقة الأساسية (50% مركز و50% اعلاف خشنة)، حيث كانت المعاملة الأولى كنترول وبدون إضافات أخرى، في حين أضيفت البروبيوتيك متعدد السلالات بمعدل 1 جرام/يوم/حيوان إلى المعاملات الأربعة الأخرى عند مستوى (x109 cfu/g2) للمعاملتين الثانية والرابعة عند مستوى (4 × ABLB4109 (cfu/g للمعاملتين الثالثة والخامسة على التوالي. كما أضيف 1 جرام/ يوم/ حيوان من الخميرة الجافة Saccharomyces cerevisiae (SC) بمستوى (2 × 107 cfu/g) للمعاملتين الرابعة والخامسة. أظهرت النتائج أن إضافة البروبيوتيك متعدد السلالات إلى النظام الغذائي الأساسي لم يكن له أي تأثير على مؤشرات الدم. بينما تحسنت البروبيوتيك مع أو بدون الخميرة الجافة في المعاملة الرابعة والخامسة من قيم GSH-Px وTAC وخفضت بشكل ملحوظ مستوى MDA مقارنة بالتحكم (P <0.001). مع تحسن طفيف في مستوى تركيز IgM وارتفاع ملحوظ في تركيز IgA وIgG ومستوى نشاط الليزوزيم في مصل الدم. في الختام، فإن إضافة البروبيوتيك متعدد السلالات مع الخميرة إلى النظام الغذائي الأساسي أدى إلى تعزيز القدرة المضادة للأكسدة والاستجابة المناعية في مصل الدم في ذكور الأغنام الصعيدي المصرية.

الكلمات الدالة: بربيوتيك متعدد السلالات (MSP)، الأغنام الصعيدي، الجلوبيولين المناعي، مضادات الأكسدة، المعلمات الدموية.