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EFFECT OF SUPPLEMENTATION WITH YEAST ALONE OR MIXED WITH BOTH SPIRULINA OR BACILLUS SUBTILIS ON THE PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF EWES

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SUMMARY

This study was performed to assess the impact of live yeast alone or with a blend of Spirulina, and Bacillus subtilis supplementation on the feed intake, nutrient digestibility, ruminal fermentation, and performance of productive and reproductive ewes. Twenty-four pregnant ewes were divided into four similar groups (six in each) and fed to cover their recommended requirements, according to the NRC (2007). Animals were fed the conventional ration containing 50% concentrate feed mixture, 15% berseem, and 35% berseem straw without additive in the T1 group (as a control) or supplemented with 3 g of LY alone, or mixed with either 3g of SP or 1.5g of BS added to CFM for the T2, T3, and T4 groups, respectively. Results indicated that all supplements didn't affect feed intake but significantly improved all nutrient digestibility except for ether extract digestibility and enhanced milk production and composition. All supplements didn't impact pH values, but significantly lower ammonia-N concentration and higher total volatile fatty acid concentrations. Supplementing probiotics didn't influence total protein, albumin, globulin, aspartate aminotransferases and alanine aminotransferases, cholesterol, and triglyceride concentrations, but increased glucose and decreased urea and creatinine concentrations significantly. Probiotic supplementation had no effect on the body weight of ewes, but improved lambs' weight at weaning, feed conversion of ewes and the economic efficiency, especially for T2 and T3. It could be concluded that supplementing yeast with Spirulina or Bacillus subtilis might have positive effects on the productive and reproductive performance of ewes and their offerpring and enhance economic efficiency and the health conditions of sheep.

Key words: *ewe, probiotics, digestibility, rumen fermentation, productive and reproductive performance.*

INTRODUCTION

In animal feeding, the European Union (EU) Commission has restricted antibiotic use for health and environmental concerns, focusing on natural alternatives such as probiotics, prebiotics, and herbal products. Probiotics are helpful microorganisms, as noted by Salminen *et al.* (2021), that help counteract environmental challenges, improve animal performance, and reduce feeding costs (Al-Khalaifah *et al.*, 2018). Also, they have a positive effect on rumen fermentation, microbiota balance, and immune systems (Amin and Mao 2020; Ban and Guan 2021), using an appropriate quantity of probiotics, either as a single or mixed additive (FAO/WHO 2002). Furthermore, probiotics hinder pathogenic growth by producing inhibitory substances (Plaza-Diaz *et al.*, 2019; Sjofjan *et al.*, 2021). On the other hand, yeast, Bacillus subtilis, and lactic acid bacteria are more commonly probiotics used in animal feeding due to their highly effective and stable nature during feed processing and storage, as well as their non-toxicity (Ohashi and Ushida, 2009; EFSA, 2013). In this regard, Bacillus spp. can survive under high temperature, bile salt, solvent, and gut acidic resistance, along with their ability to form colonies in the small intestine under aerobic and anaerobic conditions even when antibiotics are presence (Gilliland *et al.*, 1984; Abel-Santos, 2015). Moreover, Bacillus species can produce spores, anti-microbial, fungal, and viral compounds, and digestive enzymes like amylase, lipase, protease, phytase, and others, in addition to aiding immune system regulation (Latorre *et al.*, 2015; Hu *et al.*, 2018). Furthermore, yeast is a naturally occurring source of vitamins, minerals, enzymes, lipids, proteins, carbohydrates, and others that support nutrient digestibility, productive performance, and the immune system (Dhama *et al.*, 2015; Habeeb *et al.*, 2017). Additionally, Shen *et al.* (2018) reported that yeast could be a variable alternative to antibiotic for ruminant animals. Spirulina also is an effective feed supplement for different species of animals because it contains high-quality nutrients and has health-promoting characteristics (Korany *et al.*, 2019). Spirulina is enriched with proteins, fatty acids, and good vitamins and minerals (Ghaeni and Roomiani, 2016; Michalak and Mahorse 2020). Not only that, but it also contains phenolic compounds, bioactive components, like free radical scavenging enzymes, and pigments like carotene (Gabr *et al.*, 2020). Furthermore, several studies have concluded that providing animals with different probiotic species (alone or mixed) improves health status and enhances productive and reproductive performance (Jia *et al.*, 2018; Khattab *et al.*, 2020; Devyatkin *et al.*, 2021; Piovan *et al.*, 2021; Pogranichniy *et al.*, 2023; Abd El-Hamid 2024; Abdou *et al.*, 2024).

This study objective to evaluate the impact of live yeast (LY) supplementation with or without Spirulina (SP) or Bacillus subtilis (BS), on feed intake, nutrients digestion, rumen liquor activity, some blood serum parameters, and productive and reproductive performance of ewes.

MATERIALS AND METHODS

Ethical Statement:

This study was carried out in the sheep experimental unit of the Faculty of Agriculture, AL- Azhar University, Nasr City, Cairo. All efforts were made to minimize animals' distress during work. This experiment was conducted up to 120 days (one month before and three months after lambing) with a three-week acclimation stage before beginning work.

Experimental Animals and management:

Twenty-four pregnant ewes, aged 3- 4 years and average 54.96±0.5 kg live body weights (LBW) were randomly assigned to four identical groups of six each, based on their age and LBW, starting from late-month of gestation and continuing until kids weaning. All Ewes were weighed at the start of experiment and then every two weeks. The rations were provided twice a day at 8.00 a.m. and 15 p.m. All animals were vaccinated for infectious illnesses and internal and external parasites. Animals were placed in semi open pens and having continuous access to mineral block and fresh water during the experimental period. Kids were weighed within 24 hours of birth, then monthly until weaning. Kids were colostrum suckled at the first days postpartum and then allowed freely sprinked milk until they weaned at three months old.

Feeding trail:

All ewes received a basal diet consisted of 50% concentrate feed mixture (CFM), 15% fresh berseem (FBR), and 35% berseem straw (BS). The first experimental group (T1) fed the basal diet without probiotics served as control, while the T2 group was given a basal diet plus 3g yeast (LY), the T3 group was given 3 g LY plus 3g spirulina (SP), and the T4 group was given 3g LY plus 1.5g Bacillus subtilis (BS). The CFM consists of 38% yellow corn, 30% wheat bran, 15% sunflower meal, 6% soybean, 4% gluten feed 16%, 3% molasses, 2% limestone, 1% common salt, and 1% mineral and vitamin premixes. The feeding allowances were computed following NRC (2007) and adjusted according to the physiological and productive ewe (pregnancy and post-lambing) phases. Probiotics were mixed with 20 g of ground CFM just before the morning feeding. The commercial Spirulina powder was obtained from the National Institute of Oceanography and Fisheries (NIOF) in Egypt, and its chemical composition is as follows: 55.80% CP, 6.20% EE, 4.90% CF, 23.00% NFE, and 10.10% ash. Yeast contained 2x109 CFU/g and Bacillus subtilis contained 2x109 CFU/g. The chemical composition of CFM, FBR, and BS, as well as the calculated composition of the basal ration are shown in Table 1.

Digestibility trials and rumen samples:

At the end of the feeding trial, digestion experiments were conducted for 10 days (7 days as a preliminary period followed by 3 days as a collection period), using three ewes from each group. At the end of the collection period, rumen fluid samples were taken using a rubber stomach at 3 hrs post feeding to estimate pH, total volatile fatty acids (TVFA's), and ammonia concentrate (NH3-N). The samples were filtered through three layers of gauze.

Table (1): Chemical analysis of feedstuffs and the calculated composition of the basal ration.

Items	Chemical composition, % on DM basis			
	CFM*	**FBR**	**BS**	**Basal ration**
Dry matter (DM)	92.03	15.32	91.55	81.90
Organic matter (OM)	86.43	83.08	88.42	86.78
Crud protein (CP)	13.85	16.22	7.53	11.69
Crud fiber (CF)	11.94	26.10	33.30	22.05
Ether extract (EE)	2.50	2.43	2.32	2.42
Nitrogen free extract (NFE)	58.14	38.33	45.27	50.61
Ash	13.57	16.92	11.58	13.22

**CFM consisted of 38 % yellow corn, 30% of wheat barn, 15% sunflower meal, 6% soybean, 4%Gluten feed 16%, 3% molasses, 2% limestone, 1% common salt and 1% mineral and vitamins premixes.*

Blood samples were obtained from three fasting ewes in the morning. The samples were collected using centrifuging tubes with anticoagulants from the Jugular vein before and after lambing. Blood samples were stored at room temperature, then centrifuged at 3000 rpm for 20 minutes and stored in a deep freezer at - 200 C until analysis.

Productive and Reproductive traits:

The body weight of ewes (BW) was recorded at various trial phases (per and post-partum and at the weaning of lambs). Also, the actual milk yield as 4% fat-corrected milk (FCM) for each ewe was calculated based on the equation cited by Gaines (1928): as follows: 4% fat corrected milk (FCM) = 0.4 × milk yield (kg) + 15 × fat yield (kg). The milk sample (50 mL) was collected biweekly from each ewe (three ewes per group) and stored at 200C until chemical analysis by the milk scan system. The newborn lamb weights were recorded within 24 hours post-parturition and then monthly until weaning periods (three months). The weaning rate determines the number of lambs weaning/number of lambed ewes in the group*100. The twinning rate is estimated as the expressed the number of twin lambing / total lambs in the group*100. The weight gain of kids/ewe were calculated by multiplying the daily gain of lambs by the twinning rate. Calculations of the lambing rate (number of kids born/ewes lambing). The feed intake and refuse were documented, along with the feed conversion, which was estimated as kg DM per kg gain of weaned lambs.

Analytical procedure:

The official procedure of AOAC (2000) was used to estimate DM, CP, EE, CF, and ash, both for feed intake and feces samples. The pH value of rumen fluid samples was estimated immediately after straining the samples using the digital pH meter 201. The TVFAs and ammonium concentrations were measured by using the methods of Warner (1964) and Abou-Akkada and Osman, (1967), respectively. The total protein (TP), albumin (Al), urea, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), cholesterol, triglyceride, and glucose concentrations of blood serum were measured by calorimetric methods using commercial kits following the manufactory protocols. The lactose, fat, and protein levels were analyzed in milk samples by the Milkoscan system (Foss Electric Hillerod, 133B Milkoscan, Denmark).

Statistical analysis:

Data were statistically analysed by the General Linear Model's procedures of the SAS (2003) program. A one – way analysis was used to investigate the effect of different diatery treatments on the tested parameters by using the following model: $Y_{IJ} = \mu + T_i + E_{jj}$

Where: YiJ = the observation of the parameter measured, μ = the overall means, Ti = the effect of dietary treatment, (i= 1.2,3 and 4) and EiJ = the random error. Differences among means were assessed by Duncan's Multiple Range – test Duncan, (1955).

RESULTS AND DISCUSSION

Digestibility of nutrients and nutritive value:

Data in Table 2 show that dietary supplementation with probiotics had significantly (P<0.05) improved nutrient digestibility compared to the control rations, except for EE digestibility, which was unchanged.

Also, there were no significant differences between the yeast supplementation and the control groups in dry matter digestibility. Moreover, the T3, and T4 groups recorded significantly (P<0.05) higher digestibility in most nutrients than those in the T2 group. As explained by Amin and Mao (2020), several growth factors that promote the growth and activity of beneficial bacteria and inhibit harmful bacteria, which results in improved gut health, the immune system and nutrient absorption. As per yeast supplements, they can help the growth and activity of lactate-consumed and cellulolytic bacteria, leading to enhanced CF digestibility (Marden *et al.*, 2008). Also, yeast supplementation improved CP and NFE digestibility due to increased fiber and protein degradation, resulting in a higher rate of microbial nitrogen flow post-rumen, as noticed by (Uyeno *et al.*, 2015). Additionally, Olson *et al.* (1992) found that the addition of yeast improves true rumen OM digestibility, which resulted in improved microbial efficiency. Regarding the effect of BS, it can help improve the digestibility and absorption of nutrients through enhanced rumen fermentation conditions, which led to an increased total number of proteolytic and amylolytic bacteria and their ability to produce enzymes, according to Kang *et al.* (2012), Kubo *et al.* (2013), Sun *et al.* (2013), and Latorre *et al.* (2015). As for SP supplements, Gomaa *et al.* (2018) reported that SP is a method to improve nutrient digestion and ruminal fermentation because they contain unsaturated fatty acids and carotenoids, polysaccharides, and others that help to lower protozoa numbers and promote bacteria growth. It also contains high-quality components such as growth factors and β-glucan which help scavenge free radicals (Kholif *et al.*, 2017; Iwamoto, 2004). Our results are in agreement with those obtained by Deng *et al.* (2018), Mousa and Marwan (2019), Kholif *et al.* (2020), Hamdon *et al.* (2022), and Abdou *et al.* (2024), who recorded that these supplements improve nutrient digestion. Similar results were achieved by Abdou *et al.* (2018), Pazla *et al.* (2018), and Kattab *et al.* (2024) who added probiotics along with chemical, mineral, fibrolytic enzyme supplements in ruminant rations. Moreover, the inclusion of microalgae spp. in the rations of goats (Mavrommatis and Tsiplakou 2020), sheep (Stokes *et al.*, 2015; Alghonaim *et al.*, 2022), and dairy cows (Gaafar *et al.*, 2017) improved digestion of nutrients and productivity. In a similar vein, Gomaa *et al.* (2018) found that mixing Nannochloropsis oculata with sunflower oil promoted bacterial activity, which led to enhanced DM and NDF digestibility. In contrast, other studies observed that probiotic addition in ruminant rations didn't impact nutrient digestibility (Qiao *et al.*, 2010; Le *et al.*, 2016; Ferreira *et al.*, 2019). Also, Martins *et al.* (2023) noticed that supplementing yeast culture with phytonutrients didn't influence nutrient digestibility. The differences in earlier findings were related to the type and/or levels of probiotics and animal species, as reported by Whitley *et al.* (2009).

On the other hand, the feeding values of experimental rations especially TDN and DE, were significantly (P < 0.05) higher with all supplement groups than those in the unsupplemented one, which is in line with those obtained by Gaafar *et al.* (2017), Abdou *et al.* (2018), Hamdon *et al.* (2022), and Abdou *et al.* (2024). Nevertheless, the DCP value was significantly (P<0.05) higher in the T2, and T3 groups compared to the other groups. Similarly, Kewan *et al.* (2021) noticed that adding probiotics with or without garlic to lamb rations improved TDN value but had no effect on DCP value. While, Wafa *et al.* (2020) noticed that the addition of two levels of yeast culture to dairy cow rations had no effect on their nutritional value.

Table (2): Digestibility coefficients and nutritive value of the experimental rations.

Item	Experimental rations				
	T1	T2	T3	T4	±SE
Digestibility coefficients %					
Dry matter (DM)	70.27^{b}	70.97^{b}	72.18^{a}	72.37^{a}	0.32
Organic matter (OM)	71.97^{c}	73.69^{b}	74.85^{a}	75.00^{a}	0.21
Crud protein (CP	72.68^{c}	74.36^{b}	74.54^{b}	76.04^{a}	0.43
Crud fiber (CF)	53.47^{c}	55.26^{b}	57.25^{a}	56.96^{a}	0.47
Ether extract (EE)	64.43	66.39	65.67	67.14	1.15
Nitrogen free extract (NFE))	80.60^{c}	81.90^{b}	83.01^{a}	82.99^{a}	0.26
Nutritive value (%)					
Total digestible nutrients (TDN)	64.57^{c}	65.94^{b}	66.96^{a}	67.11^{a}	0.23
Digestible crud protein (DCP)	8.53^{b}	8.71^{b}	8.68^{b}	8.91^{a}	0.06
DE(Mcal/Kg DMI) A	2.85^{c}	2.91^{b}	2.95^{a}	2.96^{a}	0.01

a, b and c: Means within rows with different superscript are significantly different (P<0.05).
T1 – group = basal diet without supplementation; T2– group = basal diet supplemented with 3 g/h/d of LY; T2 group = basal diet supplemented with 3 g/h/d of LY plus 3 g/h/d of SP; T3–group = basal diet supplemented with 3 g/h/d of LY plus1.5 g/h/d of BS.

Rumen fermentation:

As shown in Table 3, dietary supplementation with probiotics had no effect on rumen pH value, as supported by the work of Peng *et al.* (2012), Kholif *et al.* (2020), and Li *et al.* (2021) after adding different probiotic species to ruminant rations. Also, the addition of B. licheniformis or yeast or mixing them into the lamb's rations had no effect on pH ruminal. Furthermore, Ghoniem *et al.* (2017) found that pH value wasn't affected when fed fattening calves' yeast alone or with mixed bentonite. In some other reports, animals fed probiotic supplements led to significantly ($P<0.05$) higher or lower pH values compared to those fed the control ration (Sun *et al.*, 2013; Le *et al.*, 2016; Dias *et al.*, 2018; Mousa and Marwan 2019; Cai *et al.*, 2021; Ngo *et al.*, 2021). Moreover, the pH value of these studies was above 6, indicating that rumen fermentation conditions were good for cellulolytic bacteria to improve (Mertens, 1977; Qadis *et al.*, 2004). On the other hand, data in Table 3 indicates that supplementation with probiotics led to a significant ($P<0.05$) reduction in ammonia-N concentrations compared to the control group, which was probably attributed to improved ammonia transport to microbial proteins and increased growth and activity of bacteria (Harrison *et al.*, 1988; Williams and Newbold, 1990) or directly lowering protein degradation (Eweedah *et al.*, 2005). Also, as reported by Ghasemi *et al.* (2012) ammonia concentrations serve as the measure of microbial protein degradation and utilization of non-protein nitrogen. These results are consistent with those obtained by Qiao *et al.* (2010), Ghoniem *et al.* (2017), Le *et al.* (2016), Mousa and Marwan (2019) and Kumprechtová *et al.* (2019). Also, Jia *et al.* (2018) found lower NH3-N concentrations and higher microbial crude protein (MCP) when adding Bacillus licheniformis or Saccharomyces cerevisiae or mixing them as an alternative to monensin (antibiotic) to the lamb rations. Reverse results were obtained by Kewan *et al.* (2021), Ngo *et al.* (2021), and Sun *et al.* (2013), who found that yeast or Bacillus amyloliquefaciens or Bacillus subtilis natto addition increased ammonia levels. In other studies, Bacillus subtilis natto product or Nannochloropsis oculata or yeast addition didn't affect ammonia levels (Peng *et al.*, 2012; Kholif *et al.*, 2020; Zhang *et al.*, 2022). Furthermore, Kholif *et al.* (2023) noted that replacing the feed concentrate mixture with 40% Moringa oleifera plus Chlorella vulgaris increased ammonia concentrations. Moreover, data in Table 3 indicates that supplementation with probiotics increased TVFA's levels, likely due to their ability to enhance the activity and growth of bacteria and protozoa in the rumen (Wallace and Newblod 1993) and improve rumen fermentation conditions (Anjum *et al.*, 2018; Crossland *et al.*, 2019; Mombach *et al.*, 2021). Also, other authors reported that the BS can support Lactobacillus bacteria growth, improve environmental rumen conditions, and increase TVFA production through their ability to degrade carbohydrates and proteins in the rumen (Sun *et al.*, 2013; Souza *et al.*, 2017). Moreover, Sun *et al.* (2016) reported that BS improves rumen fermentation conditions by increasing the total proteolytic and amylolytic bacteria number. Similar results to those obtained here were recorded by Mousa and Marwan (2019); Kholif *et al.* (2020) and Zhang *et al.* (2022). Also, Kholif *et al.* (2023) found that replacing the feed concentrate mixture with 40% Moringa oleifera with 1% or 2% level of Chlorella vulgaris led to higher TVFA concentrations, but it had no effect at a 3% level of Chlorella vulgaris. Furthermore, Kewan *et al.* (2021) stated that adding yeast, alone or with garlic to the lamb rations increased TVFAs. In contrast, Jia *et al.* (2018) found that supplementation with probiotic species alone or in combined with those in the lamb ration had no effect on TVFAs.

Table (3): Ruminal fermentation pattern in ewes fed of the experimental rations.

Item	Experimental rations				
	T1	T2	T3	T4	±SE
pH value	6.37	6.43	6.45	6.39	0.54
Ammonia-NH3 concentrations mg/100 ml	14.10[a]	12.73[b]	12.25[c]	12.16[c]	0.12
TVFA's concentrations meg/100 ml	10.57[b]	11.31[a]	11.65[a]	11.74[a]	0.18

a, b and c: Means within rows with different superscript are significantly different (P<0.05).
T1 – group = basal diet without supplementation; T2– group = basal diet supplemented with 3 g/h/d of LY; T2 group = basal diet supplemented with 3 g/h/d of LY plus 3 g/h/d of SP; T3–group = basal diet supplemented with 3 g/h/d of LY plus1.5 g/h/d of BS

Productive performance of ewes:

Feed intake and body weight of ewes:

Data in Table 4 reveals that probiotic supplementation didn't influence DMI, aligning with the findings of Ngo *et al.* (2021), Hamdon *et al.* (2022), El-Deeb *et al.* (2023), Mokhtar *et al.* (2023), and Abdou *et* *al.* (2024). Also, Jia *et al.* (2018) and Abdou *et al.* (2018) confirmed similar results when adding probiotics alone, a mixture of them, or with chemical additives. Inconsistently, Mousa and Marwan (2019), Wang *et al.* (2022), Jia *et al.* (2022), Rabee *et al.* (2022), and Zhang *et al.* (2023) showed that adding probiotics resulted in increased DMI, whereas Boeckaert *et al.* (2008) observed it decreased. On the other hand, dietary supplementation didn't affect the ewes' body weight before, after lambing and weaning among experimental groups, as shown in Table 4, consistent with results from Baranowski *et al.* (2007), Anjum *et al.* (2018), Mokhtar *et al.* (2023), and El-Deeb *et al.* (2023). While mixing Lactobacillus licheniformis and yeast as supplement in lamb rations improved their body weight, as reported by Jia *et al.* (2018). Likely, the effect of adding probiotics on body weight is linked to several factors like basal rations, dosage, viable number of yeast cells, feeding strategy, and others (Mikulec *et al.*, 2010).

Table (4): Feed intake, body weight changes (kg), milk yield and composition of ewes as affected by probiotics supplementation.

Item	Experimental rations				±SE
	T1	T2	T3	T4	
Daily feed intake (g/d/ h) on DM.:					
CFM	1150	1150	1150	1150	
Berseem	306	306	306	306	
Berseem straw	916	916	916	916	
Total DM feed intake (g/h/d) DMI	2372	2372	2372	2372	0.33
Total digestible nutrients intake TDNI	1.54[c]	1.57[b]	1.59[a]	1.59[a]	0.02
Digestible crude protein intake DCPI	0.20	0.21	0.21	0.21	0.12
Average live body weight of ewes (kg)					
Before lambing (kg)	54.27	55.27	55.33	55.00	1.99
After lambing (kg)	51.98	52.26	52.18	52.30	1.91
Live body weight (kg) at weaning period	54.86	55.51	55.63	55.30	1.95
Actual milk production (g/h/d)	457.88[d]	471.27[c]	507.43[b]	531.57[a]	3.75
4 % FCM production (g/h/d)	542.35[d]	562.25[c]	621.63[b]	658.35[a]	3.57
Milk composition (%)					
Fat %	5.23[b]	5.28[b]	5.50[a]	5.59[a]	0.06
Protein %	4.33[b]	4.35[ab]	4.48[a]	4.54[a]	0.05
Lactose %	4.12[b]	4.20[b]	4.37[ab]	4.45[a]	0.03

a*, b and c: Means within rows with different superscript are significantly different (P<0.05).*
T1 – group without supplementation; T2 – group supplemented with 3 g/h/d of LY; T3–group supplemented with 3 g/h/d of LY plus 3 g/h/d of SP; T4–group supplemented with 3 g/h/d of LY plus1.5 g/h/d of BS.

Milk yield and its composition:

Milk production of animals can be affected by several factors (breed, age, body weight, nutrition, lactation stage, number and type of offspring), and others, as reported by Chrastinova *et al.* (1997). The results in Table 4 indicate that all supplement groups were significantly (P<0.05) higher with both daily actual milk and 4% FCM yield in comparison to the control group. The highest values were obtained with T4 followed by T3 then T2, respectively. The improved rates were 2.92, 10.82 and 16.09% for actual milk production and 3.67, 14.62, and 21.39% for 4% FCM production for T2, T3 and T4, respectively for probiotic supplementation groups than the control group. Also, the groups T3 and T4 showed better milk quality in terms of milk fat, protein and lactose, due to improved nutrient digestion, increased ruminal microbial protein synthesis, and higher TVFA production, which resulted in increased energy production and an enhanced immune system (Desnoyers *et al.*, 2009; Boyd *et al.*, 2011; Dhama *et al.*, 2015; Khalifa *et al.*, 2016). It also increases propionate, which supports the synthesis of gluconeogenesis and lactose (Rigout *et al.*, 2003; Dijkstra *et al.*, 2012; Mao *et al.*, 2013). Also, microalgae have been proven to be effective in improving milk production due to their high nutritional value (Yaakob *et al.*, 2014; Kholif *et al.*, 2017). Some authors have reported that adding yeast, microalgae, and Bacillus subtilis with or without other supplements in the ruminant rations increased milk production (Kritas *et al.*, 2006; Ghoniem *et al.*, 2018; Abdou, 2018; Khalif *et al.*, 2020; Jia *et al.*, 2022; El-Deeb *et al.* 2023; Goetz *et al.* 2023; Abdou *et al.* 2024). Moreover, Kattab *et al.* (2024) observed that the inclusion of probiotics with or without fibrolytic enzymes in lactating buffalo rations improved milk production. Reverse results were obtained by (Luan *et al.*, 2015; Kafilzadeh *et al.*, 2019;

Mavrommatis and Tsiplakou 2020), Ma *et al.* (2020), and lago *et al.* (2021) who found that the milk production didn't affect by adding probiotics. Also, noticed that the addition of BS PB6 didn't affect milk yield. Meanwhile, milk fat percentage was increased by adding probiotic which may be due to their ability to enhance the growth and activity cellulolytic bacteria (Mao *et al.*, 2013). Also, probiotic supplementation enhanced milk composition, aligning with findings from Lamminen *et al.* (2019) who observed that including SP in dairy cow rations increased the milk fat and protein content without influencing milk production. Similarly, Elaref *et al.* (2020) found that the incorporation of probiotics into Sohagi ewe rations increases milk production and improves milk fat and protein content but had no effect on lactose percentage; however, this improvement wasn't consistent with the findings of Du *et al.* (2022). The increased milk lactose percentage with added BS is in agreement with those obtained by Goetz *et al.* (2024). However, yeast addition didn't affect milk content; the reason is unclear. Similarly, the addition of yeast to dairy cow fed low-forage rations by Ferreira *et al.* (2019), or yeast culture mixed with phytonutrients to dairy cow rations by Martins *et al.* (2023), had no effect on milk production and its composition. The differences in the results could be attributed to factors such as animals' age, lactation state, physiological conditions, feeding system, yeast dosage, and others (Doleźal *et al.*, 2012).

Reproductive performance of ewes:

The lamb's size at birth and weaning and their weights and twin rates increased in the T2 and T3 groups compared to the T1 and T2 groups, as shown in Table 5, may be attributed to the effect associated with probiotc supplements on the reproductive performance of ewes triats. Also, the average daily gain of lambs in supplementation groups was significantly (P<0.05) higher than those in control group. The highest total weight gain and average daily weight gain of kids per ewe were recorded in T3, followed by T4 then T2, whereas T1 had the lowest values (P<0.05). The relative improvement (%) of lamb's daily gain were 7.32, 32.96 and 23.2% for T2, T3 and T4, respectively compared to the control group (T1).

Table (5): Reproductives performance and feed conversion of dam and their offspring as affected by probiotics supplementation.

Items	Experimental rations				±SE
	T1	T2	T3	T4	
No. of dam	6	6	6	6	
No. of dam kidded	6	6	6	6	
No. lambs size	6	6	7	7	
Lambing rate %	100	100	116.66	116.66	
No. of lamb at weaning	6	6	7	7	
Weaning rate %	100	100	116.66	116.66	
Parity patterns					
No of dam born single	6	6	5	5	
Single rate %	100	100	83.34	83.34	
No of dam born twins	-	-	1	1	
Twins rate %	-	-	16.66	16.66	
Offspring performances					
Lambs' weight at birth, (kg)	4.09	4.57	4.84	4.55	0.4
Lambs' weight at weaning (kg)	18.87^{b}	20.45^{ab}	24.42^{a}	22.75^{a}	0.62
Total body weight gain (kg)	14.78^{c}	15.88^{b}	19.58^{a}	18.20^{a}	1.49
Average daily gain (g/d)	164^{c}	176^{b}	218^{a}	202^{a}	0.02
Relative improve (%)	100	107.32	132.96	123.2	
FCR (Kg feed intake/kg weight gain of kids)	14.43^{a}	13.43^{b}	9.31^{d}	10.02^{c}	1.14

a, *b and c: Means within rows with different superscript are significantly different (P<0.05).*
T1 – group = basal diet without supplementation; T2– group = basal diet supplemented with 3 g/h/d of LY; T2 group = basal diet supplemented with 3 g/h/d of LY plus 3 g/h/d of SP; T3–group = basal diet supplemented with 3 g/h/d of LY plus1.5 g/h/d of BS.

The feed conversion ratio of the dam (expressed as kg DM feed per kg weight gain of kids) was significantly (P<0.05) improved in T3, followed by T4 then T2, compared to the T1. These results revealed that dietary supplementation improved the reproductive performance, feed conversion ratio of the dam, and growth performance of suckling kids. According to Khalifa *et al.* (2016), there is a positive relationship between the feeding system and some parameters such as body weight and conditions of ewes, udder size and teat, body weight of kid's milk flow and milk energy. This finding is in agreement with those obtained by Abd Eldiam *et al.* (2018) providing pregnant ewes with SP and vitamin A enhanced their health and the brith of weightier lambs. Similar results are recorded by El-Deeb *et al.* (2023), who confirmed that ewes fed the SP ration showed an increase daily gain of their offspring. Moreover, El-Hway *et al.* (2022) reported that adding of 3% Nannochloropsis oculata improved daily gain of lambs and FCR compared to those supplemented 1.5% Nannochloropsis oculata ration or no supplementation ration.This could be due to SP is rich nutrients such as amino acid, ployunsaturated fatty acids, minereal and vitamins and others (Gabr *et al.*, 2020). Moreover, Elaref *et al.* (2020) noted that inclusion of two level of dry yeast to the Sohagi ewe rations led to a significant higher lamb birth weight and their average daily gain during suckling period. Similarly, Zhang *et al.* (2022) found increased daily gain and feed conversion of bull fed yeast culture ration. According to, Mousa and Marwan (2019), adding BS to the ration of lambs, led to improved FCR. Also, Abdou *et al.* (2024) found that FCR increased in dairy cows fed yeast rations. Similar trend, Le *et al.* (2016) reported that adding calves with probiotics enhanced health and improved weight gain and feed conversion along with a shorter weaning period. Also, the results of Hassanien *et al.* (2015) and Gaafar *et al.*, (2017) are consistent with our results. Conversely, Sales (2011) found no effect of probiotics on the daily gain and feed conversion of sheep.

Blood parameters:

Blood serum values of TP and its fraction, ALT and AST activities, cholesterol and triglycerides didn't significantly differ (P<0.05) among all groups; as shown in Table 6. However, the addition of probiotics led to significantly lower (P<0.05) blood serum creatinine, and urea concentration, while it significantly (P<0.05) increased the serum blood glucose levels. These results are consistent with earlier studies on the impact of adding different probiotics on TP and its fraction (Ghoniem *et al.*, 2018; Khalif *et al.*, 2020; Ma *et al.*, 2020; Du *et al.* 2022; Abdou *et al.*, 2024). In general, studies conducted by Kattab *et al.* (2024) showed similar trends.

Table (6): Blood parameters of ewes fed the experimental rations.

Item	**Experimental rations**				
	T1	**T2**	**T3**	**T4**	**±SE**
TP (g/dl)	7.11	7.25	7.34	7.38	0.52
Al (g/dl)	4.24	4.38	4.47	4.46	0.35
Gl (g/dl)	2.83	2.87	2.87	2.92	0.37
Urea (mg/dl)	70.83a	49.61b	53.97b	55.29b	1.27
Creatinine(mg/dl)	1.27a	0.93b	0.90b	0.90b	0.02
ALT (U/L)	12.86	12.14	11.26	13.27	1.68
AST (U/L)	33.45	33.17	34.59	35.51	1.91
Glucose (mg/dl)	60.53b	62.15b	67.88a	68.12a	1.02
Cholesterol (mg/dl)	82.90	80.40	81.62	80.27	4.39
Triglycerides (mg/dl)	31.88	30.82	33.81	30.45	3.45

a, *b and c: Means within rows with different superscript are significantly different (P<0.05).*
T1 – group = basal diet without supplementation; T2– group = basal diet supplemented with 3 g/h/d of LY; T2 group = basal diet supplemented with 3 g/h/d of LY plus 3 g/h/d of SP; T3–group = basal diet supplemented with 3 g/h/d of LY plus1.5 g/h/d of BS.

Also, the blood serum urea and creatinine levels were significantly lower by supplementing probiotics, likely due to enhancing protein metabolism and utilization. This result aligns with those reported by Mousa and Marwan (2019) and Mahrous *et al.* (2019). Conversely, Abdou *et al.* (2024) found no probiotic supplementation had any effect on them, while El-Deeb *et al.* (2023), and El-Nagar *et al.* (2021) found higher levels of blood serum urea in ruminants fed SP or lactobacillus acidophilus, but only within a normal range. Also, the current results showed that adding probiotics didn't affect AST and ALT levels, which confirmed that supplementing probiotics didn't influence liver functions, as noted by Kholif *et al.*, (2020), El-Deeb *et al.* (2023). Also, Du *et al.* (2022) didn't find any effect of adding yeast culture on liver function. Moreover, Özbek *et al.* (2003) reported that the blood analysis of AST and ALT is very necessary to evaluate the metabolic and health status of animals, SP may help in the prevention of liver and kidney diseases, according to Gutiérrez-Rebolledo *et al.* (2015). Further, probiotic supplementation increases levels of glucose compared to the control groups, as it due to improved OM digestibility and increased TVFA production as well as lowering propionic acid, which is responsible for about 73% of the glucose production in the ruminant liver (Oh *et al.*, 2015). Moreover, the current serum glucose levels confirmed the positive relationship between it and milk production in the previous studies of Kumprechtová *et al.* (2019), Kholif *et al.* (2020) and Abo El-Nor and Kholif

(1998). It might be due to enhanced gluconeogenesis and increased glucose absorption (de Valdez *et al.*, 1997). These results are in agreement with those obtained by Hussein (2014), Kholif *et al.* (2020), El-Deeb *et al.* (2023), El-Nagar *et al.* (2021). Also, in this study, the blood serum of glucose was within range for healthy ewes (Jackson and Cockcroft, 2002). Additionally, the incorporation of probiotics had no effect on cholesterol and triglyceride levels which is consistent with similar trend which also reported by Siqueira *et al.* (2022). According to Gabr *et al.* (2020), SP is rich in bioactive compounds and proteins, polyunsaturated fatty acids, excellent vitamins and minerals and others, which could lead to higher glucose, triglycerides, and cholesterol levels. However, the bloold serum parameters were within range for healthy animals (Etim *et al.*, 2013).

Economic efficiency:

The Data in Table 7 reveales that the addition of yeast alone or mixed with spirulina or with Bacillus subtilis to ewe rations enhanced the economic effectiveness of their rations. Economic efficiency improved by 106.50, 145.50, and 134.00 for T2, T3 and T4, respectively, based on the control group. Our results are consistent with those of Kewan *et al.* (2021) reported an improvement in economic efficiency with yeast plus garlic, followed by garlic, and yeast supplementation, surpassing the control ration by 42, 32, and 31%, respectively. Moreover, economic efficiency increased by adding yeast alone (Abdou *et al.*, 2024) or mixed with chemical supplements (Abdou, 2018), to the lactating ruminant rations. Also, the addition of SP enhanced productive performance in dairy goats and cows (Khalifa *et al.*, 2016; Gaafar *et al.*, 2017) and growing lambs (Hafez *et al.*, 2013), so it had the potential to be economically effective.

Table (7): Impact of dietary supplementation on the economic efficiency.

Item	Experimental rations			
	T1	**T2**	**T3**	**T4**
CFM	1.25	1.25	1.25	1.25
Berseem	2	2	2	2
Berseem straw	1	1	1	1
Total Feed Intake (kg)	284.4	284.4	284.4	284.4
Feed additive				
Yeast (g/h/d)	0	3	3	3
SP (g/h/d)	-	-	3	-
BS (g/h/d)	-	-	-	1.5
Additive cost	0	0.3	1.2	1.35
[1]Feed cost/ewe (L.E./d)	17.20	17.50	18.40	18.55
Weight gain of kids/ewe (g/d)	164	177	255	237
Cost of weight gain of kids/ewe (L.E./kg)	105	99	72	78
[2]Price of weight gain (L.E./d)	34.4	37.2	53.6	49.8
[3]Economic efficiency	2.00	2.13	2.91	2.68
[4]Improvement of economic efficiency (%)	100.0	106.5	145.5	134.0

a, b and c: Means within rows with different superscript are significantly different (P<0.05).
T1 – group = basal diet without supplementation; T2– group = basal diet supplemented with 3 g/h/d of LY; T2 group = basal diet supplemented with 3 g/h/d of LY plus 3g/h/d of SP; T3group = basal diet supplemented with 3 g/h/d of LY plus1.5 g/h/d of BS.
Diets price (L.E. per ton) at 2022 were: 12000 LE/ton of CFM and 500 LE/ton of berseem fresh, 1200LE/ton berseem straw, 100LE/kg of yeast, 300 LE/kg of SP, and 350 LE/kg of BS.
1Total feed cost (L.E/h./d)= Price of each ration ingredient x its amount consumed.
2 Price of kg live body weight was 210 L.E.
3 Economic efficiency = (price of weight gain/ feed cost).
4 Economic efficiency improvement, % = economic efficiency of T1, T2 and T3 x 100/ economic efficiency of T1.

CONCLUSION

It could be concluded that mixing yeast with Bacillus subtilis or Spirulina as a natural supplement had significantly improved ewes, productive and reproductive performance. The combination also enhanced nutrient digestibility, feeding values, milk production, feed efficiency and economic efficiency with no negative effect on ewes, health or their offerspring.

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تأثير إضافة الخميرة وحدها أومختلطة مع الاسبيرولينا اوالباسيلليس ستيليس على الأداء الإنتاجى والتناسلي للنعاج

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أجريت هذه الدراسة لتقييم تأثير إضافة الخميرة الحية وحدها أو مع مزيج من الاسبيرولينا والباسيلليس ستيليس في علائق النعاج على تناول العلف وهضم العناصر الغذائية وتخمير الكرش وأداء الإنتاجى والتناسلى النعاج. تم تقسيم أربع وعشرين نعجة حامل إلى أربع مجموعات متشابهة (ستة في كل مجموعة) وتم تغطية الإحتياجات الغذائية لها وفقًا .NRC (2007) تم تغذية الحيوانات على العليقة تحتوي على (50٪ من خليط العلف المركز و15٪ من البرسيم الطازج و35٪ من تبن البرسيم بدون إضافات في المجموعة T1 (المجموعة الكنترول)، تم إضافة 3 جرام من الخميرة الحية وحدها، أو خلطها مع 3 جرام من الاسبيرولينا أو 1.5 جرام من الباسيلليس ستيليس للمجموعات T2 و T3و T4على التوالي. أشارت النتائج إلى أن إضافة البروبيوتيك لم يكن لها أي تأثير على تناول العلف وأداء النمو لدى النعاج. ولكن حسنت معاملات هضم جميع العناصر الغذائية بشكل ملحوظ باستثناء EE كما حسنت انتاج اللبن ومكوناته. لم تؤثر إضافة البروبيوتيك على قيم الأس الهيدروجيني، في حين إنخفض تركيز الأمونيا بشكل معنوى، وكما زاد تركيز الأحماض الدهنية الطيارة معنويا مع الإضافات. لم تؤثر إضافة البروبيوتيك على البروتين الكلى والألبيومين والجلوبيولين وإنزيمات الكبد والكوليسترول والدهون الثلاثية، ولكنها زادت من مستويات الجلوكوز وخفض مستويات اليوريا والكرياتينين معنويا. لم تؤثر إضافة البروبيوتيك على وزن الجسم لدى النعاج معنويا، ولكن زاد عدد الحملان المولودة معدلات نموها خاصة بالنسبة لمجموعتين T3 و T4كما حسنت الكفاءة الاقتصادية لمجموعتين T3 و .T4يمكن الاستنتاج أن إضافة الخميرة مع SP أو BS ها تأثيرات إيجابية على الأداء التناسلي والإنتاجى والاقتصادي والحالة الصحية للأغنام.

الكلمات المفتاحية: *النعاج، البروبيوتيك، معاملات الهضم، مقاييس الكرش، الأداء الإنتاجي والتناسلى*