EFFECTS OF SENPs-ENRICHED PROBIOTIC SUPPLEMENTATION ON GROWTH PERFORMANCE, RUMINAL FERMENTATION AND BLOOD METABOLITES OF GROWING LAMBS

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SUMMARY

The aim of this study was to evaluate the effect of supplemental either only Bacillus subtilis (BS) or in the form of SeNPs-enriched Bacillus subtilis (SNEBS) on digestibility of supplemented diets, growth performance, ruminal fermentation and blood metabolites in growing Barki lambs. A total of thirty-six healthy growing male Barki lambs aged 8-10 months weighing 25.62±0.58 kg were randomly divided into three groups of 12 lambs each. The 1st group was given a control diet without additives, the 2nd group was given a control diet supplemented with 6.2 × 10⁸ CFU/g of BS alone, and the 3rd group was given a control diet supplemented with 3.9 × 10¹² CFU/g of SENPs-enriched probiotic (SNEBS) in a feeding trial that lasted for 126 days. Results indicated that the BS and SNEBS supplementations increased significantly (P < 0.05) the final body weight, total weight gain, average daily gain (ADG), when compared to the control group. The inclusion of BS and SNEBS were increased the digestibility of DM, OM, CP, CF, NDF, ADF, nitrogen absorbed and nitrogen balance (P < 0.05). There were no significant differences in pH, total and individual VFA among the experimental dietary treatments, except for the propionic acid. The concentration of NH₃-N was increased significantly (P < 0.05) in BS and SNEBS groups compared to that of control one. Supplementation of control diet with BS and SNEBS significantly increased (P < 0.05) glucose, total proteins, albumin and globulin, while, supplementation with BS or SNEBS significantly decreased (P < 0.05) serum cholesterol, triglycerides and HDL-c compared to those of control group. Therefore, inclusion BS and SNEBS to growing lambs’ diets could be monitoring and maximise the productive performance items.

Keywords: Bacillus subtilis, SeNPs, growth performance, ruminal fermentation, blood metabolites, lambs

INTRODUCTION

Antibiotics (also known as growth promoters) have been used as feed additives in various farm animals for decades to minimise the frequency of diarrhoea under certain conditions and extremely in most circumstances can be significantly increasing the productive performance of ruminant animals. Due to the dysfunction of using antibiotics as a feed additives where they could lead to a rise in bacterial antibiotic resistance, the European Union (EU) agreed to prohibit the use of them beginning of year 2006. Therefore, several efforts have been made to identify other compounds that have favourable effects on animals via modifications in the gut flora. Probiotics, prebiotics, organic acids, herbs, and essential oils are among the so-called "antibiotic alternatives" (Simon 2005). In ruminants, probiotics are used as feed additives to improve rumen fermentation and promote immunological
function and overall health issues (Seo et al., 2010). Probiotics in the rumen could be interact with rumen microbes to boost rumen fermentation and synthesis an antimicrobial compounds like bacteriocins which protect the gut from harmful pathogens (Weinberg et al., 2003). Also, they may have beneficial effects in terms of increasing the population of ruminal cellulolytic bacteria (Dawson et al., 1990), resulting in increased fibre digestibility, improved nutrient synthesis, and bio-availability of nutrients contributing to a better growth performance (Oyetayo and Oyetayo, 2005).

Selenium (Se) is an important trace element with well-documented health benefits (Brown et al., 2001) where its existence is inadequate in most areas of the world. Dietary Se deficit is thought to be a cause of aberrant myocardial matrix remodelling and dysfunction in the normal heart (Wang et al., 20019). In animals, appropriate amounts of Se supplementation can regulate nutrient substances metabolism and improve digestion, antioxidation alleviate stresses (Tufarelli et al., 2011; Tufarelli et al., 2016; Lukaski, 1999). Supplementation of the diet with tetravalent Se (IV) can be achieved using a salt of selenium, such as sodium selenite. Low molecular weight organic Se complexes that synthetize with amino acids (Jing et al., 2015) or prepared in nano forms (Lin et al., 2015), can be utilized to supplement the diet since they being higher bioavailability than inorganic forms, which are typically employed as potential dietary supplements (Brown et al., 2000). Despite the fact that Se nanocomposites have a higher bioavailability than organic sources of Se (Lin et al., 2015), their higher cost has suppressed them from being widely used. There is a need in animal husbandry to find a cheap and convenient source of selenium nanoparticles (SeNPs) for widely using in feeding practices management for ruminant animals. However, the effect of SeNPs-enriched Bacillus subtilis as a feed additive on rumen fermentation and feed utilization efficiency in ruminants has not been studied. The goal of this research was to determine the effects of Bacillus subtilis or Bacillus subtilis enhanced with SeNPs supplementations on growth performance in growing lambs, with an emphasis on rumen fermentation characteristics and nutrient utilization.

MATERIALS AND METHODS

Feeding Trial

Experimental Design and Facilities

This study was conducted at Noubaria experimental station, Animal Production Research Institute, Agricultural Research Centre, Ministry of Agriculture, Egypt. Also, this work was planned in accordance with the guidelines approved by the Institutional Animal Care and Use Committees (Protocol No. 27-1W-0521) of city of Scientific Research and Technological Applications, Alexandria, Egypt. A total of thirty-six healthy growing male Barki lambs, aged 8-10 months and weighing 25.62±0.58 kg, were randomly divided into three groups of 12 each in feeding trial that lasted a period of 126 days. The 1st group was given a control diet with no additives, the 2nd group was given the control diet supplemented with 6.2 × 10⁷ CFU/g of Bacillus subtilis alone, and the 3rd group was given the control diet supplemented with 3.9 × 10¹² CFU/g of Bacillus subtilis enriched with selenium nanoparticles (SeNPs). The isocaloric and isonitrogenous control diet used in this study was consisted of concentrate, corn silage (CS) and wheat straw (RS) (70:15:15), respectively, which were formulated according to the nutritional requirements of sheep by NRC (2007). The ingredients of concentrate feed mixture (CFM) were formulated as (g/kg DM) 347 yellow corn, 153 barley grain, 154 soybean meal, 290 wheat bran 30 molasses, 13 dicalcium phosphate, 7 limestone, 4 salt, in addition 2 g/kg feed of premix that consisted of some essential trace minerals and vitamins as presented below Table (1).

Bacillus subtilis was isolated from an environmental ecosystem in Egypt, characterized, and optimized to represent in vitro/vivo probiotic properties in order to determine the safety and efficacy as an animal feed additive. The bacterial culture was maintained at −20 °C in de Man, Rogosa and Sharpe (MRS) medium (Merck, Germany) supplemented with 20% (v/v) glycerol. The number of live Bacillus reached 6.2 × 10⁹ CFU/mL, after fermentation. The fermentation of selenium-enriched B. subtilis was prepared with sodium selenite supplemented into the culture medium until the Se concentration reached 0.35 ppm. After fermentation, SNEBS were harvested, SeNPs reached 0.35 ppm and live numbers of B. subtilis was 3.9 × 10¹² CFU/mL. Each 1 g of BS and 1 g of SNEBS were combined with 1 kg of lime stone (as a carrier), and then with 1000 kg of mass feed for compound the probiotic Bacillus for compound the probiotic Bacillus. Chemical composition of experimental feedstuffs is presented in Table 1.
Table (1): Chemical compositions of concentrate feed mixture, corn silage and rice straw.

| Chemical Composition (g/kg DM): | CFM | Corn silage | Rice Straw |
|--------------------------------|-----|-------------|------------|
| Dry matter                     | 903.4 | 317.9       | 874.3      |
| Organic matter                 | 935.2 | 925.7       | 901.7      |
| Crude protein                  | 148.8 | 86.7        | 33.8       |
| Crude fiber                    | 74.9  | 247.3       | 375.4      |
| Ether extract                  | 29.6  | 19.4        | 10.7       |
| Neutral detergent fiber        | 356.8 | 435.7       | 715.8      |
| Acid detergent fiber           | 222.9 | 271.4       | 583.7      |
| Lignin                         | 57.4  | 61.5        | 106.4      |
| Non-fiber carbohydrates \(^1\) | 415.2 | 398.1       | 157.7      |

\(^1\)non-fiber carbohydrates = 1000 - (aNDFom + crude protein + ether extract + ash), where Neutral detergent fiber expressed exclusive of residual ash (aNDFom).

Growth performance of lambs

Body weight of growing lambs was recorded before morning feeding at the beginning of the feeding trial and on a weekly basis thereafter. On a daily basis, feed intake was determined by calculating the difference between the amount of feed offered and the amount of feed refused. Moisture content of the feed offered and refused was examined on a daily basis in order to correct and recording the dry matter intake of the feed. Feed conversion ratio (FCR) was calculated for each individual lamb from total feed intake and total weight gain during the experimental period.

Digestibility and Nitrogen Balance

Twelve Barki rams (average weight 45±1.40 kg) were used in the digestibility and nitrogen balance experiment (four rams for each group). Each trial lasted for four weeks; the first three weeks were considered as a preliminary period, followed by one week for feces and urine collection. Sheep were fed twice a day at 8 a.m. and 3 p.m., and water was offered freely. Chemical composition of feed, faeces and urine were determined according to A.O.A.C (2005) methods. Samples (20%) of faeces and urine were taken once a day from each animal and at the end of collection period composited samples were prepared for each group then stored at −18 °C until proceeding of analysis. Faecal samples were oven dried at 60 °C for 72 hrs. then were ground through 1 mm screen on a Wiley mill and a sample of 50 g was obtained for examination. The crude protein (CP), crude fiber (CF), ether extract (EE) and ash content of feed and faeces samples were determined, while the nitrogen (N) content of urine samples were determined according to AOAC guidelines (2005). Cell wall constituents (NDF, ADF and ADL) were determined according to VanSoest (1991). Hemicellulose and cellulose were calculated by differences.

Rumen fermentation characteristics

Rumen liquor samples (20 mL) were collected by stomach tube for each animal of digestibility trial before the morning feeding at the end of the trial. Samples were taken at 3 h after morning feeding to determine pH, ammonia-N and Volatile Fatty Acids (VFA) (Ramos-Morales et al., 2014). The rumen samples were filtered through four layers of cheesecloth; the pH value was measured immediately using a portable pH meter (GLP 21 model; CRISON, Barcelona, Spain). The rumen samples were then kept at -20 °C for future analysis. Ruminal NH₃-N concentration was measured colorimetrically (Spectrophotometer PD-303 UV, APEL, Japan) using commercial kits (SPINREACT, Ctra. Santa Coloma, 7, Girona, Spain) as described by (Konitzer and Voigt, 1963). Volatile fatty acids (VFA) concentrations were determined by steam distillation as described by (Warner, 1964). Samples were stored at -20 °C until extra analyses. Concentration and molar proportions of individual VFA were measured by using gas chromatography with some modifications according to (Abo-Zeid et al., 2017).

Serum biochemical parameters

At the end of the feeding experiment blood samples were collected before the morning feeding from all lambs from via jugular vein puncture using a gauge needle (size 20) after clipping and sterilizing the area of collection. The blood sample was collected without anticoagulant in plain vacuum tubes. The samples were centrifuged (700×
g, 15 min.) and the serum was carefully decanted into serum vials and stored at -20 ºC until use for biochemical analysis. The serum glucose, total protein, albumin, cholesterol, triglycerides and high-density lipoprotein–cholesterol (HDL-c) were determined colorimetrically by using commercial kits produced by Biodiagnostic Co., Cairo, Egypt.

**Statistical analysis**

Data obtained from the experiment were subjected to a one-way analysis of variance (ANOVA) using SAS for Windows, and Version 9.2 (SAS Institute, USA) was used to analyse the data. Differences between treatment means were considered significant at P < 0.05 according to the following statistical model:

\[ Y_{ik} = \mu + X_i + e_{ik} \]

where: \( Y_{ik} \) = the response variable; \( \mu \) = the overall mean; \( X_i \) = the fixed effect of treatment (control and Bacillus subtilis and Bacillus subtilis enriched with SeNPs); \( e_{ik} \) = the residual error.

**RESULTS AND DISCUSSION**

**Growth performance**

Supplementation of control ration with BS or SNEBS significantly increased (P < 0.05) final body weight, total weight gain, average daily gain (ADG) in comparison with those of control one, with no significant differences between the two tested rations respecting these items (Table 2). While there were no significant variations in total feed intake among the treatment groups. Feed conversion ratio appeared to be more efficient significantly with the two tested rations (BS and SNEBS) in comparison with control one, being 6.51, 6.27 and 7.42 kg DMI/kg gain, respectively, with no significant differences between two tested groups (BS and SNEBS) respective this item. The beneficial effects of Se and probiotic bacteria on growth performance of chickens have been well-documented (Yang *et al.*, 2017; Zhang *et al.*, 2012). *B. subtilis*, a probiotic *Bacillus* strain, has a greater ability to survive in harsh conditions (low moisture, high pelleting temperature, and less nutrients) than *Lactobacillus*, which has been more widely developed as a biomedical additive (Bader *et al.*, 2012). The compound additive (SNEBS) that containing SeNPs and *Bacillus subtilis* would combine considerably favourable impacts of the two elements on the growth performance of lambs. The results indicated that growth rates were improved significantly in lambs given BS or SNEBS supplements in their rations. In agreement with the present results, Saleem *et al.* (2017) reported that growing lambs supplemented with probiotics in their diets had better growth performance in terms of final body weight, ADG and FCR. Meanwhile, *Bacillus subtilis* spores can provide an anaerobic environment for the growth and proliferation of lactobacilli species by up taking \( O_2 \) from the gut after germination (Jazi, *et al.*, 2017). In addition, *Bacillus subtilis* can be potentially disturbs pathogens growth by synthesizing antimicrobial substances including bacteriocin-like substances, bacilysin, bacillaene, amicoumacin, and bacillomycins (Suva *et al.*, 2016). This could happen through competitive adhesion and synthesis of the antimicrobial compound, immunomodulation, improved intestinal integrity and function and digestive enzyme secretion (Jayaraman *et al.*, 2017). Furthermore, these results may refer to the fact that probiotics have ability to improve the microbial ecology of the intestine, reduce passage rate of the digesta and improve the digestibility of amino acids (Biggs and Parsons, 2007). These results are agreed with El-Sagheer and Hassanein (2014) who reported that when rabbits fed basal diet supplemented with probiotic alone or mixture of probiotic and prebiotics the body weight and FCR were significantly improved than those of the control group.

Zhou and Wang (2011) indicated that final body weight, body weight gain (BWG) and FCR were enhanced significantly in nano-Se groups in comparison with the control group. Similarly, Dlouha *et al.* (2008) found that dietary supplementation of 0.3 mg/kg Se from Se-Chlorella improved BWG and FCR. The improved performance because of Se supplementation might be attributed to its involvement in regulating several enzymatic systems that interfere in energetic metabolism, and synthesising prostaglandins and metabolism of the essential fatty acid apurinic and a pyrimidinic base (Saleh, 2014). Moreover, natural antioxidants could protect intestinal mucosa against oxidative damage and pathogens (Kermauner & Laurenčič, 2008). Furthermore, Se intake appears to confer additional health benefits on the immune system and reduce inflammation (Ebeid *et al.*, 2013).
Table (2): Effects of probiotics enriched with/without SeNPs on growth performance in growing lambs.

| Items                        | Control | BS       | SNEBS    | SEM    | p Value |
|------------------------------|---------|----------|----------|--------|---------|
| Initial body weight (kg)     | 26.16   | 25.41    | 25.29    | 0.836  | 0.794   |
| Final body weight (kg)       | 48.57\(^a\) | 51.16\(^b\) | 51.77\(^a\) | 0.662  | 0.017   |
| Total weight gain (kg)       | 22.41\(^b\) | 25.75\(^a\) | 26.48\(^a\) | 1.324  | 0.011   |
| Daily gain, g                | 177.85\(^b\) | 204.36\(^a\) | 210.19\(^a\) | 11.041 | 0.004   |
| Feed intake (g DMI):         |         |          |          |        |         |
| CFM                          | 947.00  | 947.00   | 947.00   |        |         |
| Corn silage                  | 208.67  | 198.33   | 200.67   | 11.831 | 0.822   |
| Rice straw                   | 164.34  | 166.48   | 171.15   | 6.922  | 0.798   |
| Total feed intake (g DMI)    | 1320.01 | 1311.81  | 1318.82  | 8.583  | 0.827   |
| FCR (kg DMI/kg gain)         | 7.42\(^a\) | 6.51\(^b\) | 6.72\(^b\) | 0.384  | 0.012   |

\(^a\) and \(^b\) means in the same row followed by different superscripts differed significantly at (P < 0.05).

FCR: Feed conversion ratio. BS: Bacillus subtilis. SNEBS: Bacillus subtilis enriched SeNPs.

Digestibility and nitrogen balance

Results in this study indicated that rations supplemented with BS and SNEBS had higher (P < 0.05) digestibility of DM, OM, CP, CF, NDF and ADF compared with those control of one, with slightly increased in most nutrient digestibilities with SNEBS-ration than those of BS-one (Table 3). So better growth performance in lambs fed diet supplemented by BS and SNEBS was accompanied by an increase in nutrient intake and digestibility of the feed, resulting in increased nutrients availability for lambs. These findings are consistent with the Saleem et al. (2017) who reported that probiotic culture supplementation enhanced the digestibility of DM, CP, crude fibre, and nitrogen-free extract in lamb’s diet. The mode of action of BS and SNEBS is similar to that of commercial probiotic bacteria in terms of promoting dry matter intake and improving fibre degradability. This could be explained by the improvement of the concentration of cellulyolytic bacteria in the rumen of lambs fed diet with probiotic supplementation (Wallace and Newbold, 1993). The increases in rumen microorganisms would be improve the microbial protein synthesis and in turn generating a higher supply of amino acids to the postrumen, allowing lambs to gain greater body weight (Erasmus et al., 1992). In the present study, supplementation the diets with BS or SNEBS could be increased the nutrient digestibilities and feed intake that resulting an extra availability of protein and metabolizable energy and hence superior growth performance would be recognised. Furthermore, antimicrobial substances found in both BS and SNEBS probiotics; including bacteriocins and organic acids, have an inhibitory effect on numerous pathogenic microbes in the gastrointestinal tract, resulting in improved nutrient digestibilities and absorption in lambs (Choe et al., 2013). Galina et al. (2009) found that feeding a diet supplemented with probiotic Lactobacillus mixture into maturing goats had improved fibre and protein digestibilities and also increasing microbial production. According to the finding obtained by Thanh et al. (2010), using postbiotics in broiler diets had reduced the multiplication of harmful bacteria, which could increase feed consumption and thus animal performance. Amber et al. (2004), working with Lactobacillus acidophilus, got improvements in the digestibilities of energy and of most analytical fractions (DM, CP and EE), including crude fibre. The effects of probiotics, maintenance of GI health and stimulation of enzyme production by the host (Mateos et al., 2010), may possibly contributed towards improvement in nutrient digestibility in probiotic-supplemented groups. A higher digestibility of CP and fibre components (NDF and ADF) in probiotics-supplemented groups could be the result of maintaining a relatively better gastrointestinal health and gut environment that supported improvement in N utilization and growth with efficient FCR. The nitrogen intake of lambs was unaffected by the addition of BS and SNEBS to their diet. While both faecal and urine nitrogen were significant lesser (P < 0.05), but nitrogen absorbed and nitrogen balance were higher than those in the control one. Lambs fed diet supplemented with BS and SNEBS retained more nitrogen than that of control one (Table 3).

Variances in nitrogen retention values could be related to differences in amino acid content and digestibility of protein sources. According to Phillips and Rao (2001), a greater percentage of CP in the diets could be led to higher faecal and urine N excretion. Improved N might be happened due to accumulation post-ruminal amino acids absorption that appeared to be greater than tissue demands or ruminal or post-ruminal ammonia absorption (Williams et al., 1991). On the other hand, improved protein of metabolism and utilization in growing lamb diets
was associated with better digestion and NB. The value NB or NR probably refers to normal CP synthesis (Fahmy et al., 1992).

**Table (3):** Effects of probiotics enriched with/without SeNPs on nutrient intake and apparent digestibility of the experimental rations.

| Items                               | Dietary Treatments | SEM   | p Value |
|-------------------------------------|--------------------|-------|---------|
|                                    | Control            | BS    | SNEBS   |        |
| Digestion Coefficients, (%):        |                    |       |         |
| Dry matter                          | 59.84<sup>a</sup>  | 61.66<sup>a</sup> | 62.08<sup>a</sup> | 0.43  | 0.006  |
| Organic matter                       | 63.06<sup>a</sup>  | 64.89<sup>a</sup> | 65.13<sup>a</sup> | 0.19  | 0.002  |
| Crude protein                        | 62.27<sup>a</sup>  | 64.76<sup>a</sup> | 65.01<sup>a</sup> | 0.23  | 0.002  |
| Crude fiber                          | 57.38<sup>b</sup>  | 60.28<sup>a</sup> | 60.73<sup>a</sup> | 0.63  | 0.001  |
| Neutral detergent fibre              | 53.41<sup>b</sup>  | 57.73<sup>a</sup> | 57.62<sup>a</sup> | 0.11  | 0.004  |
| Acid detergent fibre                 | 41.38<sup>b</sup>  | 42.88<sup>a</sup> | 42.93<sup>a</sup> | 0.08  | 0.001  |
| Nitrogen Balance, (g/d):            |                    |       |         |
| Nitrogen intake                      | 22.46              | 22.37 | 22.41   | 0.16  | 0.849  |
| Feces nitrogen                       | 8.84<sup>a</sup>   | 8.01<sup>b</sup>  | 7.96<sup>e</sup>  | 0.11  | 0.019  |
| Urine nitrogen                       | 10.47<sup>c</sup>  | 9.86<sup>b</sup>  | 9.79<sup>b</sup>  | 0.13  | 0.004  |
| Nitrogen absorbed                    | 13.62<sup>b</sup>  | 14.36<sup>a</sup> | 14.45<sup>a</sup> | 0.09  | 0.002  |
| Nitrogen balance                     | 5.15<sup>b</sup>   | 6.50<sup>a</sup>  | 6.66<sup>a</sup>  | 0.14  | 0.001  |

*<sup>a</sup> and <sup>b</sup> means in the same row followed by different superscripts differed significantly at (P ≤ 0.05).*  
*BS; Bacillus subtilis, SNEBS; Bacillus subtilis enriched SeNPs.*

**Rumen fermentation characteristics**

There were no significant differences (P < 0.05) in respect of pH, total and individual VFA among the experimental dietary treatments, except for the propionic acid (P < 0.05) as illustrated in (Table 4). In comparison to the control group, the concentration of ruminal NH<sub>3</sub>-N was increased significantly (P < 0.05) in the rations that supplemented with BS or SNEBS. The present results respecting pH values are in agreement with the findings of Izuddin et al. (2018) who found that probiotic inclusion in the diet had no effect on rumen fluid pH in vitro. No changes in rumen pH could indicate that the rumen environment has adapted and regulated to the presence of lactic acid from probiotic supplementation in the meal. Supplementing the rations of lambs with BS or SNEBS raised the ruminal NH<sub>3</sub>-N content significantly. The rumen produces ammonia as a result of ruminal microorganisms digesting dietary protein and non-protein nitrogen. The major factors that influence the ruminal ammonia concentration are the amount of protein in the diet and the degradability of the protein in the rumen. The amount of protein in the meal and the degradability of the protein in the rumen are the two most important elements that affecting ruminal ammonia content. The ruminal concentration of ammonia rises as protein degradability rises in the rumen. In the current study, the greater NH<sub>3</sub>-N concentrations in the BS and SNEBS diets has corresponded to higher crude protein digestibility in these supplemented diets. Otherwise, the presence of lactic acid in high concentrations in postbiotics provides a constant source of lactic acid, which may promote lactic acid-using bacteria. Lactic acid-using bacteria like *Propionibacterium* spp. produce more propionic acid than the other VFAs (Seo et al., 2010) which could explain why lambs treated with BS and SNEBS had a higher molarity of propionic acid in their rumen fluid. The addition of probiotics to in vitro rumen fermentation resulted in an increase in total and major individual VFAs such acetate, propionate, and butyrate (Izuddin et al., 2018). Respecting the VFAs measurement, except for propionic acid, supplementation with BS and SNEBS in the diets of growing lambs had no effect on total and individual VFAs. These results were on line with those recorded by Astuti et al. (2018) who found that using L. *plantarum* as a probiotic in an in vitro rumen fermentation investigation had no influence on total VFA synthesis. Similarly the present findings are on line with those of Qadis et al. (2014) who found that probiotics from L. *plantarum* and *Enterococcus faecium* had no effect on total VFA in rumen fluid.
Table (4): Effects of probiotics enriched with/without SeNPs on rumen fermentation characteristics in growing lambs.

| Items                      | Dietary Treatments | SEM   | p Value |
|----------------------------|--------------------|-------|---------|
|                            | Control            | BS    | SNEBS   |
| pH (mg/100 mL)             | 6.26               | 6.19  | 6.21    | 0.08 | 0.798 |
| NH₃-N                     | 12.46ᵇ             | 13.55ᵃ | 13.43ᵃ | 0.14 | 0.002 |
| TVFA (meq/100 mL)         | 10.53              | 10.73 | 10.67   | 0.71 | 0.841 |
| Acetate (mol/100 mol)      | 60.45              | 60.77 | 60.82   | 0.84 | 0.788 |
| Propionate (mol/100 mol)   | 18.53ᵇ             | 20.46ᵃ | 20.74ᵃ | 0.27 | 0.004 |
| Butyrate (mol/100 mol)     | 9.64               | 9.04  | 8.94    | 1.05 | 0.847 |

ᵇᵃ means in the same row followed by different superscripts differed significantly at (P ≤ 0.05).

BS: Bacillus subtilis, SNEBS: Bacillus subtilis enriched SeNPs.

TVFA: Total volatile fatty acids.

Serum biochemical parameters

Supplementation the diet of growing lambs with BS and SNEBS significantly increased (P < 0.05) glucose, total proteins, albumin and globulin (Table 5). Otherwise, albumin/globulin ratio showed no significant differences, while, BS or SNEBS supplementation in the two tested rations significantly decreased (P < 0.05) the values of cholesterol, triglyceride and HDL-c compared to those of control one. In an interpretation for elevated blood glucose concentration (Khalid et al., 2011) cleared that L. Plantarum (as probiotics) could be breaking down the complex carbohydrates into simpler components such as glucose which then possibly utilized as energy for metabolic processes. Higher blood glucose levels in lambs supplemented with BS and SNEBS could be associated with increased propionic acid synthesis and absorption, as propionic acid that functioned as glucose precursor that boosts glucose production (Huntington and Eisemann, 2019). Similarly, a greater population of Propionibacterium in the rumen, especially in typical concentrate diets has alters rumen conditions by converting lactic acid to propionic acid, leading in an increased hepatic glucose production (Stein et al., 2006). In this study, lambs fed diet containing BS and SNEBS had a significant increase in total protein content. The obtained results could be a consequence of improved dietary protein digestibility due to the enzymatic activity of protease, as well as changes in the amino acid composition of the digesta due to increased microbial protein synthesis. Soren et al. (2013) found no change in albumin or globulin levels in probiotic-supplemented lambs. The increased serum albumin concentration in lambs given probiotics BS and SNEBS suggested that such supplements boost liver function and consequently improve albumin synthesis ability. In addition, probiotic administration dramatically enhanced plasma globulin concentration in newborn female calves, as recorded by Roodposhti and Dabiri (2012). The increases in globulin levels in probiotic supplemented rations might be attributed to an increase in net globulin amounts as a result of an increase in gamma globulins that generated by Kopffer cell proliferation and an increases in plasma cell numbers in the bone marrow (Raghebian et al., 2016). Serum cholesterol, triglycerides and HDL-c levels were significantly decreased by supplementing BS and SNEBS in lambs diets (Table 5) and that probably due to the hypcholesterimia effect of probiotics. Similar results are obtained by Everard et al. (2011) who reported that prebiotic treatments had anti-obesity, anti-diabetic, antioxidant, and anti-inflammatory benefits in obese mice, as well as changes in gut microbial composition. Similar findings were reported by Ooi and Liong (2010) who hypothesized that probiotic bacteria affect lipid metabolism by increasing bile salt hydrolase activity and cholesterol precipitation in some microorganisms such as Lactobacillus and Bifidobacterium, incorporating cholesterol or binding to bacteria, and producing short-chain fatty acids. Moreover, Fukushima and Nakano (1995) have proposed yet another explanation by which a probiotic can lower serum cholesterol levels. So, probiotic microorganisms were found to block hydroxymethyl-glutaryl-coenzyme A, an enzyme involved in cholesterol synthesis and in turn, resulting in a reduction in cholesterol synthesis. Similarly, probiotic supplementation may reduce cholesterol absorption and/or production in the gastrointestinal system, resulting in lower serum cholesterol in broiler hens fed a probiotic supplemented diet (Mohan et al., 1996). In addition, it was speculated that Lactobacillus acidophilus decreases the cholesterol in the blood by deconjugating bile salts in the intestine, preventing them from acting as precursors in cholesterol production (Abdulrahim et al., 1996).

With regard to plasma biochemical parameters, Table 5 shows that plasma concentrations of total cholesterol and triglycerides were significantly lower, while plasma glucose was significantly increased by dietary SNEBS. These results might be attributed to lipolysis, which was elevated by Se intake (Oppenheimer et al., 1991). Otherwise, Se-nano particle supplementation increases the levels of 15-deoxy-A-12, 14-prostaglandin J2 (Vunta et al., 2007), a known peroxisome proliferator-activated receptor-γ ligand. Activation of peroxisome proliferator-activated receptor-
γ can decrease the level of sterol regulatory element-binding protein-2, leading to depression of the cholesterol synthesis (Klopotek et al., 2006).

Table (5): Effects of probiotics enriched with/without SeNPs on blood metabolites in growing lambs.

| Items                  | Dietary Treatments | SEM | p Value |
|------------------------|--------------------|-----|---------|
|                        | Control            | BS  | SNEBS   |
| Glucose, mg/dL         | 64.34^b            | 66.32^a | 66.95^a | 0.57 | 0.021 |
| Total protein, g/dL    | 6.32^b             | 6.85^a | 7.03^a | 0.12 | 0.003 |
| Albumin, g/dL          | 3.45^b             | 3.74^a | 3.81^a | 0.08 | 0.001 |
| Globulin, g/dL         | 2.87^b             | 3.11^a | 3.22^a | 0.10 | 0.007 |
| Albumin/Globulin ratio | 1.20               | 1.20 | 1.18    | 0.07 | 0.573 |
| Cholesterol, mg/dL     | 66.17^a            | 59.55^b | 58.26^b | 1.33 | 0.006 |
| Triglyceride, mg/dL    | 43.51^a            | 39.76^b | 39.04^b | 0.57 | 0.002 |
| HDL-c, mg/dL           | 28.55^a            | 26.37^b | 26.15^b | 0.21 | 0.011 |

^a and ^b means in the same row followed by different superscripts differed significantly at (P ≤ 0.05).

BS; Bacillus subtilis, SNEBS; Bacillus subtilis enriched SeNPs.

CONCLUSIONS

The current study concluded that supplementing lambs' diets with Bacillus subtilis or Bacillus subtilis enhanced with SeNPs increased their growth performance, feed intake, and utilization of nutrient digestibilities. Also, improved rumen fermentation properties and blood metabolites. Bacillus subtilis or Bacillus subtilis enhanced with SeNPs supplements could be utilised as a feed additives for monitoring and maximize the whole productive performance items.

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