Effects of nano selenium-enriched *Bacillus subtilis* supplementation on growth performance, nutrients digestibility and blood constituents of growing rabbits

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Objectives

- Purpose of this study was to evaluate the efficiency of supplemental Bacillus in the forms of *Bacillus subtilis* alone (BS) and selenium nanoparticles-enriched *Bacillus subtilis* (SeNPsBS) as probiotic feed additives on rabbits growth performance, nutrient digestibility, nitrogen balance and blood biochemical parameters.

Background

- Both selenium (Se) and probiotic Bacillus own the virtues of regulating animal metabolism and improve the growth performance in growing rabbits.

Material and Methods

▪**Probiotic Bacteria and feed preparation:**

○*Bacillus subtilis* (BS) 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. Fermentation of nano selenium-enriched *Bacillus subtilis* was prepared with sodium selenite supplemented into the BS culture medium. After fermentation, live BS without Se was 1 × 10^9 cfu/ml, while SeNPsBS was 1 × 10^9 cfu/ml, and SeNPs reached 0.35 ppm. Each 1 g of BS and SeNPsBS were mixed with 1 kg of a carrier before being added to 1000 kg of animal feed.

▪**Animals:**

A total of 105 male New Zealand White growing rabbits aged 6 weeks, weighing 762 ± 13.8 g, were randomly distributed into five groups of 21 rabbits (7 replicates of 3 rabbits each) in a completely randomized experimental design.

▪**Treatments:**

- ○Control (with no probiotic).
- ○Groups T1 and T2 were supplemented with probiotics *Bacillus subtilis* at doses 0.5 × 10^9 CFU/kg diet and 1 × 10^9 CFU/kg diet, respectively.
- ○Groups T3 and T4 were supplemented with probiotics SeNPs-enriched *Bacillus subtilis* at doses 0.5 × 10^9 CFU/kg diet and 1 × 10^9 CFU/kg diet, respectively.

Figure 1: BS

Figure 2: SeNPsB

Figure 3: After being pelleted with animal feed

▪**Growth performance measurements:**

- ○Body weight was calculated as the difference between the final and initial rabbit weight.
- ○Feed intake was recorded daily during the experiment.
- ○Average daily gain and feed conversion ratio were calculated.
- ○Mortality rate was recorded daily and the percentage was recorded for each group at the end of the experiment.

▪**Sampling:**

- ○ At the end of the experiment, rabbits were kept in metabolic cages to measure nutrient digestibility and nitrogen balance.
- ○ Blood sample were collected for serum biochemistry at the end of the experiment. Serum biochemistry were determined according to the manufacturers' instructions using commercial assay kits (Bio-diagnostic, Cairo, Egypt).
- ○ Data were analysed by analysis of variance using the GLM procedures of SAS.

Results

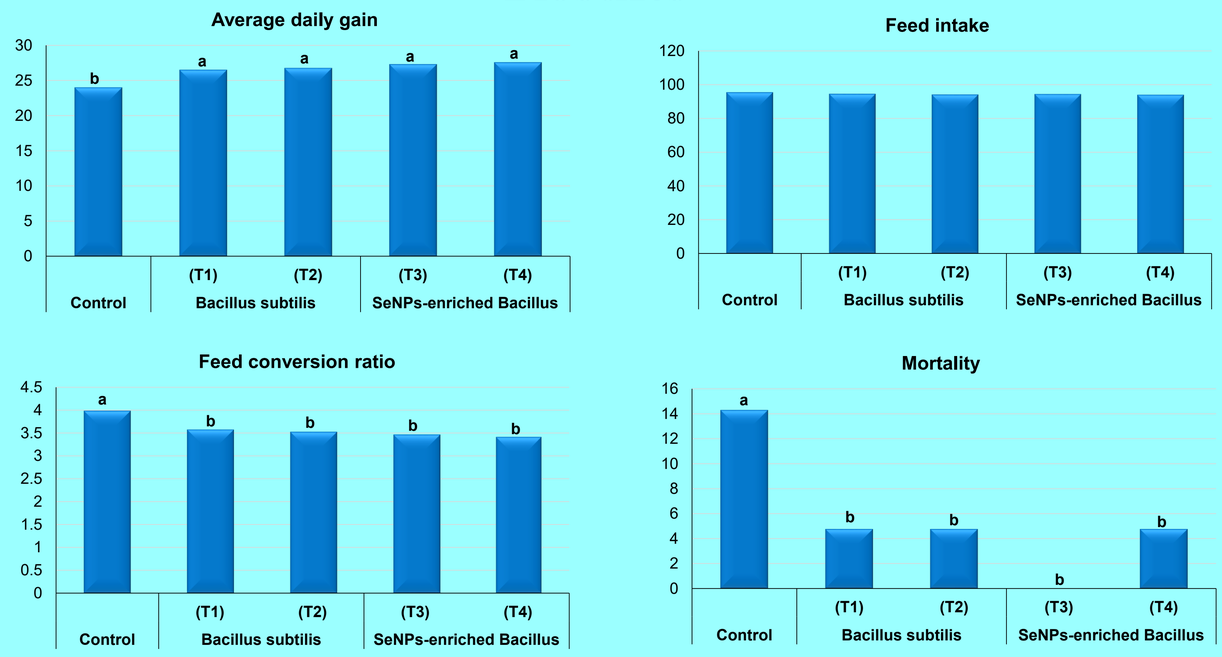

Figure (4). Effect of probiotic *Bacillus subtilis* supplementation on growth performance of rabbits.

Table (1). Effect of probiotic Bacillus subtilis supplementation on apparent nutrient digestibility and nitrogen balance of rabbits.

Items	Experimental groups					SEM	P-value
	Control	Bacillus subtilis		SeNPs-enriched Bacillus			
		(T1)	(T2)	(T3)	(T4)		
Apparent digestibility (%)							
Dry matter	64.76 [b]	66.49 [a]	66.72 [a]	67.18 [a]	67.26 [a]	0.83	0.001
Crude protein	66.39 [b]	69.03 [a]	68.83 [a]	69.22 [a]	69.14 [a]	0.41	0.017
Neutral detergent fibre	34.85 [b]	39.49 [a]	39.88 [a]	40.83 [a]	40.33 [a]	1.47	0.004
Acid detergent fibre	28.39 [b]	33.16 [a]	33.05 [a]	34.18 [a]	34.51 [a]	1.39	0.001
Nitrogen balance (g/day)							
N intake	3.07	2.99	2.96	2.97	2.92	0.19	0.845
Faces nitrogen	0.76 [a]	0.56 [b]	0.54 [b]	0.47 [b]	0.48 [b]	0.08	0.037
Urine nitrogen	0.84	0.78	0.77	0.79	0.75	0.03	0.722
N absorbed	2.31 [b]	2.43 [a]	2.42 [a]	2.50 [a]	2.44 [a]	0.08	0.041
N retained	1.47 [b]	1.65 [a]	1.65 [a]	1.71 [a]	1.69 [a]	0.06	0.006

[a-b] Means in the same row with different superscripts are significantly different (P<0.05).

Table (2). Effect of probiotic Bacillus subtilis supplementation on carcass characteristics of rabbits.

Items	Experimental groups					SEM	P-value
	Control	Bacillus subtilis		SeNPs-enriched Bacillus			
		(T1)	(T2)	(T3)	(T4)		
Pre-slaughter weight, g	2258.46	2284.33	2271.06	2294.14	2280.11	42.84	0.873
Dressing, %	57.48 [b]	60.54 [a]	61.18 [a]	61.74 [a]	61.21 [a]	1.15	0.015
Cecum weight, %	5.25 [b]	5.51 [a]	5.55 [a]	5.49 [a]	5.58 [a]	0.08	0.001
Liver, %	3.05	3.11	3.18	3.14	3.16	0.11	0.897
Kidney, %	2.44	2.31	2.41	2.33	2.37	0.16	0.853
Heart, %	0.51	0.53	0.54	0.52	0.54	0.04	0.788
Spleen, %	0.11	0.12	0.12	0.11	0.12	0.01	0.852

[a-b] Means in the same row with different superscripts are significantly different (P<0.05).

Table (3). Effect of probiotic Bacillus subtilis supplementation on serum biochemical Parameters of rabbits.

Items	Experimental groups					SEM	P-value
	Control	Bacillus subtilis		SeNPs-enriched Bacillus			
		(T1)	(T2)	(T3)	(T4)		
Total protein (g/dl)	6.43 [b]	6.89 [a]	6.97 [a]	6.94 [a]	7.06 [a]	0.15	0.004
Albumin (g/dl)	3.16 [b]	3.53 [a]	3.59 [a]	3.61 [a]	3.69 [a]	0.06	0.001
Globulin (g/dl)	3.27 [b]	3.36 [a]	3.38 [a]	3.33 [a]	3.37 [a]	0.04	0.032
Albumin globulin ratio	0.97 [b]	1.05 [a]	1.06 [a]	1.08 [a]	1.09 [a]	0.03	0.018
Cholesterol (mg/dl)	103.85 [a]	75.33 [b]	72.73 [b]	69.38 [b]	67.55 [b]	11.94	0.013
Triglycerides (mg/dl)	75.57 [a]	63.51 [b]	60.28 [b]	57.94 [b]	55.66 [b]	8.16	0.021
HDL (mg/dl)	49.64 [a]	42.55 [b]	41.39 [b]	41.21 [b]	39.13 [b]	2.06	0.016
LDL (mg/dl)	39.10 [a]	20.08 [b]	19.28 [b]	16.58 [b]	17.29 [b]	4.14	0.024
VLDL (mg/dl)	15.11 [a]	12.70 [b]	12.06 [b]	11.59 [b]	11.13 [b]	1.66	0.029

[a-b] Means in the same row with different superscripts are significantly different (P<0.05).

Conclusion

Bacillus probiotics in the forms of *Bacillus subtilis* alone, as well as SeNPs-enriched *Bacillus subtilis*, are alternatives natural additives that promote growth performance, nutrient digestibility, nitrogen balance, and lower blood lipids in rabbits. Probiotics, in both forms, are a promising natural feed supplements with favorable productive and physiological effects.

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