## Single and combined effects of Bacillus subtilis and inulin on performance of late-phase laying hens



4. Results

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1. Introduction

2. Objectives

Strength of eggshell is a vital defense line against physical damage and penetration of pathogenic microbes. Eggshell strength decrease in aged hens as they are less efficient in absorbing calcium than younger ones. The incidence of cracked eggs could exceed 20 % at the end of the laying period. Enhancing intestinal calcium availability and absorption could solve the problem. As hen gets older, the gut mucosal system becomes more susceptible to lose its integrity. *Bacillus subtilis* could improve intestinal integrity and nutrients absorption efficiency. Inulin could increase the intestinal absorption and bioavailability of calcium. *B. subtilis* and inulin are involved directly in the stabilization of intestinal microflora ecosystem and recovery of intestinal integrity. We assumed that these additives can improve the exhausted intestinal tract of aged laying hens, and thus enhance performance and eggshell quality of laying hens at the late phase of production.

To investigate the potential improvement of laying hens' performance and eggshell quality in the late phase of production by dietary inclusion of *B. subtilis*, inulin, and their combination.

## 3. Methods

Eighty hens (64 wk old) were randomly distributed into 4 treatment groups each consisting of 5 replicates of 4 hens. Birds in the groups were fed a basal diet (control) or basal diet plus 1 g/kg *B. subtilis* (2.3) ×108 cfu/g) or basal diet plus 1 g/kg inulin, or basal diet plus a synbiotic combination of *B. subtilis* and inulin for 12 wk. *B. subtilis* was provided by Kemin Industries, Herentals, Belgium (CloSTAT<sup>1M</sup>; 2.3 ×108 cfu/g). Inulin was provided by Beneo GR, Orafti Active Food Ingredients, Tienen, Belgium.

## Hens supplemented with *B. subtilis* and synbioitc combination exhibited the highest increase in egg production and eggshell density



(Figure 1). Inulin and synbiotic exhibited the highest increase in eggshell thickness and the lowest eggshell deformations (unmarketable eggs). Tibia density, and Ca content increased by inulin and synbiotic inclusions (Table 1). *B. subtilis*, inulin and their synbiotic combination increased villus height and crypt depth in all intestinal segments, compared with the control (Table 1). B. subtilis and inulin modulated the ileal and caecal microflora composition by decreasing numbers of *Clostridium* and Coliforms and increasing bifidobacteria and lactobacilli.

The present study confirmed the beneficial effects of dietary inclusion of *Bacillus subtilis*, inulin or their combination on gut health and intestinal integrity. These beneficial effects were directly associated with improvements in laying performance and eggshell quality.

**Figure 1:** Effects of dietary treatments on egg production and eggshell density

**Table 1:** Effects of dietary treatments on laying hens' performance, eggshell quality, tibia traits and intestinal villus-crypt system.

Parameters	Control	<b>B. subtilis</b>	Inulin	Synbiotic	SEM <sup>a</sup>	P value
Eggshell thickness (mm)	0.33 <sup>z</sup>	0.36 <sup>y</sup>	0.39 <sup>x</sup>	0.40 <sup>x</sup>	0.01	0.041

5. Conclusion

Egg performance and eggshell quality of aged laying hens can be improved by supplementing the diet with Bacillus subtilis or inulin. Egg performance was more affected by Bacillus subtilis, while eggshell quality was more affected by inulin. The synbiotic combined the effects of both. The intestinal colonization with beneficial microflora along with the increase in intestinal villi-crypts absorptive area were directly associated with the improvements in performance and eggshell traits of the supplemented hens.

Unmarketable eggs (%) <sup>b</sup>	8.4 <sup>x</sup>	3.5 <sup>y</sup>	1.7 <sup>z</sup>	1.5 <sup>z</sup>	0.41	0.000
Tibia density(g/cm <sup>3</sup> )	1.18 <sup>z</sup>	1.25 <sup>yz</sup>	1.32 <sup>xy</sup>	1.39 <sup>x</sup>	0.03	0.006
Tibia Calcium (mg/g of ash)	313.1 <sup>z</sup>	335.7 <sup>yz</sup>	369.8 <sup>xy</sup>	373.3 <sup>x</sup>	0.86	0.007
Duodenum Villi height (µm)	991.7 <sup>z</sup>	1098.5 <sup>y</sup>	1146.0 <sup>y</sup>	1196.0 <sup>x</sup>	16.45	0.000
Jejunum Villi height (µm)	632.9 <sup>z</sup>	655.5 <sup>y</sup>	687.0 <sup>x</sup>	690.0 <sup>x</sup>	5.22	0.006
Duodenum crypt depth (µm)	111.8 <sup>z</sup>	117.7 <sup>y</sup>	123.7 <sup>x</sup>	120.3 <sup>xy</sup>	1.43	0.012
Jejunum crypt depth (µm)	88.1 <sup>z</sup>	96.0 <sup>y</sup>	$103.3^{x}$	103.9 <sup>x</sup>	2.04	0.023

x, y, z Means in the same row with different superscripts are significantly different at P<0.05.

<sup>a</sup> SEM= Pooled standard error of the means.

<sup>b</sup> Deformed eggshells: broken, cracked, and shell-less eggs.