

A tough egg to crack

What probiotics can do for egg producers

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Large volumes of saleable eggs at the highest quality are key for egg producers. Intestinal health and optimal nutrient and mineral absorption in the digestive tract are essential for egg production, shell quality and food safety of eggs. Probiotics are known to have a beneficial effect on the intestinal microbial composition, as probiotics support the persistence of beneficial commensal microbes which assist in the digestion of feed and help to protect the host from pathogenic colonisation.

In 2016, the probiotic with the commercial name Calsporin® received a new European feed additive registration for application in laying hens. Since then, numerous new studies have been carried out that further demonstrate the beneficial effect of the dietary probiotic in layers and breeders. Calsporin® contains viable spores of *Bacillus velezensis* DSM 15544. A new taxonomic name, as the strain was formally known under the name *Bacillus subtilis* C-3102 (DSM 15544). This article provides an update of the scientific research available on the effect of probiotic *Bacillus velezensis* DSM15544 in laying hens.

Support performance of hens, young and old

Optimal feeding supports young hens in early peak production and will enable egg producers to better maintain production performance in the final stage of laying. In young hens, between 19 to 42 weeks of age, the addition of 3×10^8 CFU *B. velezensis* DSM 15544 per kg feed, resulted in a faster increase of egg weight, higher egg mass and better utilisation

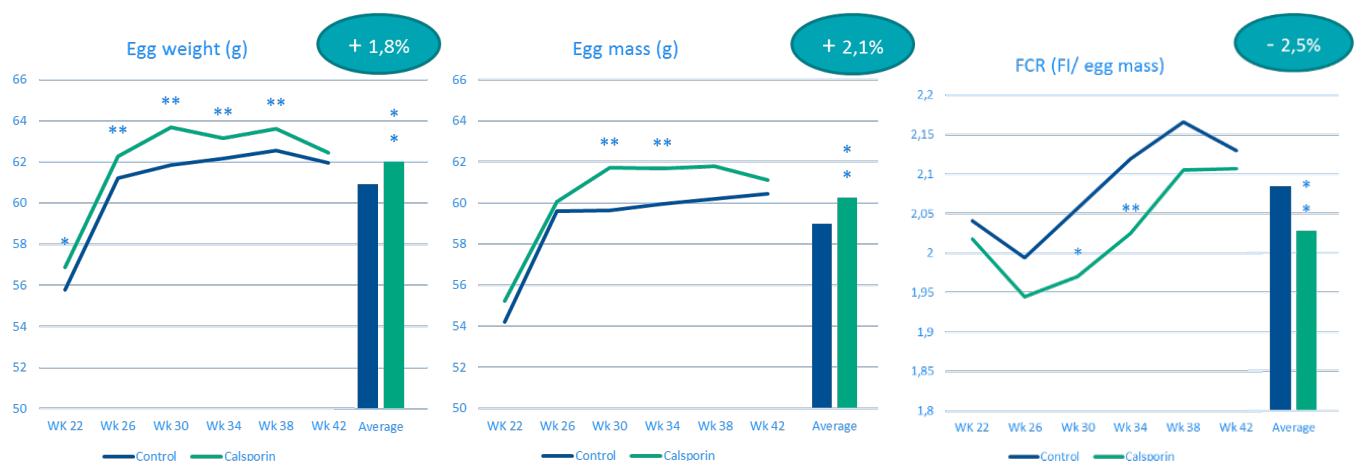
of the feed (figure 1)¹. Even with hens during peak performance, similar feed intake and higher performance did not negatively influence live hen weight in the probiotic group, reflecting an improved feed efficiency, instead of mobilisation of body reserves for egg production. In another study, following hens for a period of one year (from 19 to 70 weeks of age), the inclusion of 3×10^8 CFU *B. velezensis* DSM 15544 per kg feed improved laying rate, egg weight, egg mass and feed conversion over the whole period². The beneficial support of laying performance by the addition of Calsporin® proven in the registration and practical field trials is also reaffirmed in recently published scientific articles. In various studies, the addition of *B. velezensis* DSM 15544 improved egg production^{3,4}, egg weight⁵, egg mass³ and feed conversion ratio^{3,4}. In a study with increasing amounts of the probiotic per treatment, the addition of *B. velezensis* in the diet linearly increased egg weight⁵. Even though the fact that the probiotic is already effective at a low dosage of 30 ppm (3×10^8 CFU/ kg feed), higher concentrations can improve animal performance even more.

Freshness and colour of the egg

Haugh unit and yolk index are important indicators to evaluate the freshness of eggs. Although a trend for a higher Haugh unit was found in one trial², this could not be confirmed by another study, in which a significant positive effect on yolk index (calculated by dividing yolk height by yolk width) was found by the addition of *B. velezensis* DSM 15544⁵.

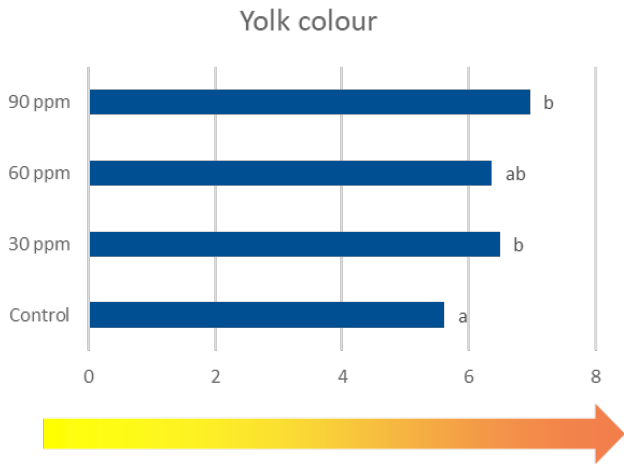
Figure 1 a-c: *Bacillus velezensis* DSM 15544 positively influenced laying hen performance in early stages of the production period¹.

* Trend for significant difference (P < 0,10), ** Significant difference (P < 0,05).



Interestingly, yolk colour was linearly and quadratically increased with increasing levels of the *B. velezensis* strain (figure 2)⁵. Enriched yolk colour might be related to better absorption of carotenoids, primarily xanthophylls, and deposition into the developing ova by the laying hen. Changes in colour were seen before in a practical field trial with brown laying hens, where the addition of the probiotic resulted in a higher percentage of more dark coloured eggshells.

Figure 2: *Bacillus velezensis* DSM 15544 linearly and quadratically increased yolk colour⁵. a, b: Bars with different superscript are significantly different between treatments (P<0,05)



Robust eggshells

Eggshell formation is a classical and rapid biomineralization process. In a span of less than 20 hours, about 2 grams of calcium deposits as calcium carbonate in the eggshell gland. As already mentioned, hens receiving *B. velezensis* DSM 15544 in their diet have a higher egg weight compared to hens receiving the same diet without the probiotic^{1,2,5}. In practice, an increase in egg size is often negatively

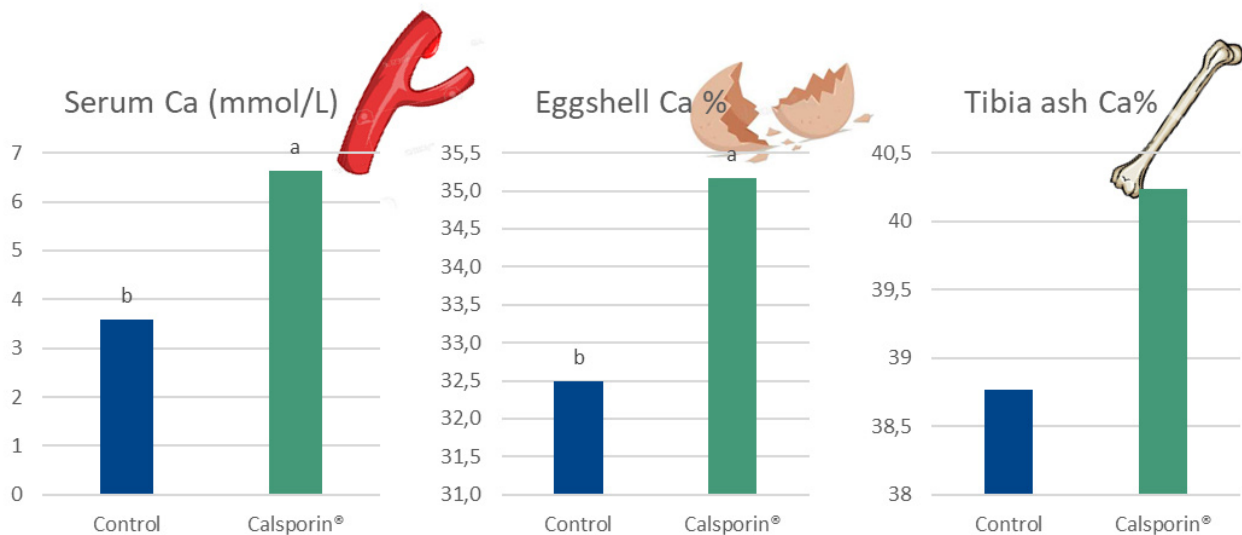
correlated with shell quality. With the probiotic, however, in all cases, larger eggs did not result in thinner eggshells or compromise the eggshell strength. In contrast, even thicker eggshells⁵ and fewer cracked eggs² were noticed when egg size was increased.

Calcium absorption declines in older laying hens and can partly explain the reduced eggshell quality during the late production phase. Dietary probiotics can help to maintain eggshell quality and supplementation enables the egg producers to prolong the laying period. In aged laying hens, the addition of *B. velezensis* DSM 15544 resulted in higher shell weight, an increase in eggshell thickness and as a result, improved eggshell strength^{4,6}. The eggshell breaking potential reduced from 3-4% in the control group to less than 1% in the Calsporin[®] treated group⁶.

Support nutrient and calcium absorption

Improved performance and eggshell quality can be explained by better feed utilisation by the hens, as it was shown that the apparent digestibility of protein, fibre and ash was significantly higher in 70-week old birds receiving *B. velezensis* DSM 15544². Recently, a new trial confirmed the improved apparent digestibility of calcium in 79-weeks old laying hens⁴. Increased absorptive area (higher villi and villi-crypt ratio)^{4,7} and transcellular transport by CALB1 (a major component of transcellular Ca transport in the duodenum and upper jejunum)⁴ could explain the higher calcium absorption by the use of Calsporin[®]. Composition of the microbiota could also have an influence on the solubility and absorption of calcium. By the stimulation of the lactic acid production of commensal lactobacilli⁶, probiotics have the ability to decrease the luminal pH, beneficial for the dissolution and uptake of calcium. Higher calcium absorption in laying hens was also confirmed by higher calcium levels in the serum and increased calcium in the eggshell content, without affecting calcium levels stored in the tibia (figure 3)⁴.

Figure 3: Addition of *B. velezensis* DSM 15544 increased the calcium in serum, eggshell and tibia of aged laying hens (week 79)⁴. a, b: Bars with different superscript are significantly different between treatments (P<0,05)



Bacillus velezensis to fight pathogens

Layer health and also the food safety of eggs are related to intestinal health and the composition of the microbiota. Probiotics support the presence of beneficial gut bacteria and can suppress the development of opportunistic pathogens. Local bacteria in the digestive tract compete with pathogenic species for epithelial binding sites and nutrients, positively support the host intestinal immune response and are able to produce metabolites to control the growth of opportunistic pathogens. In young pullets, a combination of Calsporin® and yeast cell walls increased the microbial diversity of the immature microbiota and simultaneously reduced the *Salmonella* Enteritidis infection⁸. *Salmonella* Enteritidis (SE) is one of the most reported serotypes causing foodborne illness in humans, where laying hens can serve as a SE reservoir and eggs are at a high risk of contamination. In aged laying hens after moulting, *Bacillus velezensis* DSM 15544 elevated the number of beneficial microbes (e.g. *Lactobacillus*) and decreased *Clostridium perfringens*, another opportunistic pathogen⁶. Supporting the immune status of the laying hens is another strategy to fight against various infections. In laying breeders, both serum IgM and serum antibody levels against avian influenza after vaccination linearly increased by increasing levels of *B. velezensis* in the diet⁵.

Microbiota and inflammatory responses

Opportunistic pathogens can also trigger energy demanding immunological pathways in the gut. Harmful microbes are recognised by receptors on immune cells and can trigger pro-inflammatory responses.

Addition of *B. velezensis* DSM 15544 to the diet of laying hens modulated the TLR4/MyD88/NF-kB signalling pathway, resulting in a reduced release of the pro-inflammatory cytokines IL-1 β and TNF- α ⁷. *Escherichia coli* and *Salmonella* especially exhibit structural characteristics that can be recognised by toll-like receptors (TLRs), particularly TLR4, and activate a cascade of inflammatory signals. Lower numbers of *E.coli*, *Salmonella* and *Clostridium* were confirmed in the small intestine and can explain that the suppression of pro-inflammatory cytokines might be attributed to an improved microbiota. Antioxidant status of the hens was evaluated by total antioxidant capacity, catalase, total superoxide dismutase and glutathione peroxidase malondialdehyde in the intestinal mucosa, plasma, spleen and liver. Both, the intestinal and overall antioxidant status of the laying hens improved with the supplementation of Calsporin®⁷. Energy savings related to an improved anti-inflammatory and antioxidant status can also contribute to the ameliorated egg production and feed efficiency in laying hens.

Probiotics as a nutritional solution

Bacillus velezensis DSM 15544 has proven to be an effective feed solution to support laying hen performance, egg quality and food safety of the eggs. The low inclusion level makes the probiotic cost attractive and the heat resistance of the spores enables excellent survival of the probiotic in pelleted layer feed.

References are available on request from: info@orffa.com

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