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Feed supplementation with β-Asp-Arg dipeptides via stable co-expression cyanophycin and cyanophycinase in plants

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Livestock diets can be supplemented with dipeptides in order to promote optimal growth and wellbeing. Due to the dual role of arginine as building block for proteins and regulator of physiological functions, pronounced effects were observed after addition of β-aspartate-arginine (β-Asp-Arg) dipeptides to feed. Currently, β-Asp-Arg is generated *in vitro* from the cyanobacterial storage polymer cyanophycin (CGP) via incubation with the cyanophycinase enzyme (CGPase) which are both produced in *E. coli*. Because of the high costs and limited scalability, bioreactor-based production is commonly used for the synthesis of high-value but not for cost-sensitive products such as supplements for animal diets.

Alternatively, recombinant low-value products can be produced in plants in an economic manner using existing agricultural infrastructure and farming practices. We already established the production of CGP in plastids of tobacco and potato, yielding up to 9.4% of the dry weight (dw) in stably transformed plants. We also demonstrated that CGPases can be transiently co-expressed in the cytosol of CGP-producing tobacco via the MagnICON system without affecting the CGP accumulation in intact chloroplasts. Amongst different CGPases, CphE showed the highest yield and was able to degrade CGP in homogenized leaf tissue when the spatial separation of cytosol and plastid stroma was destroyed. Oral administration of feed pellets, which contained purified CGP and CGPase to mice, showed that the released dipeptides were absorbed into the blood.

Now we could demonstrate that CGP degradation is not only possible after transient expression of CphE in the cytosol of CGP-producing tobacco, but also when the CGPase is introduced into these plants via stable transformation. Constitutive expression of CphE in parallel to CGP synthesis does not affect the accumulation of CGP in leaf chloroplasts and degrades CGP completely after decomposition of cells. The transfer of this system to feed plants would therefore provide an easy and cheap system to directly release the β-Asp-Arg dipeptides in the intestine of animals in the digestive process.