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1. Executive summary

Riboflavin (Vitamin B2) is an essential vitamin for all non-ruminant livestock animals. Also poultry feed formulations need to contain riboflavin, in order to avoid disorders in energy metabolism, oxidation protection of lipids, growth and neuronal control of the limbs. With increasing growth or laying performance of modern genotypes, these requirements raise due to higher metabolic turnover. This holds true also for organic poultry. In organic systems, however, low inputs of artificial or isolated substances to animal feed are aspired. Furthermore, the ban of GMO-based substances in organic production cycles requires separate GMO-free production lines for the microbiological production of riboflavin. Such production lines are on smaller scales and with less efficient organisms, thus, more expensive.

Given this background, riboflavin supplements to organic poultry should be as low as possible, with safe margins regarding animal health, welfare and productivity. Specific recommendation figures for organic systems do not exist, however, there are reasons to assume somewhat lower requirements than in conventional systems, due to slightly lower growth rates and other feed formulations. Furthermore, the scientific basis of current conventional recommendations are rather outdated. For these reasons, the goal of the present work was to generate experimental evidence for safe lower levels of riboflavin supplementation to organic poultry feed formulations. These implied in separate approaches fattening broilers, laying hens, as well as breeding hens and their offspring.

At the same time, a new European GMO-free riboflavin product has replaced the previously widely used product, which had been produced in China and was recently taken off market. We therefore also tested and approved the equivalence of the new product with conventional commercially available GMO-based riboflavin.

Broiler diets were tested in four different experiments with slow-growing genetical strains fattened with organic feed formulations during 55-63 days. A total of 12 different riboflavin supplementation schedules were tested, ranging from zero to 9.6 mg supplementation per kg of feed, always applying the European product Ecovit R (Agrano GmbH, Riegel, Germany). Impaired growth rates as a sign of riboflavin deficiency were found with supplementation levels 0, 2.5, and 3.5 mg/kg, in different experiments. However, in other experiments, also supplementation levels below 3.5 mg/kg performed without causing deficiency symptoms. Note that all biological feed components contain intrinsic riboflavin (range 1.5-3.5 mg/kg), which adds to the supplemented. Altering levels of intrinsic contents caused different responses supplementation levels. Considering these alterations of native riboflavin contents, we conclude 4.0 mg/kg supplementation to be a generally safe level for growing chicken. Supplementation graded by phases proved to perform well, however practical applicability of grading schedules appears less likely.

With respect to laying hens, including breeders, we tested five levels of supplementation from 1.5 to 4.5 mg/kg. None of these supplementation levels caused any depressions in laying performance nor impaired health scores. However, with supplementation of 1.5 mg/kg, we found reduced riboflavin concentrations in egg yolks and livers, which are considered as first signs of riboflavin deficiency. Since no such differences occurred between supplementation levels of 4.5, 3.0 and 2.5 mg/kg, we conclude a homeostatic equilibrium in this range. Therefore, 3.0 mg riboflavin / kg feed are considered a safe supplementation level for laying hens fed organic diets.

Breeder hens were examined with respect to their own performance and health, including egg fertility, hatchability and subsequent growth development of the hatched broiler chicks. We compared supplementation levels 4.0 and 2.5 mg/kg in one experiment. Although no signs of deficiency occurred with the hens, the fertility and the hatchability, growth performance of chicks from low-supply parents was clearly impaired. Chicks from the control group (4.0 mg/kg) developed according to the usual growth rates of the genetic strain investigated (Hubbard S757), but chicks from low-supply parents showed less than 90% of the growth rate with clear statistical significance. A similar effect was found with a low supplementation level for the chicks themselves, when their parents had been supplied normally. An additive depressive effect of low supplementation of parents and offspring was not found. Health impacts due to the different supplementation levels of breeding hens were generally not found. The conclusion is that for breeding hens a supplementation with 4.0 mg riboflavin per kg feed is sufficient but necessary in order to prevent growth depressions of the offspring.

Full detailed information on all experiments is provided in four scientific publications (section 2.2). Two manuscripts are still confidential until online publication by the journals.

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2. Dissemination activities related with the Deliverable

2.1 Presentation of the results at scientific conferences

In 2019, we firstly presented the results on riboflavin supply to broilers at the Annual Conference of the European Association of Animal Science (EAAP) in Ghent, Belgium. In 2020, we presented our results on riboflavin supplementation of organic laying hens at the Annual Conference of the Society of Nutrition Physiology in Göttingen, Germany, March 5-7, 2020. Furthermore, we contributed with two talks on riboflavin supplementation to organic broilers and organic parent hens to the Annual Conference of the European Association of Animal Science (EAAP), online, December 1-5, 2020.

2.2 Publication of the results in peer-reviewed journals

We published the following peer-reviewed full papers:

Lambertz, C., Leopold, J., Damme, K., Vogt-Kaute, W., Ammer, S., Leiber, F. 2020. Effects of a riboflavin source suitable for use in organic broiler diets on performance traits and health indicators. *Animal* 14: 716-724.

Lambertz, C., Leopold, J., Ammer, S., Leiber, F., Thesing, B., Wild, C., Damme, K. 2021. Demand-oriented riboflavin supply of organic broiler using a feed material from fermentation of Ashbya gossypii. *Animal* 15:100003.

The following two publications are in preparation:

Leiber, F., Holinger, M., Amsler, Z., Maeschli, A., Maurer, V., Früh, B., Lambertz, C., Ayrle, H.2021. Riboflavin for laying hens fed organic winter diets: effects of different supplementation rates on health, performance and egg quality. Revised version submitted to *Biological Agriculture and Horticulture*.

Leiber, F., Amsler, Z., Bieber, A., Maurer, V., Früh, B., Lambertz, C., Ayrle, H.2021. Effects of reduced riboflavin supplements for organic broiler parent stock and their chicks on health, performance and fertility. In preparation for submission to *Biological Agriculture and Horticulture*.

2.3 Publication of the results in conference abstracts

We published the following conference abstracts:

Lambertz, C., Leopold, J., Damme, K., Vogt-Kaute, W., Ammer, S., Leiber, F. 2019. Einsatz einer ökokonformen Riboflavinquelle bei langsam wachsenden Mastbroilern. In: Nachhaltigere Tierernährung: Erfolgreiche Fütterung, Ökonomie, Biodiversität und Umwelt im Einklang. Tagungsband, 57. Jahrestagung der Bayerischen Arbeitsgemeinschaft Tierernährung, 162-166.

Lambertz, C., Leopold, J., Damme, K., Vogt-Kaute, W., Ammer, S., Leiber, F. 2019. Effect of riboflavin dosage and source on growth, slaughter traits and welfare of broilers. Book of Abstracts of the 70th Annual Meeting of the European Federation of Animal Production EAAP, Ghent, Belgium. p. 156.

Leiber, F., Holinger, M., Maeschli, A., Früh, B., Lambertz, C., Maurer, V., Ayrle, H. 2020. Effects of riboflavin concentration in premixes on performance, egg quality and health indicators in laying hens. Proceedings of the Society of Nutrition Physiology, 29, 88.

Leiber, F., Amsler. Z., Leubin, M., Baki, C., Eppenstein, R., Lambertz, C., Maurer, V., Ayrle, H. 2020. Riboflavin requirements in organic poultry: graded supplementation to layers and parent hens. Book of Abstracts of the 71st Annual Meeting of the European Federation of Animal Production EAAP, online conference. p. 593.

Lambertz, C., Leopold, J., Ammer, S., Thesing, B., Wild, C., Damme, K., Leiber, F. 2020. Requirement-oriented supply of organic broilers with riboflavin from fermentation of Ashbya gossypii. Book of Abstracts of the 71st Annual Meeting of the European Federation of Animal Production EAAP, online conference. p. 433.

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2.4 Presentation of the results to stakeholders of the relevant industry

In 2019, FiBL presented the current developments on vitamin B2 recommendations for poultry to Swiss farmers, poultry industry and feed mill stakeholders with a presentation at a conference on 24.1.2019 in Frick. Further we updated German farmers, policy makers, feed mill representatives, and consultants in two (one) workshops: "Bedarfsgerechte ökologische Fütterung von Geflügel: „Neue Quellen für Riboflavin – Vitamin B2-Fütterung beim Mastbroiler" at Arbeitskreis ökologische Geflügelhaltung, Freising, 15.11.2019, and at Geflügelberatertagung, Rot am See, 18.09.2019. On 24 September 2019 we presented the current status on vitamin B2 to an internal meeting of the Swiss poultry industry, and on 20 August 2020 we contributed with a presentation on riboflavin supplementation to a training event for German and Swiss veterinarians and organic certifiers in Frick.