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## Effects of riboflavin concentration in premixes on performance, egg quality and health indicators in laying hens

Einfluss der Riboflavinkonzentration im Prämix auf Leistung, Eiqualität und Gesundheitsindikatoren bei Legehennen

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In organic animal feeds, the use of genetically modified organisms is prohibited. Therefore, B-vitamins have to be produced with alternative, more expensive strains of microorganisms and the dosage of added riboflavin is economically relevant (1). Since riboflavin requirement data for poultry are comparably old, the requirements for laying hens with a typical organic diet were reassessed. The aim was to find safe lower thresholds of riboflavin supplementation without impact on animal health and performance.

Methods: Basis and control was a usual Swiss organic layer feed with 4.5 mg riboflavin/kg feed added via the premix (R4.5). Identical feed was produced with 3.0 and 1.5 mg riboflavin/kg feed added (R3.0 and R1.5, respectively). Diets were applied to respectively three groups (pens) of 15 Lohmann Brown-classic hens at 25 weeks of age, when the experiment started. Ad libitum feeding of the diets lasted for 18 weeks. Feed consumption and laying performance were recorded daily, by pen. Individual body weight was determined and assessments for motility behavior, and scores for lesions, plumage, leg integrity and keel bone integrity were carried out seven times throughout the experiment. With the same frequency, eggs (20 per pen) were collected and analysed for yolk riboflavin content, colour, shell strength and weights of yolk, albumen and shell. In experimental weeks 11 and 18, respectively two hens from each pen were slaughtered and livers were analysed for riboflavin concentration. Statistical analyses were performed with R (version 3.5.3; R Core Team 2019). Fixed effects were week and treatment, including their interaction. Random effect was pen. For body weight and scorings, nested random effects were animal within pen. Riboflavin analyses had been carried out on composite samples (two per pen) and respective data were therefore only compared by Students t-test.

Results: Effective total riboflavin concentration at the start of the of the experiment was 6.1, 5.3, and 3.3 mg/kg feed for R4.5, R3.0, and R1.5, respectively. Concentrations in all diets declined during the first eight weeks of the experiment, and subsequently stayed stable at 5.0, 4.5, and 3.0 mg/kg for R4.5, R3.0, and R1.5, respectively. Neither laying performance, nor feed intake, body weight or any health and welfare indicator differed between the three feeding treatments. Egg quality parameters (weight, egg shell stability and yolk colour) were not affected by the level of riboflavin supplementation. However, riboflavin concentrations in egg yolks of the R1.5-treatment declined in experimental week 7 by approximately 20% compared to the other two treatments. Subsequently, the values remained stable until the end of the experiment in week 18 (0.58, 0.56 and 0.43 mg/100g fresh matter for R4.5, R3.0, and R1.5, respectively). Also in the livers of R1.5 animals, we found lower riboflavin concentrations in experimental weeks 11 and 18 (8-10% decline) compared to R4.5 and R3.0 among which no difference occurred (week 18: 2.18, 2.20 and 1.98 mg/100g fresh matter for R4.5, R3.0, and R1.5, respectively).

Conclusions: Supplementation of only 1.5 mg riboflavin/kg feed led to reduced levels in egg yolk and liver, indicating a metabolic deficiency of this water soluble vitamin. Although no impacts on feed intake, body weight, performance and clinical scores were found, this dosage should be considered as too low. The addition of either 3.0 or 4.5 mg did not result in any differences, suggesting that both dosages are above the lower critical threshold. Regarding the effective total riboflavin concentration of diets R3.0 and R1.5, this threshold was between 4.5 and 3.0 mg/kg, which is well in line with former literature (2). The addition of 3.0 mg riboflavin/kg feed is suggested as safe in organic layer nutrition.

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