Abstract of the doctoral dissertation of Kinga Szczepanik, M.Sc., entitled:

Effects of *Hermetia illucens* fly larvae meal and astaxanthin on production performance, health status, and gastrointestinal structure and function in pigs

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Weaning is a critical phase in pig breeding, often associated with an increased risk of intestinal disorders, oxidative stress, and infections. In the context of climate change and the need for alternative protein sources, this study investigated the effects of a full-fat meal derived from *Hermetia illucens* (HI) larvae—rich in amino acids and bioactive compounds (antimicrobial peptides, chitin, lauric acid)—on piglet immunity. Additionally, the impact of astaxanthin (AST), a potent natural antioxidant, on oxidative balance and overall health status was evaluated. The study was designed to assess the effects of HI larvae meal and AST supplementation on the production performance and health status of weaned piglets. A total of 48 piglets were assigned to six groups (n = 8 per group) with diets supplemented with HI meal (0%, 2.5%, or 5%) and/or AST (0 or 0.25 mg/kg). In addition to evaluating basic production parameters, a series of analyses were conducted, including biochemical and hematological blood tests, immunoenzymatic, immunohistochemical, histological, and genomic assessments. Chemical analyses of meat and fat composition were also performed.

The results indicate that the inclusion of HI and AST meal in the feed ration had no negative effect on growth performance, weight of organs and gastrointestinal tract sections (calculated as % of body weight), as well as feed consumption and average daily weight gain in the piglets participating in this study. Although statistically significant differences were observed between groups, hematological and biochemical blood parameters were within physiological norms, suggesting that HI and AST meal supplementation does not adversely affect piglet health. Analysis of lard stored frozen for 3 months showed that the addition of AST to the feed along with HI meal reduced the level of the TBARS index. This result indicates that these additives can be used as an effective antioxidant to reduce the effects of lipid peroxidation in adipose tissue. In the case of meat, no such clear effect was obtained. Histological analyses showed that simultaneous administration of AST along with HI meal at 2.5% levels significantly increased the width of the muscularis and its layers in the duodenum, which could potentially contribute to better peristalsis. In contrast, 2.5% HI meal increased the length and width of the villi, and when administered together with AST, improved the ratio of villi length to crypt depth. In the jejunum, a beneficial effect of AST was observed, which increased the width of the mucosa, the thickness of the longitudinal muscle layer, the number of goblet cells and lengthened the villi, while a positive effect of HI meal on the absorption surface was seen in both the jejunum and ileum. In addition, there was increased cell proliferation in the crypts of the duodenum and jejunum, particularly with 2.5% HI meal supplementation. Analysis of

the expression of tight junction proteins suggested that the highest expression was in the control group, not subjected to 13 supplementation with supplements. As part of the implementation of the study, the effect of astaxanthin on the health status of the piglet liver was also evaluated, taking into account both the histology of the liver tissue and the expression of genes related to its function. In the experiment conducted, significant histological differences were observed between animals receiving AST supplementation in the feed mixture and those in the control group. In the AST - supplemented group, there was a significant reduction in the deposition of collagen fibers in liver tissue, indicating a potential role for AST in protecting against excessive collagen accumulation. Gene expression analysis showed that the liver of piglets receiving AST - supplemented feed had reduced expression of the *NR1H3* gene, whose inhibition may promote hepatocyte regeneration after liver steatosis. In contrast, expression of the *CYP7A1* gene, which is key to cholesterol metabolism and excretion via bile acid synthesis, was increased. These changes suggest that AST may promote detoxification processes and lipid homeostasis in the liver. In addition, reduced expression of *NOTCH1* and *CREB* genes was observed, although their role in alleviating liver fibrosis remains unclear.

In conclusion, it can be concluded that the supplementation of piglet feed during the peri-weaning period with HI and AST meal can be a valuable addition to piglet diets, improving the structure and function of the intestine and liver, promoting health status, and favorably influencing the oxidative stability of adipose tissue, without adversely affecting animal production rates.