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improvement. Nutritional strategies aimed at modulating the microbiota—host relationship can improve chickens' immunological status and metabolic fitness. Here, we present the results of a pilot trial aimed at analyzing the effects of a nutritional strategy involving vitamin B₂ supplementation on the ileum, caeca and litter microbiota of Ross 308 broilers, as well as on the metabolic profile of the caecal content. Three groups of chickens were administered control diets and diets supplemented with two different dosages of vitamin B₂. Ileum, caeca, and litter samples were obtained from subgroups of birds at three time points along the productive cycle. Sequencing of the 16S rRNA V3-V4 region and NMR metabolomics were used to explore microbiota composition and the concentration of metabolites of interest, including short-chain fatty acids. Vitamin B₂ supplementation significantly modulated caeca microbiota, with the highest dosage being more effective in increasing the abundance of healthpromoting bacterial groups, including Bifidobacterium, resulting in boosted production of butyrate, a well-known health-promoting metabolite, in the caeca environment.

Cultivar selection and nitrogen fertilization on wheat protein composition and on the expression of toxic epitopes studied by proteomic analysis

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Soft wheat is one of the main diet component for several bakery products with high organoleptic properties. In addition to the fundamental technological role, gluten proteins are also the primary cause of the celiac disease (CD) and other gluten-related diseases (1). It is essential taking into account several factors as genotypes, crop agronomic conditions, baking process, aiming to create a cereal closer to a "gluten-disorders safe" one (2). The aim of this study is to evaluate the impact of N fertilization rate on the variation of gluten peptides in different wheat cultivars (cv): old variety (landrace), modern cv, and a tritordeum cv (hybrid of wild barley and durum wheat). Refined flours of each genotype X N rate combination were analysed through mono (SDS-PAGE) and two-dimensional electrophoresis (2-DE) coupled with mass spectrometry analysis, reversed-phase

high-performance liquid chromatography (RP-HPLC). ELISA-R5 test was used to quantify the immunotoxicity of samples. An *in vitro* digestion was performed on flours to observe the resistance of toxic and immunodominant epitopes. Gluten content (GC) was higher in landraces as the higher amounts of toxic CD (33-mer, 25-mer) and allergenic epitopes compared to modern ones, while Tritordeum presented lowest values for gluten R5 immunotoxicity. Although N fertilization strongly impact on GC, the effect of N rate on gluten composition and occurrence of gluten epitopes was limited, unlike the genetic effect.

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Short-chain fatty acids as biomarkers monitoring diet and environment effect on gut microbiota

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Short-Chain Fatty Acids (SCFAs) are important gut microbiota metabolites known for their beneficial effects on the host organism. They can be considered as biomarkers of the health state of an individual since their production is associated with a good microbiota state, intestinal transition time and with an optimal diet, rich in dietary fibers and low in fats [1]. The presence of SCFAs in the intestinal tract has demonstrated several advantages, e.g. lowering luminal pH, favoring nutrients absorption and reducing the formation of some pathogens associated with diseases such as bowel disorders, colon cancer and metabolic problems [2]. An analytical method has been optimized, including a rapid sample preparation with ethyl ether extraction of SCFAs after acidification of the fecal sample with sulfuric acid, followed by gas chromatographic analysis [3]. The method is being applied to several projects dealing with the assessment of the effect of specific diets or mobility and environment, on gut microbiota. The method and preliminary results from the applications will be presented.

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