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The composition and enzymatic activity of gut microbiota in laying hens fed diets supplemented with blue lupine seeds

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ABSTRACT

The composition and activity of gut microbiota were analyzed in laying hens fed for 24 weeks a control diet containing soybean meal (diet C) and two experimental diets supplemented with 10% or 20% blue lupine seed meal used as a substitute for soybean meal (diets L_{10} and L_{20}, respectively). In comparison with soybean, lupine seeds had a higher content of neutral detergent fiber (NDF) and raffinose family oligosaccharides (RFOs) (25.4% *vs.* 9.61% and 7.98% *vs.* 5.91%). The dietary 20% lupine seeds increased the content of NDF and RFOs in the ration, from 10.5% to 12.54% and from 0.77% to 2.08%, respectively. The final body weights of layers, and the number and weight of eggs laid were similar in all groups. In comparison with group C, group L_{20} hens were characterized by a lower dry matter concentration of digesta ($P = 0.024$), and higher activity of mucosal sucrase in the small intestine ($P = 0.006$). The following differences were noted between groups with respect to the counts of bacteria identified in the cecal digesta: total bacterial counts—$C < L_{10}$ and L_{20} ($P = 0.004$), *Lactobacillus*/*Enterococcus*—$C < L_{20}$ ($P = 0.014$), *Bifidobacterium* sp..—$C < L_{10} < L_{20}$ ($P = 0.001$), *Escherichia coli*—$C > L_{10} > L_{20}$ ($P = 0.001$). Group L_{20} birds, compared with group C, had higher activity levels of cecal bacterial glycolytic enzymes, α- and β-glucosidase, α- and β-galactosidase and α-arabinopyranosidase ($P = 0.001$ in all cases), and an increase close to significance ($P = 0.105$) in the cecal pool of short-chain fatty acids. Cecal ammonia concentrations were lower in both lupine-fed groups than in control group ($P = 0.004$), and digesta pH was lower in group L_{20} than in the other two groups ($P = 0.001$). The results of our study indicate that the inclusion of blue lupine seeds at 20% of the layer diet, and to lower extent at the 10% dietary dosage, had a positive effect on the cecal microbiota. In regard to cecal microbiota composition, the shift toward higher percentage of potentially beneficial bacteria groups (*Bifidobacterium* sp., *Lactobacillus* and/or *Enterococcus*) was manifested upon 20% of lupine seeds in a diet and both dietary lupine levels decreased the percentage of the potential pathogenic bacteria *Bacteroides*, *Prevotella* and *E. coli*. The above positive changes in the composition of cecal microbiota might prevent a deterioration in the laying performance of hens fed diets supplemented with lupine seeds which considerably increased the content of NDF and RFOs in the ration.

Abbreviations: ADF, acid detergent fiber; BW, body weight; C, control diet containing soybean meal; CP, crude protein; DM, dry matter; FCR, feed conversion ratio; GIT, gastrointestinal tract; L_{10}, 10% blue lupine seed meal used as a substitute for soybean meal in the daily ration; L_{20}, 20% blue lupine seed meal used as a substitute for soybean meal in the daily ration; NDF, neutral detergent fiber; NSP, non-starch polysaccharide; RFO, raffinose family oligosaccharide; SCFA, short-chain fatty acid; SEM, standard error of the mean.

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1. Introduction

Soybean meal >is the most common vegetable protein source in poultry diets. Therefore, the cost and availability of soybeans, in particular conventional non-GMO soybeans, have a significant effect on the economics of the global poultry production (Jezierny et al., 2010). The world's total area under non-genetically modified soybeans has been gradually decreasing with expanding the area under transgenic varieties (James, 2011). Other by-products of vegetable oil extraction, such as sunflower meal (Jankowski et al., 2011) and rapeseed meal (Mikulski et al., 2012), have also been considered as alternative protein sources in poultry diets. Attempts have also been made to use low-alkaloid varieties of yellow, white and blue lupines as substitutes for soybean meal in animal nutrition. Relatively high level of quinolizidine alkaloids in traditional high-alkaloid lupine varieties has been recognized to be responsible for poor feed utilization (Zdunczyk et al., 1998).

The seed yield of low-alkaloid blue lupine varieties has reached 30 dt/ha, and it is substantially higher than the seed yield of low-alkaloid yellow lupine varieties (Dolata and Wiartr, 2008). The alkaloid content of seeds is 290–390 mg/kg and 100–170 mg/kg in blue lupine and yellow lupine, respectively. The relatively high alkaloid content of blue lupine seeds is undesirable since monogastric animals, in particular young pigs, have lower tolerance to the alkaloids of blue lupine (including lupanine) than to the alkaloids of yellow lupine (including gramine) (Gdala et al., 1996). A high alkaloid content of Polish blue lupine varieties is the major factor limiting the use of lupine seeds in animal diets. Lupines with a lower alkaloid content have been tested in other experiments conducted in Europe (Van Barneveld, 1999; Jezierny et al., 2011).

Previous research findings, reviewed by Van Barneveld (1999), show that the optimum production of laying hens could be maintained with the use of between 10% and 20% lupine grain/kg diet, provided that amino acid supplements were included in the ration. In a study by Perez-Maldonado et al. (1999), the inclusion of lupine seeds at 25% of layer diets supported good performance (above 90%). The same amount of lupine seeds in organic layer diets, without the addition of amino acids, decreased egg production and egg quality (Hammershøj and Steenfeldt, 2005).

The levels of tryptophan, lysine and threonine in cereal–lupine diets are insufficient to meet the amino acid requirements of poultry (Nalle et al., 2011). Lupine seeds can be used as a protein source in diets supplemented with crystalline amino acids. Research carried our in the last decade of the 20th century revealed that apart from a relatively high protein content, lupine seeds contain high concentrations of non-starch polysaccharides (NSPs) that reduce their nutritional value (Gdala and Buraczewska, 1996; Van Barneveld, 1999). According to Nalle et al. (2011), narrow-leafed lupines are a good source of protein, but a poor source of metabolizable energy and sulfur-containing amino acids.

High levels of water-soluble NSPs are closely correlated with the viscosity of small intestinal digesta, thus affecting the rate of food passage (Hetland and Svihus, 2001), microbial growth and GIT health (Santos et al., 2006). The results of some experiments indicate that dietary NSPs significantly increase the intestinal populations of pathogenic bacteria at the expense of beneficial microbiota (Langhout, 1999). The findings of other authors (Persia et al., 2002; Lan, 2004) show that dietary fiber is beneficial to intestinal microbial ecology, and may suppress the colonization of enteric pathogens that adversely affect the health and welfare of animals. In view of the above, evaluations of the effects of grain legumes in poultry diets should account for a potential influence of increased NSP concentrations on the intestinal ecosystem.

The objective of this study was to determine whether different inclusion levels of blue lupine seeds (0%, 10% and 20%) in layer diets induce significant changes in the counts and enzymatic activity of intestinal microbiota and affect laying performance.

2. Materials and methods

2.1. Birds and housing

The study was conducted at the Department of Poultry Science, University of Warmia and Mazury, Olsztyn, Poland. The protocol for this study was approved by the local Animal Experimentation Ethics Committee.

A total of 150 eighteen-week-old Lohmann Brown laying hens were used in a feeding trial. Before the experiment, all birds were weighed individually and were placed in individual cages (30 × 60 cm) where they were kept during the first 20 weeks of the laying period (from 18 to 38 weeks of age). The replicates were equally distributed among three cage levels to minimize the cage level effect. Room temperature was set at 28 °C on the day of placement, and it was subsequently reduced by 2 °C per week. Air exchange was maintained at 0.4–0.5 m^3/h/kg of body weight (BW). The hens had free access to water and diets in meal form. At the termination of the experiment, the hens were weighed and selected birds were sacrificed by cervical dislocation to retrieve the test material.

2.2. Experimental design and diets

Laying hens were randomly assigned to three dietary treatments (50 replicates in each, 1 hen = 1 replicate), and they were fed diets whose composition and calculated nutritional value are given in Table 1. Iso-protein and iso-energetic diets contained similar concentrations of amino acids, including arginine, lysine and methionine, and similar levels of minerals, including calcium and available phosphorus.

Feed mixtures were prepared in the "Agrocentrum" feed mill in Kaleczyn, Poland. The main cereal grain in layer diets was wheat (at 54.5%, 49.4% and 40.3% in diets C, L_{10} and L_{20}). The inclusion levels of triticale (10%) and rapeseed meal (4%)