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Effect of Dietary Lupin (*Lupinus albus*) on the Gastrointestinal Microbiota Composition in Broiler Chickens and Ducks

Martina Geigerová[1], Roman Švejstil[1], Eva Skřivanová[1], Eva Straková[2], Pavel Suchý[3]*

[1]Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic

[2]Department of Animal Nutrition, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Science Brno, Brno, Czech Republic

[3]Department of Animal Husbandry and Animal Hygiene, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Science Brno, Brno, Czech Republic

**Corresponding author: skrivanovae@af.czu.cz*

ABSTRACT

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The purpose of the study was to evaluate the amount of raffinose-series oligosaccharides (RSO) in soybean meal (SBM), whole white lupin seed meal (WLM), sunflower meal (SFM), and rapeseed oil meal (ROM) and to determine whether partial or complete dietary WLM replacement affected the numbers of bacteria in selected groups in the microbiota of broiler chickens and ducks without inducing any weight loss. Total counts of anaerobes, lactobacilli, bifidobacteria, and *Escherichia coli* in caecal samples from both ducks and broiler chickens, as well as in a crop chyme, in broiler chickens, were determined. Live weights before slaughter were determined. Both broiler chickens and ducks were fed a control diet with SBM (L_0) or diet containing 50% or 100% WLM as a substitute for SBM (groups L_{50} and L_{100}, respectively). In comparison with SBM, WLM contained significantly higher amounts of RSO, and the amounts of oligosaccharides in SFM (1.73 ± 0.26 g/100 g) and ROM (1.79 ± 0.14 g/100 g) were negligible compared to those in WLM (8.26 ± 0.14 g/100 g) and SBM (6.96 ± 0.21 g/100 g). The inclusion of lupin in chicken diets did not significantly affect the monitored bacterial groups in crop chyme, but a complete replacement of SBM with WLM (L_{100} group) in chicken diets significantly ($P \leq 0.05$) increased the counts of lactobacilli in caecal samples. Partial (L_{50} group) and complete (L_{100} group) lupin supplementation in the duck diet significantly ($P \leq 0.05$) increased counts of lactobacilli and bifidobacteria by at least one order of magnitude. *E. coli* counts in poultry were not affected by changes in diet. The results of our study indicate that partial dietary replacement of SBM with WLM did not significantly affect the live weight of broiler chickens and ducks, but that complete replacement of SBM with WLM may lead to weight loss.

Keywords: bacterium; poultry; prebiotic; white lupin; raffinose-series oligosaccharides

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From an ecological point of view, animal production and the nutrition feed required for these animals, represent an undeniable environmental burden. Therefore, there is an effort, especially in European countries, to select locally sourced feed ingredients, if possible. In the last decade, soybeans became the most common source of vegetable protein in monogastric animal diets (Chaudhary et al. 2015; Heger et al. 2016); however, a majority of soybeans are imported from overseas. In addition to reducing the need for these imports, feeds based on non-genetically modified plants are currently desirable in developed countries (Frewer et al. 2013). Potential alternative sources of dietary protein can be obtained from by-products of vegetable oil extracted from sunflower or rapeseed meal (Dadalt et al. 2016; Liermann et al. 2016). Other protein sources include a pea protein isolate and potato or corn protein concentrate (Froidmont et al. 2009; Wiltafsky et al. 2009; Dadalt et al. 2016). However, there are certain nutritional limitations (e.g., alkaloid content, trypsin inhibitors, and tannins) which must be considered. Based on these requirements, low-alkaloid varieties of sweet lupin (*Lupinus albus*, *Lupinus angustifolius*, *Lupinus luteus*) are considered promising for use in animal feed. Its advantage is that lupin can be used to completely replace soybean meal (Zraly et al. 2008; Hernandez and Roman 2016). The use of lupin as an alternative source of vegetable protein for the production of animal feed is increasing rapidly. There are many studies analyzing the impact of replacing soya with lupin in animal diets. Many authors confirmed that lupin is a suitable protein component for use in animal feed, based on production parameters and nutrient digestibility in animals (Zraly et al. 2008; Volek and Marounek 2009; Zdunczyk et al. 2016; Zwolinski et al. 2017). In addition to proteins, important growth-promoting factors in lupin seeds include their significant amounts of saccharides, including raffinose-series oligosaccharides (RSO). These oligosaccharides are not digested in the upper gastrointestinal tract (GIT) of monogastric animals. Without changing their structures, they pass to the intestine, where are fermented by gut microbiota to produce short-chain fatty acids and gas. This can lead to flatulence and abdominal discomfort (Guillon and Champ 2002). However, RSO have been identified as prebiotic agents. In *in vitro* studies, particularly, they have been shown to promote the growth of health-promoting bacteria such as bifidobacteria and inhibit the growth of *Escherichia coli* in the gut (Hernandez-Hernandez et al. 2011; Wongputtisin et al. 2015). Therefore, the aim of our study was to determine the amounts of oligosaccharides in selected meals serving as a potential source of protein and to assess whether the inclusion of *Lupinus albus* instead of soya in broiler chicken and duck diets could induce changes in selected bacterial groups.

MATERIAL AND METHODS

Quantitative determination of raffinose series oligosaccharides in experimental meals. Four meals were selected as a potential source of protein in animal nutrition including soybean meal (SBM), white lupin seed meal (WLM), sunflower meal (SFM), and rapeseed oil meal (ROM). The amount of RSO was determined by an enzymatic method – the Megazyme Raffinose/Sucrose/Glucose Assay Kit (Megazyme International, Ireland) using α-galactosidase and invertase was applied according manufacturer's instructions. The method does not distinguish between raffinose, stachyose and verbascose; their quantities were measured as a group. Three replicates were used to determine the amounts of RSO per meal.

Birds and housing. The study was conducted at an accredited experimental barn of the Department of Animal Nutrition at the University of Veterinary and Pharmaceutical Sciences Brno. The protocol for this study was approved by the local ethic committee.

In this study, a total of 240 one-day-old broiler chickens (ROSS® 308) and 180 one-day-old ducks (Cherry Valley) were purchased from International Poultry Testing MTD Ústrašice, Czech Republic. Animals were placed in pens with deep litter, and each experimental group was maintained separately. For broiler chickens and ducks, a 23 : 1 h light : darkness lighting regime was used throughout the experiment. The temperature was set at 21–31°C for broiler chickens and 8–30°C for ducks, depending on their ages.

Experimental design and diets. Broiler chickens were randomly assigned to three dietary treatments (80 replicates each), and each treated group was divided by sex for 40 males and 40 females. During the study period, broiler chickens were fed

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Table 1. Selected nutrients of experimental diets (g/kg, dry matter) for broiler chickens containing a different amount of white lupin seed meal

Nutrition value	1–14 days			15–29 days			30–35 days		
	L_0	L_{50}	L_{100}	L_0	L_{50}	L_{100}	L_0	L_{50}	L_{100}
Crude protein	254.5	265.1	248.7	226.9	217.3	223.3	205.1	215.5	203.6
Fat	63.9	57.7	60.5	66.4	68.1	69.8	81.4	66.5	80.9
Crude fibre	24.3	35.1	52.3	24.9	49.4	57.5	36.3	35.9	45.2

L_0 = diet with soybean meal (SBM), L_{50} = diet containing 50% of white lupin seed meal (WLM) as a substitute for SBM, L_{100} = diet with WLM

a control diet based on SBM (L_0) or one of two diets containing 50% or 100% WLM as a substitute for SBM (groups L_{50} and L_{100}, respectively). The chickens were fed for three experimental periods over 35 days (i.e. days 1–14, 15–29, and 30–35). The composition and calculated nutritional values of these diets are shown in Table 1. Analogously, ducks were separated by dietary treatment and sex into six groups (30 ducks per group) and were fed diets containing SBM meal as a control or diets with 50% or 100% WLM as a replacement for SBM (groups L_0, L_{50}, and L_{100}, respectively). The ducks were fed for 40 days in four periods (i.e. days 1–10, 11–19, 20–35, and 36–40). The composition of their diets is presented in Table 2.

The control feed mixture was prepared by ZZN Pelhřimov, Czech Republic, and the test feed mixtures containing whole white lupin seed were prepared by MTD Ústrašice, Czech Republic. The poultry had free access to water and feed mixtures and were fed through feeder drop tubes *ad libitum*. At the end of the experiment, the poultry were weighed. To monitor intestinal bacteria, 18 broiler chickens and 18 ducks (6 birds per group) from each treatment group (L_0, L_{50}, and L_{100}) were randomly selected. Immediately after slaughter, samples from caeca from both kinds of birds and crop chyme from broiler chickens were collected directly into tubes containing Wilkins-Chalgren broth (Oxoid, UK). Samples were kept on ice until the microbiological analysis.

Microbiological analysis. Counts of total anaerobic bacteria, bifidobacteria, lactobacilli, and *Escherichia coli* were determined by cultivation. The obtained samples were homogenized and serially diluted in Wilkins-Chalgren broth (Oxoid) under anaerobic conditions. Wilkins-Chalgren agar (50 g/l; Oxoid) supplemented with soya peptone (5 g/l; Oxoid), L-cystein (0.5 g/l; Sigma-Aldrich, USA), and Tween 80 (1 ml/l; Sigma-Aldrich) was used for enumeration of total anaerobic bacteria. Bifidobacteria were enumerated on the same agar as total anaerobes with the addition of glacial acetic acid (1 ml/l) and the antibiotic mupirocin (100 mg/l; Oxoid), according to a method reported by Rada and Petr (2000). These plates were incubated in anaerobic jars (Anaerobic Plus System, Oxoid) at 37°C for 72 h. To enumerate lactobacilli, Rogosa agar (82 g/l; Oxoid) adjusted to pH 5.4 ± 0.2 with glacial acetic acid was used. Lactobacilli were cultivated for 72 h under micro-aerophilic conditions using the double-layered pour-plate method. Counts of *E. coli* were determined using TBX-agar (Oxoid), with plates incubated aerobically at 37°C for 24 h.

Statistical analysis. The amounts of RSO in meals, live weight, and bacteria enumeration were analyzed statistically using STATISTICA software

Table 2. Selected nutrients of experimental diets (g/kg, dry matter) for ducks containing a different amount of white lupin seed meal

Nutrition value	1–10 days			11–19 days			20–35 days			36–40 days		
	L_0	L_{50}	L_{100}	L_0	L_{50}	L_{100}	L_0	L_{50}	L_{100}	L_0	L_{50}	L_{100}
Crude protein	238.2	247.9	265.8	213.7	214.5	229.8	180.1	182.8	175.8	178.7	170.4	180.9
Fat	39.5	42.9	57.7	37.9	44.5	55.5	37.6	43.0	52.4	43.3	46.3	49.4
Crude fibre	26.1	48.3	53.3	30.3	38.0	63.5	31.4	39.3	46.1	36.1	35.1	37.7

L_0 = diet with soybean meal (SBM), L_{50} = diet containing 50% of white lupin seed meal (WLM) as a substitute for SBM, L_{100} = diet with WLM

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(Version 12.0, 2013). Amounts of RSO and numbers of bacteria are presented as mean values ± standard deviations (SD). Live weights are presented as mean values with pooled standard errors of the mean (SEM). A one-way analysis of variance (ANOVA) was performed to determine whether values differed among the treatment groups, and $P \leq 0.05$ was considered statistically significant. Prior to the statistical analysis data were checked for normality (Shapiro-Wilk test).

RESULTS

Amounts of RSO in experimental meals. The quantities of RSO in experimental meals are shown in Table 3. The amounts of oligosaccharides found in meals ranged from 1.73 to 8.26 g/100 g. Relatively low amounts of RSO were found in SFM and ROM compared to those in WLM and SBM. WLM contained the highest amount of RSO of all the tested meals.

Growth performance. The live weights of broiler chickens and ducks were determined. The final body weights of broiler chickens are shown in Table 4. No statistical differences were found in the live weights of male broiler chickens fed different diets. The means of live weights of broiler chickens in the L_0 group and L_{50} group were almost identical. The average live weight of female broiler chickens in the L_{100} group (2.19 kg) was significantly lower than that in the L_{50} group (2.33 kg), but no statistical difference was found between live weights in the L_0 and L_{100} groups. Complete lupin replacement in duck diets also negatively affected their final body weights (Table 5). Significant differences in live weights were found between the L_{100} group and L_{50} group of female ducks, and between the L_{100} group and L_{50} group of male ducks, and moreover between the L_{100} group and L_0 group of male ducks.

Table 3. The amount of raffinose series oligosaccharides (RSO) (g/100 g) in soybean meal (SBM), white lupin seed meal (WLM), sunflower meal (SFM), and rapeseed oil meal (ROM)

Meal	Amount of RSO
SBM	6.96 ± 0.21^{A}
WLM	8.26 ± 0.14^{B}
SFM	1.73 ± 0.26^{C}
ROM	1.79 ± 0.14^{C}

$^{A–C}$means with different superscripts significantly differ ($P \leq 0.05$)

Table 4. Live weight (kg) before slaughter of broiler chickens fed diets based on soybean meal (SBM) and/or white lupin seed meal (WLM)

	L_0	L_{50}	L_{100}	SEM
Male	2.53^{A}	2.55^{A}	2.46^{A}	0.32
Female	2.30^{AB}	2.33^{A}	2.19^{B}	0.23

L_0 = diet with SBM, L_{50} = diet containing 50% of WLM as a substitute for SBM, L_{100} = diet with WLM, SEM = standard error of the means

^{A,B}means in the same row with different superscripts significantly differ ($P \leq 0.05$)

Bacteria enumeration. Counts of selected bacterial groups in caecum and crop samples collected from broiler chickens are shown in Table 6. The average numbers of total anaerobic bacteria, bifidobacteria, lactobacilli, and *E. coli* isolated from crop chyme in all three experimental groups were not significantly different. The amount of lupin in diets did not affect the number of these bacteria. Although statistically significant differences were not found, counts of bifidobacteria and lactobacilli were the highest in the group in which soya was completely replaced with lupin. Conversely, in the same group (L_{100}), the counts of *E.coli* were the lowest. In the caeca of broiler chickens, lactobacilli counts were significantly higher in the L_{100} group than in the L_{50} and L_0 groups. This is the only statistically significant difference that was found in the faecal microbiota of broiler chickens. The highest counts of bifidobacteria as well as *E. coli* were detected in the L_{50} group.

Considerably higher bacterial diversity was observed in the faecal microbiota of ducks than that of chickens (Table 7). Bifidobacteria and lactobacilli

Table 5. Live weight (kg) before slaughter of ducks fed diets based on soybean meal (SBM) and/or white lupin seed meal (WLM)

	L_0	L_{50}	L_{100}	SEM
Male	3.26^{A}	3.14^{A}	2.94^{B}	0.31
Female	3.11^{AB}	3.19^{A}	2.98^{B}	0.25

L_0 = diet with SBM, L_{50} = diet containing 50% of WLM as a substitute for SBM, L_{100} = diet with WLM, SEM = standard error of the means

^{A,B}means in the same row with different superscripts significantly differ ($P \leq 0.05$)

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Table 6. Bacterial counts (log CFU/g ± SD, *n* = 6) in caecal samples and crop chyme of broiler chickens fed diets based on different amounts of white lupin seed meal (WLM)

		Total anaerobes	Bifidobacteria	Lactobacilli	*E. coli*
Caecum	L_0	9.33 ± 0.38^A	8.34 ± 1.34^A	8.76 ± 0.68^A	7.99 ± 0.39^A
	L_{50}	9.56 ± 0.27^A	9.14 ± 0.27^A	8.21 ± 0.68^A	8.57 ± 0.65^A
	L_{100}	9.77 ± 0.29^A	8.95 ± 0.46^A	9.55 ± 0.49^B	8.22 ± 0.23^A
Crop	L_0	8.72 ± 0.54^A	5.66 ± 0.37^A	8.28 ± 0.51^A	6.20 ± 1.14^A
	L_{50}	8.31 ± 0.54^A	5.42 ± 0.56^A	8.04 ± 0.69^A	6.29 ± 1.38^A
	L_{100}	9.21 ± 0.56^A	6.29 ± 1.24^A	8.93 ± 0.69^A	5.44 ± 0.50^A

L_0 = diet with soybean meal (SBM), L_{50} = diet containing 50% of white lupin seed meal (WLM) as a substitute for SBM, L_{100} = diet with WLM

^{A,B}means with different superscripts in columns from the same type of sample significantly differ ($P \leq 0.05$)

counts were significantly higher in both experimental groups in which soya was replaced with lupin (L_{50} and L_{100}), as compared to the control (L_0) group. The numbers of bifidobacteria in the L_{50} and L_{100} groups were higher by at least one order of magnitude. The number of lactobacilli in the L_{50} group was higher by two orders of magnitude. No statistically significant differences were found among counts of total anaerobic bacteria, and the amounts of *E. coli* were approximately equal in all three groups.

DISCUSSION

Members of the raffinose family of oligosaccharides are present in various plant sources (Andersen et al. 2005). High amounts of RSOs are mainly found in legumes, and their levels in seeds vary by species and based on environmental factors (Martiinez-Villaluenga et al. 2005). The aforementioned oligosaccharides are not digested by the monogastric animals, and are therefore available for bacterial fermentation to produce short-chain fatty acids and gas (Guillon and Champ 2002). High levels of oligosaccharides, in particular α-galactosides, can by certain means be even considered as antinutritional factors, as their fermentation in monogastric animals can lead to the increased fluid retention, hydrogen production, and can impair the utilization of nutrients (Saini and Gladstones 1986). Therefore, the particular source and concentration of these compounds should always be tested *in vivo*.

Generally, all lupin species are good sources of RSOs and can be used for the isolation of oligosaccharides. According to Martiinez-Villaluenga et al. (2005), white lupin seeds contain RSO amounts ranging from 5.46 to 8.51% dry matter (DM). In our experiment, WLM contained comparatively high levels of RSO (8.26 ± 0.14 g/100 g). High amounts of RSOs were also found in SBM, but these levels were lower than those in lupin, which corroborates the findings of other authors (Kumar et al. 2010; Svejstil et al. 2015). According to Zdunczyk et al. (2014), the inclusion of blue lupin seeds (20%) to a layer diet can increase the RSO content (from 0.77 to 2.08% DM). The amount of oligosaccharides in SFM and ROM was similar, and RSO contents in these meals were negligible compared to those with WLM and SBM.

Table 7. Bacterial counts (log CFU/g ± SD, *n* = 6) in caecal samples of ducks fed diets based on different amounts of white lupin seed meal (WLM)

		Total anaerobes	Bifidobacteria	Lactobacilli	*E. coli*
Caecum	L_0	9.54 ± 0.45^A	6.93 ± 0.74^A	4.53 ± 0.40^A	7.21 ± 0.29^A
	L_{50}	9.96 ± 0.42^A	8.55 ± 0.44^B	6.55 ± 0.98^B	6.97 ± 0.86^A
	L_{100}	9.93 ± 0.32^A	8.15 ± 0.53^B	6.06 ± 0.46^B	7.13 ± 0.28^A

L_0 = diet with SBM, L_{50} = diet containing 50% of WLM as a substitute for SBM, L_{100} = diet with WLM

^{A,B}means with different superscripts in columns significantly differ ($P \leq 0.05$)

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The commensal microbial community plays a major role in poultry health and digestion and its composition can be influenced by diet. Currently, there is limited information available in the literature on whether crop microbial composition can be affected by feed. The crop is the first major defence against pathogens in broiler chickens. One of the barriers of the crop against pathogens is an acidic pH. A lower pH can be promoted by lactic acid fermentation performed by lactobacilli. Lactobacilli are the dominant bacterial group in the crops of broiler chickens (Kieronczyk et al. 2016), as shown also in our results. The numbers of lactobacilli in crops were similar to the numbers of total anaerobic bacteria in all three groups (L_0, L_{50}, and L_{100}). Besides lactobacilli, among the health-promoting bacteria belong also bifidobacteria. In our study, bifidobacteria were found at approximately 10^6 CFU/g in poultry crops, which was a lower order of magnitude than described by Petr and Rada (2001). The highest numbers of lactobacilli and bifidobacteria were found in the L_{100} group, relative to those in the L_{50} and L_0 groups, but these differences were not significant. *E. coli* counts in the crops of broiler chickens were not affected by diet. Undigested oligosaccharides in the upper part of the GIT are fermented in the intestines of birds by the gut microbiota (Patterson and Burkholder 2003). The presence of RSO in diets may result in increased numbers of bacteria in certain populations (Jozefiak et al. 2004). Higher counts of total anaerobes in the caeca of broiler chickens and ducks were found in the L_{100} group, relative to that in the L_0 group. However, these differences were not significant because both diets contained some RSO. Dietary RSO has been shown to increase the numbers of lactic acid bacteria, as well as to increase visible bacteria attached to cell walls in the caecum (Lan et al. 2007). Complete replacement of SBM with WLM in the diets of broiler chickens affected the numbers of lactobacilli in caeca samples; however, the other investigated bacterial groups were not affected. Similarly, differences in the composition of duck diets positively affected lactobacilli and bifidobacteria counts. The inclusion of whole white lupin seeds in the experimental diets caused appropriate changes in the amounts of probiotic bacteria. Increased numbers of bifidobacteria and lactobacilli can have a positive effect on poultry by regulating the intestinal microbial balance (Buclaw 2016). Similar results were described by Zdunczyk et al. (2014), who observed increased counts of bifidobacteria and lactobacilli in laying hens fed a diet supplemented with 20% blue lupin seeds. In contrast, the addition of yellow lupin seed meal to the feed of turkeys did not increase the numbers of lactobacilli (Zdunczyk et al. 2016). *E. coli* is a common intestinal bacterium, and most of strains are commensal; however, some strains can cause disease. The counts of *E. coli* in faecal samples of both types of poultry were not affected by differences in the composition of diets.

In addition to monitoring quantitative changes in selected bacterial groups, final body weights were determined. As suggested in the introduction, the replacement of SBM with lupin meal in the diets of various monogastric animals, including rabbits, turkeys, chickens, and pigs, does not necessarily reduce weight gain (Wu et al. 2004; Zraly et al. 2008; Volek and Marounek 2009; Zdunczyk et al. 2016). However, there have been some reports of weight loss with this replacement (Olkowski et al. 2005; Smulikowska et al. 2014). Our results showed that partial inclusion of lupin in diets did not significantly affect the body weights of broiler chickens or ducks, but that complete replacement of SBM with WLM reduced their live weights.

CONCLUSION

The present study shows that WLM contains higher levels of RSO than SBM, and supplementation of diets had a positive influence on the intestinal microbiota composition of broiler chickens and ducks. Partial and complete replacement of SBM with lupin in duck diets significantly increased counts of lactobacilli and bifidobacteria. Further, a significant increase in the numbers of lactobacilli in broiler chicken caecum was observed only when SBM was fully replaced with WLM. The obtained data showed that a diet containing 50% whole white lupin had a positive effect on the composition of the intestinal microbiota in ducks, and that this addition had neither negative nor positive effects on the live weights of ducks and broiler chickens.

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