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Effects of increased dietary inclusion of yellow lupins and enzyme supplementation on performance, ileal digestibility of nutrients and microbial status of large intestine in broiler chickens

Auswirkungen steigender Anteile an gelben Lupinen und eines Enzymzusatzes im Futter auf die Mastleistung, die ileale Verdaulichkeit der Nährstoffe und den mikrobiellen Status im Dickdarm bei Broilern

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Introduction

Lupin seeds, especially from the "sweet" varieties, such as yellow lupins (*Lupinus luteus* L.) are important sources of good quality protein for non-ruminant animals, but they contain also relatively large amounts of non-starch polysaccharides (NSP) that are characterized by their anti-nutritional effect. The anti-nutritive activity of NSP is well known. It is recognized that these polysaccharides depress the digestion of starch, protein and lipids in the digestive tract of broiler chickens (Ward and Marquardt, 1987; Van Der Klis et al., 1993; Jamroz and Piech-Schleicher, 1982). The average concentration of NSP varied from 270 to 350 g/kg dry matter (DM) in yellow lupin seed. About 5-10% of them constitutes water-soluble fraction (Gatel, 1994; Gdala and Buraczewska, 1996; Evans et al., 1993).

Lupins contain about 8% in DM of certain oligosaccharides, especially raffinose, stachyose, and verbascose (Gatel, 1994). Oligosaccharides of the raffinose family are not digested due to the lack of alpha-1,6-galactosidase capable of hydrolyzing the alpha-1,6-galactosidic linkages in the intestinal mucosa. As a consequence, these saccharides are not absorbed into the blood stream and are metabolized by the microbes (mainly *Clostridia*) in the hindgut. This process results in production of large amounts of carbon dioxide and hydrogen, which are contributory factors to the flatulence problem (Nowak and Steinkraus, 1988).

Utilization of lupin seeds as a protein source in monogastric animal diets has been limited depending on the content of toxic alkaloids. However, the development of low-alkaloid cultivars of lupins has expanded the potential use of lupin seeds in poultry diets. The suitability of various levels of lupin seeds in broiler diets were evaluated in several studies, but there is no consensus with respect to the maximum level of lupins that can be included in broiler rations. Anti-nutritive effects of high levels of lupins in the diet were described to decrease feed consumption and to reduce growth rate (Brenes et al., 1993; Olver and Jonker, 1997). Constraints on the level of inclusion of lupins in broiler diets may be due to anti-nutritive activity of NSP and alkaloids as well, but some fructooligosaccharides of legume plants could have prebiotic properties (Bailey et al., 1991; Choi et al., 1994).

A way to counteract possible anti-nutritive effect is supplementing diets with enzymes (carbohydrases) or application of chemical or physical methods - dehulling, autoclaving etc. (Richter et al., 2001; Flis et al., 1996).

The purpose of this study was to examine whether addition of commercial carbohydrase enzymes to the wheat-barley rich diets could have a positive effect also when simultaneously different levels of yellow lupins will be used in the diet. The performance, carcass characteristics, ileal digestibility of nutrients and amino acids and content of selected bacteria in the large intestine were determined as the basic parameters.

Material and methods

Animals and feeding

The experiment was carried out with "Ross 308" broiler hybrids. A total of 1,200 one-day old chickens (sex ratio = 1:1) with an average body weight of 36 g were randomly divided into six dietary treatments, each comprising 200 birds. Each treatment was replicated with four replication groups of 50 animals. The chickens were kept on wood shavings litter. The environmental temperature inside the room was gradually reduced from 29-30°C to 21°C. The lighting program was as follows: 24 hours light until 10 days of age, and later 19 hours light and 5 hours darkness.

The diets were based on cereal grains (barley 25/30% and wheat 31/35%) and contained yellow lupins, var. Juno (0.8 g/kg DM alkaloids) at amounts of 0, 5, 10% in the starter and 0, 10, 20% in the grower diets growth trial (for composition see Table 1). These diets rich in barley and wheat (in total 56-61%) were fed either without or with the addition of the enzyme preparation Ronozyme VP* at the inclusion level of 400 mg/kg. Enzyme supplementation was confirmed by in-feed analyses. The used premixes did not contain any feed antibiotic, but the anti-

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*Ronozyme VP, enzyme product marketed by DSM Nutritional Products, contains an enzyme complex derived from Aspergillus aculeatus (CBS58994), consisting of endo-1,3:1,4-beta-glucanase (EC 3.2.1.6) min. 50 FBG (Fungal beta glucanase units)/g, and other pentosanase and hemicellulase activities.

Table 1. Composition of the experimental diets
Zusammensetzung der Futterrationen

Diets	Diets					
	Starter			Grower		
	without lupins	5% lupins	10% lupins	without lupins	10% lupins	20% lupins
Feed ingredients (%)						
Barley	25.0	25.0	25.0	30.0	30.0	30.0
Wheat	33.3	32.2	31.1	35.7	33.4	31.1
Soyabean oil meal	31.3	27.1	23.0	25.1	16.9	8.7
Lupins var. Juno	-	5.0	10.0	-	10.0	20.0
Soya oil	6.5	6.7	7.0	5.6	6.1	6.6
Dicalcium phosphate	1.73	1.75	1.77	1.45	1.48	1.51
Limestone	0.58	0.57	0.55	0.62	0.62	0.59
Salt	0.30	0.30	0.31	0.36	0.37	0.37
Premix for broilers without growth promoters	1.00	1.00	1.00	1.00	1.00	1.00
DL - methionine	0.196	0.207	0.218	0.156	0.179	0.201
L - lysine	0.102	0.099	0.097	0.012	0.007	0.002
Metabolizable energy (MJ/kg)[2]	12.54	12.51	12.48	12.47	12.52	12.45
Estimated nutrients						
Crude protein (g/kg)	215.9	214.7	214.2	194.3	195.2	195.0
Crude fibre (g/kg)	45.3	50.3	55.3	47.5	57.5	67.6
Calcium (g/kg)	9.34	9.38	9.38	9.03	9.01	9.02
Available phoshorus (g/kg)[3]	4.37	4.38	4.37	3.90	3.91	3.90
Sodium (g/kg)	1.38	1.37	1.38	1.59	1.58	1.60
Polysaccharides and their constituents (g/kg)						
Arabinose	21.9	2.7	19.5	22.4	19.9	17.4
Xylose	31.8	30.7	29.5	34.3	31.9	29.6
Galactose	13.1	11.6	20.1	11.1	28.1	30.0
Non-starch polysaccharides – total	135.4	126.3	117.3	134.3	116.1	98.0
Soluble NSP	36.5	32.9	31.4	35.9	30.8	25.8
Insoluble NSP	66.4	62.3	58.2	66.8	58.7	50.5
Beta-glucans	11.7	11.6	11.5	13.7	13.5	13.4

[1] supplied per kg of diet (mg): retinylpalmitate 5.5; cholecalciferol 0.05; dl-α-tocopheryl-acetate 20; menadion 3; thiamin 2.5; riboflavin 4.5; pyridoxine 4; cyanocobalamin 0.015; nicotinic acid 25; Ca-pantothenate 8; folic acid 1.2; choline chloride 450; Mn 74; Fe 30; Zn 45; Cu 4; Co 0.4; I 0.3; diclazuril; DL-methionine 1.0.
[2] calculated from the estimated chemical composition of the diets and according to formula of European Tables of Energy Values of Feeds for Poultry WPSA,1989
[3] calculated on basis of Requirement of poultry for nutrients,1996

coccidial Diclazuril was used. The experimental diets in meal form were provided *ad libitum* (days 1-21: starter diet; days 22-42: grower diet). The concentration of crude protein reached 21.5% in the starter and 19.5% in the grower diets (Table 1).

Analytical methods

The proximate chemical analysis in components (for optimalization) and next in the whole diets were conducted according to standard methods (AOAC, 1990): the nitrogen content was determined by Kjeldahl-method using a Kjeltec 2300 Foss Tecator apparatus, crude protein by multiplying the N-content by 6.25, crude fat by ether extraction, crude fiber by the HENNEBERG-STOHMANN method using a Fibertec Tecator apparatus. Phosphorus was analyzed after previous mineralization by the ammonium vanadomolybdate method using a Specol 11 (Carl Zeiss Jena) spectrophotometer at a wave length of 470 nm. Calcium (Ca) and sodium (Na) were determined by atomic absorption spectrophotometry using AAS-3 EA-30 type apparatus (Carl Zeiss Jena).

For determination of amino acids in the diets the feed samples were hydrolyzed with 6N HCl for 22 hrs at 105°C, amino acids were separated using an Analysator 231 XL Gilson according to the MOORE-STEIN method (MOORE and STEIN, 1963). For determination of the sulphur containing amino acids the feed samples were oxidized (0°C, 24 hrs) with formic acid + hydrogen peroxide (H_2O_2) 9:1 before the HCl hydrolysis. Tryptophan was measured colorimetrically at a wave length of 590 nm after alkaline hydrolysis with lithium hydroxide (LiOH); 110°C, 16 hrs) and

Table 1a. Amino acid composition of experimental diets (g/kg)
Aminosäurenzusammensetzung der Futterrationen

Amino acids	Diets					
	Starter			Grower		
	without lupins	5% lupins	10% lupins	without lupins	10% lupins	20% lupins
Asp	19.36	19.24	19.13	16.81	16.59	16.36
Thr	7.96	7.77	7.59	7.15	6.78	6.40
Ser	8.61	8.73	8.84	7.62	7.86	8.09
Glu	39.17	40.45	41.73	36.58	39.12	41.68
Pro	19.21	18.30	17.39	17.69	15.87	14.05
Cys	3.82	3.96	4.10	3.72	4.00	4.27
Gly	8.22	8.20	8.17	7.45	7.40	7.36
Ala	8.75	8.61	8.48	7.90	7.64	7.37
Val	9.80	9.48	9.17	8.81	8.19	7.56
Met	4.63	4.61	4.64	4.11	4.10	4.07
Ileu	9.21	8.96	8.71	8.15	7.65	7.15
Leu	11.51	12.19	12.88	10.56	11.93	13.30
Thy	9.02	8.51	8.01	7.80	6.79	5.78
Phen	9.10	8.94	8.78	8.21	7.89	7.56
His	6.37	6.30	6.24	5.66	5.54	5.41
Lys	11.46	11.47	11.52	9.33	9.30	9.33
Arg	12.09	13.32	14.55	10.62	13.08	15.54
Try	2.59	2.45	2.31	2.25	1.97	1.69

The amino acid content was determined in compounds; lysine and methionine were optimalized in mixture and analitically confirmed

4-dimethyloaminobenzaldehyde (DMAB) according to LANDRY and DELHAYE (1992).

The energy value of the diets was calculated in components of diets on the basis of analytical values of nutrients content and regression formula according to the European Tables of Energy Values of Feeds for Poultry (WPSA, 1989) and next optimalized in the mixtures. NSP content was calculated on the basis of our earlier analysis of barley, wheat and lupins and data from BACH KNUDSEN (1997).

Performance

Body weight, feed intake and mortality of chickens were determined at day 1, 21 and 42. At the end of experiment 12 birds from each dietary treatment were selected for further evaluations of the carcass quality. Chickens were selected after weighing of all animals. Six ♀ and 6 ♂ were chosen on the basis of average body weight of birds in particular groups and then slaughtered after 12 hrs starvation and carcass (empty body) characteristics were determined.

Ileal digestibility

The apparent ileal digestibility of nutrients and amino acids was determined in the period between 21-28 days of life. During the adaptation period of 5 days, chromium oxide (Cr_2O_5, 5 g/kg feed) was added to the diets given to all animals in two replication-groups per each treatment group, each comprised 50 birds, as an indicator. On day 28, two hrs after feeding ten birds at the average body weight per group were randomly selected, killed (without plucking) and the gastrointestinal tracts were immediately from the warm carcass removed and the intestine prepared. The content of ileum (20 cm from and to ileo-caecal junction) was collected for the nutrients and amino acids analyses. In fresh material the nutrients and chromium was determined according to FENTON and FENTON (1979). The digestibility coefficients were calculated based on differences in relation between concentration of Cr and amino acids in the feed and intestinal content.

Microorganisms in the large intestine (rectum) content

From the animals killed for the digestibility determination the intestine was taken and the rectum content from each bird was collected separately using distilled water for *E. coli* and *Clostridium perfringens* assay. Ninety-nine ml of buffered peptone water was added 1:100 to one gram of fresh material and subsequent dilutions were prepared. *Clostridium perfringens* was cultured on TSC Agar Merck under anaerobic conditions at + 37°C for 24 hours. *Escherichia coli* was cultured on Chromocult Coliform Agar (Merck) under the same conditions. The presence of *E. coli* was determined using KOVAC'S preparation for indol estimation. *Lactobacillus* spp. were cultured on MRS at temperature of incubation of +30°C during 72 hrs.

Statistical analysis

All obtained data were evaluated statistically using SAS procedures for one- and two-factorial analysis of variance. Differences between treatment means were tested according to Duncan's multiple range test (DUNCAN, 1985). The data are shown as average values (± SD).

Results

Increased content of yellow lupins in starter diets for broiler chickens resulted in marginal effects on **body weight** in the first period of growth. Addition of enzyme preparation also was without visible effects. At the end of trial, the

highest body weight was achieved in treatments which received diets without lupins (I and II), with an average for both sexes of 2,596 g (Table 2). Birds fed the diets containing 5/10% of lupins (treatments III and IV) reached an average final body weight of 2,512 g. Average weight of birds receiving the diets with 10/20% of lupins (treatments V and VI) was reduced to 2,426 g ($P < 0.05$). The increasing dietary proportion of yellow lupins resulted in a linear decrease of final live weight by 3.2 and 6.5%, respectively. This effect was even more pronounced in male chickens. All treatments fed the diets supplemented with enzymes resulted in higher final weights than the respective unsupplemented controls, however, the effect of enzyme supplementation was not statistically significant. Some significant differences between treatments were noted for male chickens only, showing in two cases significant improvement of final weight due to enzyme supplementation (see Table 2).

Feed conversion ratio was similar in all treatments and both lupin inclusion into the diets and enzyme supplementation were without significant effects on the feed and protein conversion.

Relatively high **mortality** and culling rate in the first growth period was caused mainly by an incidentally too low environmental temperature in the broiler house as an effect of some problems with the heating system. In the second growth period the losses of birds were very low. For interaction between lupin level and enzyme supplementation insignificant P-values were calculated.

The **apparent ileal digestibility** of dry matter was not affected by lupin level, also enzyme supplementation did not significantly influence this parameter (Table 3). Lupin content in the diets increased the digestibility of ash and crude protein ($P < 0.05$) while enzyme supplementation improved the ash (by 3.4%-units) and crude protein (by 2.5%-units) digestibility ($P < 0.01$). In fat and N-free extract no differences were observed.

Ileal digestibility of amino acids was significantly affected by increased lupin level (Table 4). Thus significantly higher digestibility coefficients were found for Cys, Val, Met, Ile, Leu, Tyr, Lys, His in birds fed the diets containing higher level of lupins, but the digestibility coefficients determined for total amino acids content were similar in all treatments (Table 4). Use of the enzyme complex at 400 ppm improved the ileal digestibility of numerous amino acids and for total amino acids this value was better by 1.6%-units ($P < 0.05$). Interaction lupins x enzymes for all amino acids was high.

Concerning the **carcass composition,** a clear and partly significant ($P < 0.01$) decrease of breast muscle percentage was noted as the response to the increasing dietary level of lupins (Table 5). There was also a tendency for simultaneous increase in the percentage of abdominal fat. Enzyme supplementation had no significant effect on the carcass characteristics in broiler chickens. The share of breast muscles and abdominal fat in carcass were significantly ($P < 0.05$ and $P < 0.01$) higher in female broilers and for all levels of interactions the P-values were high.

Table 2. Average body weight (x_w), feed and protein conversion and mortality for broiler chickens (means, ± SD) (two factorial ANOVA)
Durchschnittliche Körpermasse (x_w), Futter- und Proteinverwertung sowie Mortalität der Broiler (Mittelwerte ± SD, zwei-faktorielle ANOVA)

Parameters	Treatment – diets					
	Lupins %			Enzymes (ppm)		Interaction p-value lupins x enzymes
	0	5/10	10/20	0	400	
Body weight (g)						
at day 1	35.9 ± 1.1	36.0 ± 0.9	35.9 ± 0.6	35.7 ± 0.5	36.2 ± 1.1	0.699
at day 21	548 ± 10	541 ± 32	527 ± 26	544 ± 26	538 ± 21	0.967
at day 42 (♂+♀)	2596 a ± 182	2512 ab ± 71	2426 b ± 120	2466 ± 113	2549 ± 154	0.793
- male	2861 Aa ± 228	2656 b ± 63	2553 B ± 162	2622 a ± 159	2733 b ± 222	0.206
- female	2404 ± 130	2396 ± 100	2329 ± 146	2350 ± 92	2406 ± 149	0.863
Feed conversion (kg feed/kg BW gain)						
days 1-21	1.52 ± 0.11	1.65 ± 0.15	1.68 ± 0.14	1.61 ± 0.11	1.61 ± 0.16	0.967
days 22-42	1.96 ± 0.12	2.09 ± 0.11	2.01 ± 0.13	2.04 ± 0.09	2.01 ± 0.16	0.629
days 1-42	1.87 ± 0.08	2.01 ± 0.12	1.95 ± 0.13	1.95 ± 0.08	1.94 ± 0.15	0.734
Protein conversion (g protein/kg BW gain)						
days 1-21	327 ± 24	354 ± 33	360 ± 31	346 ± 24	347 ± 35	0.967
days 22-42	382 ± 24	408 ± 22	392 ± 25	398 ± 18	392 ± 32	0.629
days 0-42	372 ± 15	401 ± 24	390 ± 25	389 ± 16	387 ± 31	0.745
Mortality and culling (%)						
days 1-21	6.67 ± 4.13	11.53 ± 6.54	7.00 ± 3.85	6.42 ± 3.11	9.83 ± 6.28	0.983
days 22-42	0.00	0.93 ± 1.85	0.58 ± 1.08	0.25 ± 0.79	0.42 ± 0.93	0.893
days 1-42	6.67 ± 4.13	12.28 ± 7.10	7.50 ± 3.96	6.62 ± 3.41	10.19 ± 6.22	0.993

Values on the same line with different superscript a, b are significantly different at value of $p < 0.05$;
Values on the same line with different superscript A, B are significantly different at value of $p < 0.01$

Table 3. Coefficients* of apparent ileal digestibility of nutrients in broilers of 28 days of age (%) (means, ± SD) (two factorial ANOVA)
Koeffizienten der scheinbaren ilealen Verdaulichkeit der Nährstoffe bei Broilern am 28. Lebenstag (%; Mittelwerte ± SD; zwei-faktorielle ANOVA)

Parameters	Treatment - diets					
	Lupins %			Enzymes (ppm)		Interaction p- value lupins x enzymes
	0	5/10	10/20	0	400	
Dry matter	67.3 ± 3.6	66.1 ± 4.7	66.1 ± 3.1	65.9 ± 4.6	67.1 ± 2.7	0.824
Crude ash	27.4 a ± 10.5	33.6 ab ± 10.4	42.7 b ± 11.1	32.9 ± 12.2	36.3 ± 12.2	0.528
Crude protein N x 6,25	78.6 a ± 3.8	81.3 b ± 2.6	81.7 b ± 1.4	79.3 A ± 3.6	81.8 B ± 1.7	0.446
Crude fat	81.2 ± 9.5	82.5 ± 4.4	81.9 ± 2.6	81.2 ± 2.7	82.6 ± 8.2	0.915
N - free extract	71.5 ± 4.2	69.0 ± 6.2	67.7 ± 3.3	69.3 ± 5.9	69.5 ± 3.8	0.878

Values on the same line with different postscripts a, b are significant1y different at a value of p<0.05;
Values on the same line with different postscripts A, B are significant1y different at a value of p<0.01
* Estimated using indicator method

Table 4. Coefficients* of apparent ileal digestibility of amino acids in broilers on 28 day of age (%) (means ± SD) (two factorial ANOVA)
Koeffizienten der scheinbaren ilealen Verdaulichkeit der Aminosäuren bei Broilern am 28. Lebenstag (%; Mittelwerte ± SD; zwei-faktorielle ANOVA)

Aminoacids	Treatments - diets					
	Lupins %			Enzymes (ppm)		Interaction p-value lupins x enzymes
	0	5/10	10/20	0	400	
Asp	75.9 ± 3.2	77.7 ± 3.1	77.0 ± 2.0	75.5 A ± 2.9	78.2 B ± 2.0	0.567
Thr	60.0 ± 7.1	63.6 ± 5.9	63.2 ± 2.9	60.3 a ± 5.9	64.2 b ± 4.8	0.738
Ser	74.2 ± 3.7	74.0 ± 3.9	73.7 ± 2.4	73.3 ± 3.9	74.6 ± 2.4	0.989
Glu	86.6 ± 2.0	88.5 ± 2.6	88.5 ± 1.1	87.3 ± 2.5	88.4 ± 1.6	0.359
Pro	91.4 ± 2.3	90.7 ± 2.5	89.8 ± 2.7	91.2 ± 2.1	90.0 ± 2.8	0.001
Cys	74.9 a ± 3.7	73.4 a ± 5.0	79.2 b ± 4.2	74.4 a ± 5.6	77.3 b ± 3.6	0.310
Gly	66.9 ± 4.5	68.0 ± 4.6	67.8 ± 3.0	65.2 A ± 3.7	69.9 ± 2.6	0.748
Ala	67.8 ± 4.2	64.9 ± 6.6	70.3 ± 5.9	65.5 ± 7.0	69.8 ± 3.8	0.269
Val	67.7 A ± 6.3	72.4 B ± 3.5	78.9 C ± 5.2	71.4 ± 5.9	74.6 ± 7.5	0.164
Met	85.9 a ± 3.4	85.9 a ± 3.3	88.9 b ± 3.0	85.6 a ± 3.4	88.2 b ± 3.0	0.848
Ile	67.4 Aa ± 5.2	75.7 b ± 2.7	79.7 B ± 2.5	73.4 ± 6.6	75.1 ± 6.0	0.691
Leu	76.7 A ± 2.7	79.3 B ± 2.4	81.2 B ± 2.5	78.2 ± 3.5	79.9 ± 2.5	0.852
Tyr	81.5 A ± 3.2	79.3 A ± 2.7	76.4 B ± 2.5	780.0 ± 3.9	80.1 b ± 2.7	0.645
Phe	77.7 ± 3.4	78.7 ± 3.2	78.1 ± 1.7	77.6 ± 2.8	78.8 ± 2.7	0.753
His	80.9 a ± 2.7	81.4 a ± 2.8	78.5 b ± 1.5	79.1 A ± 2.6	81.5 B ± 2.2	0.681
Lys	72.9 a ± 4.1	76.8 b ± 2.9	76.0 b ± 3.3	74.1 ± 3.4	76.4 ± 3.9	0.716
Arg	82.7 ± 5.0	86.3 ± 1.9	84.1 ± 4.5	85.5 ± 2.5	83.3 ± 5.2	0.534
Trp	77.1 ± 3.2	75.6 ± 5.2	73.5 ± 4.9	73.5 a ± 4.8	77.3 b ± 3.6	0.441
Total	79.2 ± 2.1	80.6 ± 2.4	80.9 ± 1.5	79.4 a ± 2.3	81.0 b ± 1.7	0.952

Values on the same line with different superscripts a, b are significant1y different at a value of p < 0.05; Values on the same line with different superscripts A, B are significant1y different at a value of p < 0.01
* Estimated using indicator method

Increased dietary proportion of lupins resulted in a significantly lower concentration of *E. coli* and decrease of *Lactobacillus* proliferation in the large intestine (rectum). Beneficial effect of enzyme application was shown in a reduced *E. coli* CFU number and significantly (0.01) higher Lactobacillus sp. concentration in large intestine content. It confirms the significant (P < 0.05 and P < 0.01) interaction between lupin content and enzyme application in mixtures.

Discussion

The beneficial effect of glucosidases addition to broiler diets rich in cereal NSP has been confirmed in numerous investigations (Annison et al., 1996; Bergh et al., 1999; Kocher et al., 2000; Nahas and Lafrancois, 2001; Jamroz et al., 2002). Commercial enzyme preparations containing mainly xylanases, beta-glucanase and cellulases were also useful when legume seeds were included in diets

Table 5. Carcass composition (in % of empty body weight) (means, ± SD) (three factorial ANOVA)
Schlachtkörperzusammensetzung (in % des Nüchterngewichts, Mittelwerte ± SD; drei-faktorielle ANOVA)

Parameters	Treatments - diets							p-values for interactionn			
	Lupins %			Enzymes (ppm)		Sex		lupins x enzymes	lupins x sex	enzymes x sex	lupins x enzymes x sex
	0	5/10	10-20	0	400	Female	Male				
Dressing percentage in % of net body weight	74.7 ± 1.0	74.9 ± 1.2	75.0 ± 1.1	74.8 ± 1.0	75.1 ± 1.2	75.2 ± 1.2	74.7 ± 0.9	0.471	0.607	0.201	0.995
Breast muscles (without skin)	23.9 A ± 2.2	22.7AB ± 2.2	21.7B ± 1.7	22.6 ± 2.2	22.9 ± 2.2	23.6 A ± 2.0	21.9B ± 2.1	0.519	0.663	0.814	0.718
Liver	2.30 ± 0.22	2.31 ± 0.26	2.37 ± 0.27	2.30 ± 0.28	2.35 ± 0.21	2.33 ± 0.27	2.32 ± 0.23	0.986	0.822	0.678	0.800
Pancreas	0.24 ± 0.05	0.25 ± 0.07	0.25 ± 0.05	0.24 ± 0.05	0.25 ± 0.06	0.26 ± 0.06	0.23 ± 0.04	0.480	0.744	0.720	0.471
Gizzard,	2.21 ± 0.29	2.25 ± 0.30	2.37 ± 0.21	2.24 ± 0.31	2.31 ± 0.24	2.37 ± 0.25	2.18 ± 0.27	0.018	0.038	0.696	0.522
Heart	0.63 ± 0.07	0.65 ± 0.09	0.66 ± 0.09	0.66 ± 0.07	0.64 ± 0.09	0.63 ± 0.08	0.67 ± 0.08	0.213	0.293	0.191	0.530
Edible giblets (heart, gizzard, liver)	5.14 a ± 0.31	5.21ab ± 0.41	5.40 b ± 0.32	5.20 ± 0.37	5.30 ± 0.36	5.33 a ± 0.35	5.17 b ± 0.37	0.061	0.193	0.711	0.867
Abdominal fat	1.22 ± 0.64	1.33 ± 0.55	1.49 ± 0.58	1.33 ± 0.65	1.36 ± 0.53	1.55 A ± 0.57	1.14B ± 0.55	0.610	0.412	0.824	0.551

Values on the same line with different superscripts a, b are significantly different at a value of $p < 0.05$;
Values on the same line with different superscripts A, B are significantly different at a value of $p < 0.0l$

(Roth-Maier and Kirchgessner, 1999; Gdala et al., 1997). Lupine seed contains arabinoxylans and β-galactans calculated in sugar residues and also mannose (11-18 g) and galactose (on average 141 g/kg DM) (Bach Knudsen, 1997). Most of the currently used enzyme preparations do not contain galactosidases or other enzymes degrading oligosaccharides (Carre et al., 1995).

In present study increased content of lupins share affected the body weight of 21 day old chickens slightly negatively. The carbohydrases addition to the feed given to very young chickens was without visible effects. At the age, when the birds had higher feed intake during the grower phase (on 42 d of life) increased lupin levels decreased the body weight by 3.4% (5/10% lupin) or by 6.6% (10/20% lupin) in comparison to control, without lupins in the diet. However, supplementation of diets with enzymes improved the birds' growth by in mean 4.2% (male) ($P < 0.05$) and by 2.4% (female) ($P > 0.05$). It may be speculated that the increase of galactose content (from 5.0 up to 20 g/kg; 98-126 g/kg total NSP) resulting from the increased lupin level in mixtures was without any significant effects on the performance. Similar results were presented by Kocher et al. (2000). However, in the work by Brenes et al. (1993) improvement of performance indices was noted.

Lupin level and enzyme supplementation did not significantly affect the analyzed performance parameters (calculated in two-factorial analysis of variance). Protein and numerous amino acids ileal digestibilities were improved with lupin inclusion into diets and by enzyme addition, too ($P < 0.05$). In dissection parameters the significant differences were stated only for breast muscle and edible giblets shares related to the empty body weight.

Lupin seeds are relatively rich in oligosaccharides, which show prebiotic properties in poultry (Bailey et al., 1991; Choi et al., 1994). The total amount of oligosaccharides reached in yellow lupine about 83 g/kg dry matter. In other varieties – *L. albus* or *L. angustifolius* these amounts reach about 62 and 48 grams, respectively (Kluge et al., 2002). It could be the explanation of significant ($P < 0.01$) reduction of E. coli CFU in content of large intestine (rectum) when the diets containing increased lupine levels were offered to the birds. Carbohydrases, through the grain' NSP degradation also reduced the *E. coli* number and additionally stimulated the *Lactobacillus* spp. proliferation ($P < 0.01$). On the basis of performances, carcass composition, apparent digestibility of nutrients and reduced E. coli number in rectum content it could be stated that the share of 5/10% yellow lupine is the beneficial level of these seeds in the diet for broiler. Obtained positive changes of microbial status in large intestine content in chickens are particularly interesting as they suggest, that lupins may be not only a protein source but also could be recognized as a prebiotical supplement of broiler diets.

Summary

The experiment was carried out with Ross 308 broiler hybrids. One-day old chickens were allocated to six treatments, each treatment consisting of four replications with

Table 6. Concentration of microorganisms in rectum intestine of chickens on 28 day of age (CFU/g) (means, ± SD) (two factorial ANOVA)
Konzentration der Mikroorganismen im Rektum der Broiler am 28. Lebenstag (CFU/g; Mittelwerte, ± SD; zwei-faktorielle ANOVA)

Parameters	Treatment - diets					
	Lupins %			Enzymes (ppm)		Interaction p-value lupins x enzymes
	0	5/10	10/20	0	400	
Escherichia coli (Log_{10} CFU/g)	6.41 A ± 0.48	5.39B ± 0.20	4.62 C ± 0.33	5.65 A ± 0.87	5.30 B ± 0.76	0.016
		5.01±0.48				
Lactobacillus sp. (Log_{10} CFU/g)	6.95 A ± 0.44	5.99B ± 0.33	6.13 B ± 0.40	6.17 A ± 0.41	6.55 B ± 0.66	0.034
		6.06±0.36				

Values on the same line with different superscripts A, B are significantly different at a value of $p < 0.01$

50 birds each. The diets were based on cereal grains (wheat 31/35% and barley 25/30% in starter/grower diets, respectively), and contained 0, 5, 10% (starter) and 0, 10, 20% (grower) of yellow lupin seeds var. Juno. Each experimental diet was fed either unsupplemented or supplemented with enzymes (Ronozyme VP) at the level of 400 mg/kg. Performance was determined on days 21 and 42. Apparent ileal digestibility of nutrients and amino acids was determined in the period between days 21 to 28. At the end of experiment, carcass composition and number of *Lactobacillus* and *Escherichia coli* in the content of large intestine (rectum) were determined.

In comparison to the diets without lupins, increased dietary content of yellow lupins slightly affected the body weight in the first period of chick growth. Enzyme supplementation did not show any significant influence. On day 42, the body weight decreased with increasing proportion of lupins; the highest body weight was observed in birds fed diets without lupins (2,546 g), then in animals fed the diets containing 5/10% lupins (2,512 g) and 10/20% of lupins (2,426 g). Effects of enzyme addition differed insignificantly. However, birds fed diets with enzymes were heavier (on average by 83 g) than control ones. All described differences were statistically significant for male chickens only. Enzyme supplementation and dietary lupin level did not influence feed and protein conversion. The apparent ileal digestibility of crude protein and ash was higher, when diets with increasing lupin content were supplemented with enzymes ($P < 0.05$). Both experimental factors had no significant effect on ileal digestibility of dry matter, crude fat and NFE. Increased lupins level and presence of carbohydrases resulted in significantly higher coefficients of ileal digestibility determined for Thr, Glu, Cys, Gly, Val, Ile, Leu, Lys and Arg. Regarding carcass composition, increasing dietary content of lupins negatively influenced the percentage of breast muscle ($P < 0.01$). For interaction lupins x enzymes the high P-values were calculated.

Increased dietary content of lupins significantly decreased CFU numbers of *E. coli* and *Lactobacillus* spp., but enzyme addition reduced CFU of *E. coli* and increased *Lactobacillus* proliferation ($P < 0.01$). The share of 5/10% of yellow lupine could be found as optimal level.

Key words

Broiler, nutrition, lupin, NSP, enzyme, growth, ileal digestibility, microbial status

Zusammenfassung

Auswirkungen steigender Anteile an gelben Lupinen und eines Enzymzusatzes im Futter auf die Mastleistung, die ileale Verdaulichkeit der Nährstoffe und den mikrobiellen Status im Dickdarm bei Broilern

Die Untersuchung wurde mit 1.200 Broilern der Herkunft Ross 308 durchgeführt. Die Eintagsküken wurden auf 6 Behandlungen verteilt, wobei jede Behandlung aus 4 Wiederholungen a 50 Tieren bestand. Die Versuchsrationen basierten in erster Linie auf Getreide (31/35% Weizen, 25/30% Gerste in Starter/Grower) und enthielten 0, 5 und 10 (Starter) bzw. 0, 10 und 20% (Grower) gelbe Lupinen der Sorte *Juno*. Jede Versuchsration wurde sowohl mit als auch ohne Zusatz von Ronozyme VP (400 mg/kg) an die Versuchstiere verfüttert. Die Leistung wurde am 21. und am 42. Masttag ermittelt. Die scheinbare ileale Verdaulichkeit wurde im Zeitraum 21. bis 28. Masttag bestimmt. Am Ende des Versuchs wurden die Schlachtkörperzusammensetzung und die Keimzahlen für *Lactobacillus* und *E. coli* im Rektum ermittelt.

Im Vergleich zu den Rationen ohne Lupinen wurden beim Einsatz von gelben Lupinen etwas höhere Körpergewichte im ersten Mastabschnitt erzielt. Dagegen hatte der Enzymzusatz keinen signifikanten Effekt. Am 42. Masttag wurden mit zunehmenden Gehalten an Lupinen in den Rationen geringere Körpergewichte beobachtet. Das höchste Körpergewicht wurde in den Behandlungen ohne Einsatz von Lupinen (2.546 g), das zweithöchste bei einem Einsatz von 5/10% Lupinen (2.512 g) und das geringste bei 10/20% Lupinen (2.426) in der Ration registriert. Der Enzymzusatz führte zu geringfügigen, nicht signifikanten Differenzen zwischen den Behandlungen. In der Tendenz waren die Tiere, die in der Ration das Enzym erhielten, 83 g schwerer als die Kontrolltiere. Alle beschriebenen Unterschiede waren aber nur für die männlichen Tiere signifikant. Weder der Enzymzusatz noch der Einsatz von Lupinen wirkte sich auf die Futter- und die Proteinverwertung aus. Die scheinbare, ileale Verdaulichkeit des Rohproteins und der Asche war bei höheren Gehalten an Lupinen und dem Zusatz des Enzyms in den Rationen höher ($P < 0,05$). Beide Versuchsfaktoren wirkten sich aber nicht auf die ileale Verdaulichkeit der Trockensubstanz, des Rohfettes und der NFE aus. Dagegen wurden bei höheren Lupingehalten und dem Zusatz der Carbohydrasen (Ronozyme VP) signifikant höhere Koeffizienten für die ileale Verdaulichkeit von Thr, Glu, Cys, Gly, Val, Ile, Lay und Arg ermittelt. Der Gehalt an Lupinen in den Rationen hat sich negativ auf den

Brustfleischanteil ($P < 0,01$) ausgewirkt. Es lagen signifikante Interaktionen zwischen dem Lupinengehalt und dem Enzymzusatz vor. Bei höheren Gehalten an Lupinen in den Rationen wurden signifikant geringere CFU Werte für *E. coli* und *Lactobacillus spp.* registriert, während der Enzymzusatz die CFU für *E. coli* verminderte und für *Lactobacillen* erhöhte ($P < 0,01$). Der Einsatz von 5% Lupinen im Starter und 10% im Grower erwies sich als am günstigsten.

Stichworte

Broiler, Fütterung, Lupine, NSP, Enzym, Wachstum, ileale Verdaulichkeit, mikrobieller Status

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