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Effect of enzyme supplementation of white lupin (*Lupinus albus* var. *Butan*)-containing diets on performance, nutrient digestibility, viscosity, pH, and passage rate of digesta in broiler chickens[*1]

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ABSTRACT

A wheat-based diet containing 300 g/kg white lupin seeds (L) was fed either unsupplemented or supplemented with one, two or three commercial enzymes. Enzyme B contained primarily xylanase activity, enzyme E primarily pectinase and β-glucanase activity, enzyme A primarily α-galactosidase activity. Each diet was fed to a group of 24 eight-day-old broiler females kept individually. During the first two weeks performance was recorded, next the passage rate was measured in 6 birds, and the digestibility of nutrients was evaluated in 10 birds from each group. On day 25-26 of life, 14 birds per group were slaughtered and viscosity and pH of digesta from different parts of the digestive tract were determined.

Body weight gain, feed conversion ratio, metabolizable energy of diet, viscosity of digesta in the jejunum and distal ileum, pH of digesta in the caeca, and excreta moisture content were not affected by enzyme supplementation of lupin diets. In group LB the digestibility of dietary fat increased ($P<0.05$) in comparison with group L. In group LBE, apparent protein digestibility and organic matter retention decreased and digesta viscosity in the caeca increased (from 2.4 and 5.6 to 21.3 mPas·s, respectively) in comparison with groups L and LB ($P<0.05$). Supplementation of the LBE diet with enzyme A caused a decrease in caecal digesta viscosity to 9.4 mPas·s and an increase in the rate of passage of digesta during the first 4 h.

KEY WORDS: white lupin, enzyme supplementation, nutrient digestibility, digesta viscosity, broiler chickens

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INTRODUCTION

Low-alkaloid lupin seeds are used as a protein source in broiler diets. However, their metabolizable energy value for broilers is not high (Alloui et al., 1994), as non-starch polysaccharides (NSP) constitute from 320 (*L. luteus*) to 400 (*L. albus* and *L. angustifolius*) and oligosaccharides, from 76 (*L. angustifolius*) to 110 (*L. albus*) or 120 (*L. luteus*) g/kg of seed dry matter (Gdala and Buraczewska, 1996). α-Galactosides of the raffinose series, mainly stachyose, predominate among the oligosaccharides of lupins, whereas among NSP, pectic polysaccharides called rhamnogalacturonans prevail. They are complex branched structures containing α-arabinian and β-galactan side chains attached to a pectin-like main chain of rhamnose and galacturonic acid linked by β-(1-4) and α-(1-2) bonds (Carré et al., 1985; Cheetham et al., 1993; Gdala and Buraczewska, 1996). Neither oligosaccharides nor NSP are hydrolysed by the digestive enzymes of chickens, but they may be fermented by the microflora in the digestive tract. Theoretically, they may negatively affect the productive performance of chickens fed diets containing lupin, but this depends on the physico-chemical characteristics of carbohydrates, which may differ greatly between the species and even varieties of lupin. Hughes et al. (2000) reported that increased amounts of soluble NSPs of *L. angustifolius* in the diet progressively increased digesta viscosity and excreta moisture levels, which points to an osmotic effect in the digestive tract of chickens. This effect was associated with a reduced apparent metabolizable energy (AME) value of the diet and depressed bird performance. Annison et al. (1995) and Kocher et al. (2000) reported that ileal viscosity in chickens increased 3-4 times after inclusion 30-35% of *L. angustifolius* seeds into the diet, however, in the last study only a 75% increase of ileal viscosity was observed after inclusion of 35% of *L. albus* seeds into the diet. Alloui et al. (1994) found that among 6 varieties of lupin belonging to 3 species grown in Poland, only one cultivar of *L. angustifolius* and one of *L. luteus* contained NSPs of antinutritional activity. Increasing the level of these cultivars from 15 to 30% in diets of equalised energy, protein and major amino acid content caused a decrease in body weight gain (BWG), while increasing the level of the remaining cultivars did not affect BWG or feed to gain ratio (FCR) significantly.

The high content of NSPs and oligosaccharides in lupin seeds and their potential anti-nutrient activity prompted attempts to improve the nutritive value of lupin diets with enzyme preparations having pectinase and α-galactosidase activities, but the effects of the trials were contradictory. Brenes et al. (1993) reported that after supplementation of a diet containing white lupin seeds with certain combinations of enzymes, increases of up to 18% in BWG and 10% in FCR were noted, while in another report (Brenes et al., 2002), BWG improved by 5.5% and FCR worsened by 1.7% after enzyme supplementation of white lupin diets. Alloui et al. (1994) found that Energex (pectinase and β-glucanase) added to lupin seeds *in*

vitro increased the solubility of protein, but not cell-wall constituents, *in vivo* it increased the AME value of 4 cultivars from among 6, but did not positively affect performance of chickens. Also Annison et al. (1995) and Kocher et al. (2000) reported that some enzymes added to diets with *L. angustifolius* significantly increased ileal soluble NSP and ileal viscosity, while they did not affect the AME value and performance of broilers.

This study was designed to investigate the effects of 3 commercial enzyme products added to a diet with white lupin seeds on the performance, nutrient digestibility, viscosity, pH, and passage rate of digesta in broiler chickens.

MATERIAL AND METHODS

Material and diets

The seeds of white lupin var. *Butan* harvested in 1999 year were used. Four isoproteinous and isoenergetic diets containing 300 g lupin seeds per kg were prepared (Table 1). The control diet (L) was unsupplemented, the remaining 3 diets supplemented with the following enzymatic preparations (further designed as enzymes): diet LB with Bio Feed Wheat (B - xylanase, 1000 FXU/g), diet LBE

TABLE 1

Composition of diets, g/kg

Component	Diet[1]			
	L	LB	LBE	LBEA
White lupin seeds	300.00	300.00	300.00	300.00
Wheat	330.90	329.90	328.90	327.40
Constant [2]	369.10	369.10	369.10	369.10
Enzyme preparation B[3]	-	1	1	1
Enzyme preparation E[3]	-	-	1	1
Enzyme preparation A[3]	-	-	-	1.5

L - lupin containing diet, LB, LBE and LBEA – lupin diets with added enzyme preparations

[1] in digestibility trial 3 g Cr_2O_3 per kg, in passage rate determination 20 g Cr_2O_3 per kg was added as a marker on top of diets

[2] constant components (g/kg): soyabean meal, 168; maize, 100; rapeseed oil, 60; limestone, 12; dicalcium phosphate, 16; NaCl, 3; DL-Met, 2.1; L-Lys (0.78%), 1.64; L-Thr, 1.14; L-Trp, 0.22 and vitamin-mineral mixture, 5 [supplied per kg diet: vit. A, 1350 IU; vit. D_3, 3500 IU; mg: vit. E, 40; vit. K_3, 4; vit. B_1, 3; vit. B_2, 8; vit. B_6, 5; vit B_{12}, 0.03; niacine, 60; Ca pantotheate, 15; folic acid, 1.75; biotine, 0.1; choline, 500; Mn, 80; Zn, 70; Fe, 70; Cu, 15; J, 1; Se, 0.2; flavomycine, 5; salinomycine, 60 and Ca, 1.075 g]

[3] enzymes: B - Bio Feed Wheat CT (xylanase, 1000 FXU/g); E - Energex CT (pectinase, 5000 PSU/g and β-glucanase, 50 FBG/g), A - Alpha-Gal (α-galactosidase, 900 GALU/g)

- with B and Energex (E - pectinase, 5000 PSU/g and β-glucanase, 50 FBG/g), diet LBEA with B, E and Alpha Gal (A - α-galactosidase, 900 GALU/g). All enzymes were produced by Novo Nordisk, the enzyme activities were given as stated by the producer; all of the preparations may have some side activities. The diets were formulated to meet the requirements of broiler chickens for amino acids and contained 208 g of crude protein and 12.1 MJ ME per kg (Table 1). Each diet was divided into three batches, the first batch was unsupplemented, 3 g Cr_2O_3 per kg was added as a marker on top of the second batch, 20 g Cr_2O_3 per kg, on top of the third batch; the diets were then mixed and cold pelleted.

Growth experiment

The experiment was carried out on 96 Starbro females that were maintained in battery cages on a standard Starter diet during the first week of life. On the 8th day of life the birds were deprived of feed for 4 h, weighed and randomly allocated into 4 groups, 24 chickens each. The average initial body weight of birds was 131±10 g. The birds were transferred to individual metabolism cages and given the experimental diets *ad libitum*; on 22 day of age after 4 h of feed deprivation, the birds were weighed, feed intake measured, and feeding of the experimental diets resumed.

Digestibility experiment

On 22 day of age, 10 chickens of average body weight 779±62 g were selected from each group and fed respective balance diets (Table 1) containing 3 g/kg of Cr_2O_3 for 6 days. A balance experiment was performed according to Bourdillon et al. (1990). Feed intake was registered and the droppings voided during the last 4 days of feeding and following 17 h starvation period were quantitatively collected and stored at -18ºC.

Passage rate determination

On day 23 day of life, 6 chickens of average body weight 807±49 g were selected from each group. The birds were deprived of feed for 2 h, then placed in clean individual metabolism cages and given 50 g of their respective diets containing 20 g Cr_2O_3 per kg (Table 1). After 2 h the uneaten remnants of the diet were weighed and the chickens were given 80 g of their respective diets without Cr_2O_3 (Table 1). After the next 14 h the uneaten diet was weighed and the same diets were given *ad libitum*. Droppings from each bird were collected quantitatively every 2 h (starting 2 h after the end of feeding Cr_2O_3 diet) into separate containers, immediately frozen and stored at -18ºC for later analyses.

Viscosity and pH measurements

Fourteen chickens fed *ad libitum* from each group were slaughtered on days 25-26 of life, the abdominal cavity was opened and the contents of the jejunum (from the duodenum to Meckel's diverticulum), proximal and distal part of the ileum (last 15 cm of the ileum before the ileocaecocolic junction), and caeca were collected. Digesta from 2 or 3-4 (distal ileum only) birds were pooled. Digesta from the proximal ileum and caeca were mixed with deionised water at a proportion of 1:1 w/w. Digesta from the jejunum, distal ileum and caeca were centrifuged at 10,000 × g for 10 min using a Beckman centrifuge (model J2-21 with J-20 rotor) at 4ºC, and the viscosity of the supernatant (0.5 mL aliquot) was immediately measured with the use of a Brookfield Digital cone/plate viscometer (model LVDV II+, Brookfield Engineering Laboratories, Stoughton, MA, USA) at 40ºC. Readings were expressed in centipoise (1cP=1 mPas·s). pH in digesta from ileum and caeca was measured by a digital pH-meter (hand-Held Meter, model WTW pH/340, Germany) using pH standard WTW D-82362 Weilheim (model STP4) at room temperature.

Chemical analyses

Prior to analysis, lupin seeds and diets were ground to pass a 1 mm sieve. Droppings were thawed, homogenised, and moisture content measured. They were then dried in a fan-forced drier for 12 h at 60ºC, kept uncovered for 48 h and ground to pass a 1 mm sieve. Dry matter, total nitrogen, crude ash and crude fibre were determined according to AOAC (1990). Cr_2O_3 was analysed spectrophotometrically according to Hinsberg et al. (1953), crude fat in diets and droppings was determined by diethyl ether extraction following acidification with 4M hydrochloric acid, gross energy content was determined using a Parr adiabatic oxygen bomb calorimeter (KL-11). Faecal N in droppings was determined according to Ekman et al. (1949).

Calculations and statistical analysis

BWG and FCR were calculated for the first two weeks of the experiment. Apparent digestibility of protein, fat, organic matter retention and metabolizable energy of diets were calculated relative to the content of Cr_2O_3 in balance diets and droppings. The metabolizable energy value of diets was calculated according Hill and Anderson (1958) and corrected to zero nitrogen balance using 34.39 kJ/g nitrogen retained. Means and pooled standard error of means (SEM) were calculated by standard procedures according to Statgraphics Plus ver. 5.1, the results were subjected to statistical analysis using one-way analysis of variance.

Duncan's multiple range test was used to separate means when significant effects ($P<0.05$) were detected by analysis of variance. For determination of the passage rate in each bird, the Cr content in excreta voided in each interval were expressed as a fraction of total Cr intake. Cumulative excretion curves of Cr were then calculated according to Vergara et al. (1989).

RESULTS

The white lupin seeds used in the study contained (in g/kg DM): crude protein 361, crude fat 95, crude ash 39, crude fibre 165. Chickens fed the LB diet ate significantly ($P<0.05$) less feed than chickens from the unsupplemented group L, but due to better FCR their BWG was not significantly different from group L. Supplementation of the LB diet with the remaining enzymes did not significantly affect feed intake, BWG or FCR (Table 2).

TABLE 2

Performance of broilers from 7 to 21 day of life

Dietary treatment[1]	Body weight gain g	Feed intake g	Feed to gain ratio g/g
L	655	887[b]	1.36
LB	625	830[a]	1.33
LBE	670	878[ab]	1.31
LBEA	651	867[ab]	1.33
Pooled SEM	13	16	0.02

[1] L - lupin-containing diet; B, E, A - added enzymes described in Table 1

[a,b] - values within column with no common superscripts are significantly different at $P\leq0.05$

The apparent fat digestibility in group LB was significantly ($P<0.01$) higher than in group L, but neither the apparent protein digestibility, organic matter retention nor metabolizable energy value of the LB diet was significantly different from diet L (Table 3). Supplementation of the LB diet with enzymes E had no significant effect on fat digestibility, while decreased protein digestibility and organic matter retention ($P<0.01$) and numerically lower metabolizable energy value of the LBE diet were found in comparison with diet LB.

Neither enzyme had a significant effect on the excreta moisture content (Table 3). The viscosity of digesta from the jejunum and distal ileum was, respectively, 3.21 and 7.05 mPas·s on diet L, and numerically (but insignificantly) decreased after supplementation of the diet with enzymes (Table 4). In contrast, digesta viscosity in the caeca increased with enzyme supplementation of the diets, mean viscosity in group LBE being 21.3 mPas·s [significantly ($P<0.05$) higher than in groups L

TABLE 3

Apparent protein and fat digestibility, organic matter retention, metabolizable energy of diets and DM content in droppings in broilers 24-30 day of age

Dietary treatment[1]	Apparent digestibility, %		Organic mater retention, %	AME_N MJ/kg DM	Excreta moisture, %
	crude protein	crude fat			
L	88.0^{bcAB}	87.7^{A}	70.5^{b}	14.23	74.3
LB	89.1^{cB}	90.9^{B}	71.0^{b}	14.17	73.9
LBE	85.9^{aA}	90.4^{B}	68.4^{a}	13.95	73.4
LBEA	87.1^{abAB}	89.4^{AB}	68.9^{a}	14.09	74.7
Pooled SEM	0.33	0.67	0.55	0.11	1.3

$^{a,b,.A,B}$ - values within column with no common superscripts are significantly different at:
^{a,b} $P \leq 0.05$; ^{A,B} $P \leq 0.01$
[1] L - lupin-containing diet; B, E, A - added enzymes described in Table 1

TABLE 4

Viscosity of jejunal, ileal and caecal digesta (mPas·s) and pH of ileal and caecal digesta in broilers 25-26 days old

Dietary treatment[1]	Viscosity of digesta from			pH of digesta from	
	jejunum	distal ileum[2]	caeca[3]	proximal ileum[3]	caeca[3]
L	3.21	7.05	2.41^{a}	7.38^{a}	5.74
LB	3.06	5.80	5.62^{a}	7.39^{ab}	5.62
LBE	2.95	6.87	21.29^{b}	7.88^{b}	5.69
LBEA	2.86	5.59	9.43^{ab}	7.74^{ab}	5.59
Pooled SEM	0.33	1.44	4.75	0.17	0.11

[1] L - lupin-containing diet; B, E and A - added enzymes described in Table 1
[2] the last 15 cm before ileocaecocolic junction
[3] digesta were mixed with deionised water (1:1 w/w) prior to measurements
^{a,b} - values within column with no common superscripts are significantly different at $P \leq 0.05$

and LB], however, very large individual variations in caecal viscosity were noted within treatments (Table 4).

In chickens fed diet LBE, the pH of digesta in the proximal ileum was significantly higher than in group L ($P<0.05$), but enzyme supplementation did not significantly influence the pH of caecal digesta.

Figures 1 and 2 show the transit time of Cr_2O_3 (insoluble marker) in the digestive tract of chickens. After 8 h from start of feeding, about 80% of consumed Cr_2O_3 was excreted (Figure 2), however, during the first 4 h the rate of Cr_2O_3 excretion was lower in group LBE, than in group LBEA (65 vs 78% of the marker; $P<0.05$). At the end, after 16 h from beginning of feeding (14 h of collection), birds from group LBE excreted more Cr_2O_3 (86%), while in the other groups, about 80% (Figure 2).

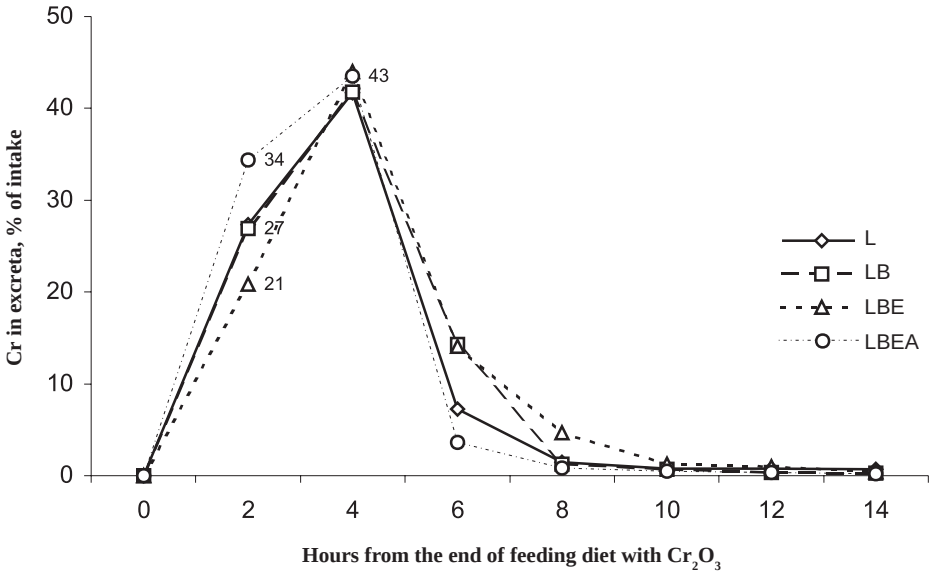

Figure 1. Noncumulative data of Cr_2O_3 excretion

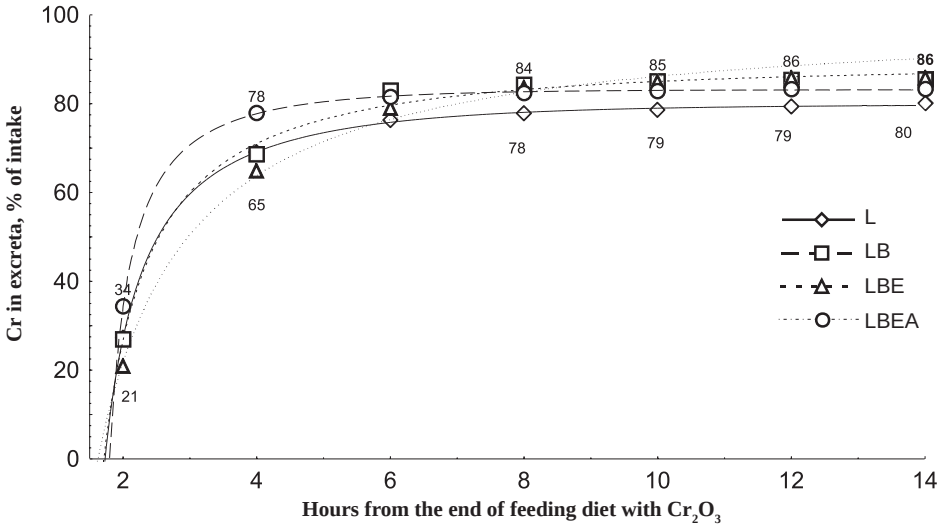

Figure 2. Cumulative excretion curves of Cr as in the function $y = 1- e^{-n(t-to)}/1+ke^{-n(t-to)}$, where y is the amount of excreted Cr, t is time in hours, to is a parameter equivalent to the first appearance of the marker, and n and k are other parameters that define the function

DISCUSSION

The trial was designed to study the effects of enzymes on the lupin component of the diet, however, as wheat was the basal cereal, the diets were also supplemented with xylanase. Xylanase cleaves the arabinoxylan polysaccharides of wheat, which in chickens is accompanied by diminished digesta viscosity and increased fat digestibility (Chesson, 2001). Both effects were also noted in the present trial. In this experiment, in the control group fed the diet with 30% of white lupin the digesta viscosity was 3.2 in the jejunum and 7 mPas·s in the distal ileum. After supplementation with xylanase it decreased to 3.06 and 5.80 mPas·s, respectively. These values were higher than the 1.92 and 2.55 mPas·s measured in broilers fed a xylanase-supplemented wheat-soyabean meal diet in our laboratory (Smulikowska et al., 2003). Also, in a control sorghum-casein based diet Kocher et al. (2000) observed jejunum viscosity of 2.07 cP, while in broilers fed a diet with 350 g/kg of white lupin cv Kiev, it increased to 3.4 cP; the respective values in the ileum were 3.03 vs 5.32 cP. This indicates that the NSPs of white lupin have viscous properties, but they are less viscous than the NSPs of *L. angustifolius* and less susceptible to hydrolysis by pectinase. Annison et al. (1995) and Kocher et al. (2000) obtained an increase of ileal digesta viscosity from 3 to over 11 mPas·s after inclusion of 300 or 350 g/kg of *L. angustifolius* seeds into a sorghum-based diet, in both experiments supplementation of the lupin diet with enzymes containing pectinase activity caused a further increase of ileal viscosity to about 34 mPas·s, which was accompanied by a significant increase of ileal soluble NSP. Also Hughes et al. (2000) noted that after supplementation of a diet containing NSPs extracted from *L. angustifolius* with an enzymatic cocktail, ileal viscosity increased significantly and was connected with an increase in ileal soluble NSPs, but the free sugar concentration was not affected. In the present experiment the digesta viscosity in the jejunum was insignificantly lower after addition of one, two, or all 3 enzyme preparations. Due to supplementation of the LB diet with Energex the viscosity of the ileal contents increased from 5.8 to 6.9 and that of the caecal contents increased from 5.6 in group LB to 21.3 mPas·s in group LBE ($P<0.05$). That, and the highest pH of ileal digesta in chickens fed the LBE diet indicated that pectinase may hydrolyse some bonds within white lupin NSP, making them more soluble and more prone to bacterial fermentation. Pectic substances of white lupin cell walls contain a high proportion of neutral sugar chains, composed mainly of poorly branched β-(1→4)galactan associated with the rhamnogalacturonan moiety, which are quite labile and highly susceptible to fermentative breakdown (Carré et al., 1985). Carré and Leclercq (1985) showed that the apparent digestibility of these substances in adult cockerels is nearly zero. Also Alloui et al. (1994) reported that Energex added to white lupin had a negligible effect *in vitro* on the solubility of protein or NDF and ADF fractions.

In pigs, Gdala et al. (1997) observed a positive effect of exogenous α-galactosidase added to a lupin diet on the digestibility of dry matter and the raffinose family of oligosaccharides. Brenes et al. (2003) stated that raffinose and stachyose of white lupin were poorly digested in the ileum or upper section of the gastrointestinal tract of chickens; the use of an enzyme exhibiting α-galactosidase activity enhanced their digestibility, while it did not improve chicken performance. The results of the present experiment suggest that supplementation of a lupin diet with α-galactosidase did not stimulate the release of nutrients that can be utilized by birds, as the organic matter retention and metabolizable energy value of the LBEA diet was lower than that of the unsupplemented one. The shorter digesta passage through gastrointestinal tract in chickens than in pigs may result in less effective utilization of the fermentation products.

Supplementation of the diet with enzymes aimed to hydrolyse NSPs and α-galactosides of lupin did not significantly improve BWG or FCR in the present study. Incorporation of hemicellulase (Perez-Escamilla et al., 1988), enzyme complexes containing among others cellulase, hemicellulase and pectinase (Kocher et al., 2000), or multi-enzyme preparations containing pectinase (Roth-Maier and Kirchgessner, 1994), into a diet with white lupin did not significantly improve the BWG of chickens, while FCR was improved by 5% in the last report only. The substantial increase in BWG up to 18 and 10% improvement in FCR, reported by Brenes et al. (1993) was rather the effect of enzymes with protease activity, not enzymes added to hydrolyse NSPs or α-galactosides of white lupin. In the later study (Brenes et al., 2002) reported that after supplementation of the white lupin diet with a multi-enzyme preparation, the BWG in broilers increased by 5%, while FCR slightly decreased.

Kocher et al. (2000) reported that excreta moisture increased in chickens fed lupin-containing diets, less with *L. albus*, more with *L. angustifolius*, added enzymes had no effect on excreta moisture. Also Roth-Maier and Kirchgessner (1994) reported that the supplementation of a white lupin diet with an enzymatic cocktail containing pectinase activity did not increase the moisture of droppings. In the present study, the water content of excreta did not change significantly after enzyme supplementation.

It seems that the soluble NSPs of white lupin are less viscous than the NSPs of *L. angustifolius*. Supplementation of a lupin diet with enzymes did not affect growth or performance of broilers, which indicates that enzymes were not able to liberate nutrients that may be utilized by chickens as an energy source.

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STRESZCZENIE

Wpływ uzupełniania enzymami diet zawierających łubin biały (*Lupinus albus* odm. *Butan*) na wydajność odchowu, strawność składników pokarmowych, lepkość i pH treści oraz tempo przepływu treści pokarmowej u kurcząt brojlerów

Dietę pszenną zawierającą w 1 kg 300 g nasion łubinu białego (L), uzupełniono jednym, dwoma lub trzema preparatami enzymatycznymi. Preparat B zawierał ksylanazę jako główny enzym, preparat E pektynazę i β-glukanazę, preparat A α-galaktozydazę. Diety podawano 4 grupom kurek brojlerów od 8 dnia życia. Grupy liczyły po 24 ptaki utrzymywane w indywidualnych klatkach. W ciągu pierwszych 2 tygodni określono wydajność odchowu, następnie w każdej grupie u 6 ptaków zmierzono tempo przepływu treści pokarmowej, a u 10 ptaków strawność składników pokarmowych. W 25-26 dniu życia 14 ptaków z każdej grupy ubito i zmierzono lepkość oraz pH treści pokarmowej.

Przyrost masy ciała, wykorzystanie paszy, wartość energii metabolicznej diety, lepkość treści w jelicie czczym i końcowym odcinku jelita biodrowego, pH treści jelit ślepych i zawartość wody w odchodach nie zmieniały się istotnie pod wpływem dodawanych enzymów. W grupie LB strawność tłuszczu była większa w porównaniu z grupą L ($P<0,05$). W grupie żywionej dietą LBE pozorna strawność białka i retencja masy organicznej były mniejsze, a lepkość treści jelit ślepych zwiększyła się (z 2,4 i 5,6 do 21,3 mPas·s, odpowiednio) w porównaniu z grupami L i LB ($P<0,05$). Uzupełnienie diety LBE enzymem A spowodowało obniżenie lepkości treści jelit ślepych do 9,4 mPas·s i zwiększenie tempa przepływu treści pokarmowej w pierwszych 4 godzinach po karmieniu.