THE EFFECT OF OLIGOSACCHARIDES AND ALKALOIDS CONTAINED IN YELLOW AND BLUE LUPINE SEEDS ON FEED INTAKE, BODY WEIGHT AND FERMENTATION PROCESSES IN THE CECUM OF RATS

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Abstract. The aim of this study was to determine the effect of different levels and composition of alkaloids and oligosaccharides contained in yellow and blue lupine seeds on growth rate and cecum function in rats. Experimental diets were supplemented with the seeds of three yellow lupine cultivars (Mister, Markiz, Taper) in the amount of 24.3%, 25.0% and 25.4%, and the seeds of three blue lupine cultivars (Sonet, Boruta, Elf) in the amount of 25.1%, 25.5% and 26.5%. The control diet contained casein as a protein source and cellulose as a fibre source. Diets supplemented with lupine seeds did not reduce feed intake, but they limited the growth rate of rats, and contributed to a significant increase in the weight of the cecum (0.66 g vs. 0.80 - 0.93 g) and cecal digesta (2.42 g vs. 3.29–4.25 g). The activity levels of bacterial glycolytic enzymes in the cecal microflora increased, and the pH of cecal digesta and ammonia concentrations in the cecum decreased in experimental groups. Desirable changes in the concentrations and profile of volatile fatty acids in the cecal digesta were noted. The values of the above parameters were not affected by lupine cultivar.

Keywords: yellow lupine, blue lupine, alkaloids, carbohydrates, rats, feed intake, body weight, cecal, fermentation.

OLIGOSACHARIDŲ IR ALKALOIDŲ, ESANČIŲ GELTONŲJŲ IR MĖLYNŲJŲ LUBINŲ SĖKLŲ SUDĖTYJE, POVEIKIS PAŠARO SĄNAUDOMS, KŪNO MASEI IR FERMENTACIJOS PROCESAMS ŽIURKIŲ AKLOJOJE ŽARNOJE

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Santrauka. Tyrimo tikslas buvo nustatyti skirtingo kiekio bei sudėties alkaloidų ir oligosacharidų geltonųjų ir mėlynųjų lubinų sėklose poveikį žiurkių augimui ir aklosios žarnos funkcionavimui. Eksperimentiniai pašarai papildyti trijų geltonųjų lubinų veislių (‘*Mister’, ‘Markiz’, ‘Taper’*) sėklomis 24,3 proc., 25,0 proc. ir 25,4 proc. bei trijų mėlynųjų lubinų veislių (‘*Sonet’, ‘Boruta’, ‘Elf*’) sėklomis atitinkamai 25,1 proc., 25,5 proc. ir 26,5 proc. Kontrolinio pašaro sudėtyje kazeinas buvo kaip proteinų šaltinis, o celiuliozė – kaip ląstelienos šaltinis. Racionai, papildyti lubinų sėklomis, pašaro sąnaudų nesumažino, bet ribojo žiurkių augimo spartą ir darė įtaką aklosios žarnos svorio (0,66 g palyginti su 0,80–0,93 g) ir aklosios žarnos turinio svorio (2,42 g palyginti su 3,29–4,25 g) didėjimui. Tiriamų grupių žiurkių aklosios žarnos mikrofloroje padidėjo bakterijų glikolitinių fermentų aktyvumas, o aklosios žarnos turinio pH ir amoniako koncentracija aklojoje žarnoje sumažėjo. Pageidautinų lakiųjų riebalų rūgščių koncentracijos ir profilio pokyčių aklosios žarnos turinyje nepastebėta. Lubinų veislės įtakos šių rodiklių vertėms neturėjo.

Raktažodžiai: geltonieji lubinai, mėlynieji lubinai, alkaloidai, karbohidratai, žiurkės, pašaro sąnaudos, kūno masė, akloji žarna, fermentacija.

Introduction. Numerous *Lupinus* species, including the most common yellow lupine (*L. luteus*), blue lupine (*L. angustifolius*) and white lupine (*L. albus*), are grown in the temperate regions of both hemispheres. They are characterized by a high protein yield per unit area and different soil and climatic requirements, and therefore they can be cultivated in many parts of the world. The results of long-term research show that due to their high nutritional value (Crépon et al., 2010; Jezierny et al., 2010) lupine seeds are a good source of protein and energy (Bach Knudsen, 1997; Muzquiz, 1999; Salgado et al., 2002). Lupine seeds have the highest protein content of all legume seeds, and the quality of lupine protein is comparable to that of soy protein. The nutritional value of lupine seeds is determined by alkaloids which affect nutrient metabolism in the body (Olkowski, 2002). Based on alkaloid concentrations, lupine seeds are classified as low-alkaloid (0%–0.05% alkaloids) or high-alkaloid (0.05%–4% alkaloids). Intensive breeding work has been carried out to find the gene responsible for alkaloid levels in lupine seeds and to develop new lupine varieties with reduced alkaloid content, thus limiting the impact of

alkaloids on the nutritive value of lupine seeds. However, the alkaloid content in lupine seeds remains an important consideration. The currently grown lupine varieties have relatively low alkaloid concentrations which do not pose health risks to humans and animals. The views on the role of anti-nutritional factors (ANFs) have changed over time; when present in low concentrations, they do not always exert adverse effects (Champ, 2002). Other naturally occurring components of lupine seeds are oligosaccharides, poorly absorbed in the small intestine. Oligosaccharides are not digested by enzymes in monogastric animals due to the absence of α-1,6 galactosidase capable of releasing galactose from polysaccharide molecules. Oligosaccharides undergo bacterial fermentation in the cecum. The process is accompanied by the production of gases that cause discomfort in animals (flatulence, diarrhea, nausea), which is why those compounds have been classified as ANFs. Recent studies suggest, however, that oligosaccharides may have a beneficial influence on the large intestine ecosystem in pigs and rats. Raffinose family oligosaccharides may cause digestive problems in humans and animals (Gdala and Buraczewska, 1996), but they can also stimulate digestion processes in the large intestine (Fooks and Gibson, 2002). As a result, lupine varieties have not been selected to decrease the concentrations of raffinose family oligosaccharides in seeds, although those compounds are regarded as ANF_s (Blőchl et al., 2007).

A growing interest in the health-promoting properties of food products has prompted research into the physiological effects of non-digestible oligosaccharides (Campbell et al., 1997; Zduńczyk et al., 2004, Zduńczyk et al., 2006). Lupines, similarly as other grain legumes, offer a variety of benefits (Poetsch, 2006; Badgley et al., 2007) and play an important role in organic farming (Rochester et al., 1998; Evans et al., 2001).

In view of the above, the objective of this study was to determine the effect of different levels and composition of alkaloids and oligosaccharides contained in yellow and blue lupine seeds on growth rate and cecum function in rats, considered an important animal model in studies focused on humans and monogastric animal species.

Materials and methods. The experiment was performed on 56 young male Wistar rats. The animal protocol used in this study was approved by the Local Institutional Animal Care and Use Committee in accordance with resolution No. 07/2011, and the study was carried out in accordance with EU Directive 2010/63/EU for animal experiments. The animals, divided into seven groups, were kept in individual cages and were fed semi-synthetic isoprotein diets. In the control diet, the sole protein source was casein (11.25%) which in experimental diets was replaced with the seeds of one of three yellow lupine cultivars (Mister, Markiz, Taper) or blue lupine cultivars (Sonet, Boruta, Elf). Lupine cultivars were selected based on their nutritional value determined, among others, by the levels and composition of oligosaccharides and alkaloids. In the control diet, the source of fibre was cellulose, and the following components were used in all diets: oil, mineral and vitamin supplements, and corn starch. The composition, alkaloid and oligosaccharide content of diets are presented in Table 1.

Chemical analysis. Lupine seeds were assayed for proximate chemical composition (AOAC, 2003), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL), according to the methods proposed by Van Soest and Wine (1967), and Van Soest (1973), using the Fibertec M system. ADL was determined by ADF hydrolysis with 72% sulfuric acid. The content of saccharose and α-galactosides (raffinose, stachyose, verbascose) was estimated as described by Gulewicz et al. (2000). The levels and composition of alkaloids were determined by capillary gas chromatography and gas chromatography coupled with mass spectrometry (Wink et al., 1995). Feed intake and the body weight gains of rats were monitored throughout the experiment.

Procedures. Conditions of housing rats were described in details by Zduńczyk et al. (1998). Body weight and feed intake of rats were determined individually every week. After four weeks of feeding experimental diets, the animals were euthanized (Close et al., 1997) and their ceca were removed. The weight of the ceca and cecal digesta was determined. The cecal digesta was assayed for ammonia concentrations – by the method of Conway, dry matter content, pH - using a Hanna Instruments pH-meter (model 301) and a microelectrode, activities of bacterial glycolytic enzymes – by the method modified by Juśkiewicz and Zduńczyk (2002). The activity levels of α- and β-glucosidase, α- and β-galactosidase, and β-glucuronidase were measured with a colorimeter, as the amount of p- or o-nitrophenol released from the respective substrates. The concentrations of volatile fatty acids were determined by gas chromatography (Schimadzu GC-14A chromatograph).

Statistical analysis. The obtained results were verified statistically by a one-factor analysis of variance (ANOVA). The statistical significance of differences between the mean values of the analyzed parameters in experimental groups was estimated by Duncan´s multiple range test, using STATISTICA PL 9.0 software.

Results and discussion. Yellow lupine seeds of all cultivars had high protein content, at 387.5–398.5g/kg DM (Table 2). Blue lupine seeds contained lower and different levels of protein (279.9, 300.7 and 317.9 g/kg DM). The levels of crude fibre and NDF varied insignificantly in the studied lupine cultivars, and the seeds of yellow lupine cv. Taper had a relatively low content of ADL and hemicelluloses (7.7 and 13.3 g/kg DM, respectively). The proximate chemical composition of lupine seeds determined in the present study is comparable to that reported by Gdala and Buraczewska (1997), Crépon et al. (2010) and Jezierny et al. (2010), except for the lower concentrations of NDF, ADF and ADL.

Distinct differences in the concentrations of raffinose family oligosaccharides were noted between yellow and blue lupine seeds (Table 3). Higher levels of those

compounds were observed in the dry matter of yellow lupine seeds, where α-galactosides accounted for approximately 90% of raffinose family oligosaccharides, with stachyose as the predominant compound (ca. 50%). The total concentrations of α-galactosides in the analyzed lupine cultivars remained within a narrow range of 108–108.9 g/kg DM. Insignificant differences were observed in the levels of individual α-galactosides. Lower concentrations of oligosaccharides (65.4 – 67.6 g/kg DM), with a lower share of α-galactosides (ca. 70%), including stachyose and verbascose, were noted in blue lupine seeds, at 65.4–67.6 g/kg DM.

The seeds of yellow and blue lupine differed significantly with respect to alkaloid content, which was relatively low in yellow lupine, at 0.007–0.009 mg/100 mg DM (Table 4). Certain differences were noted in alkaloid composition. Sparteine was the only alkaloid present in cv. Mister (100%), in cv. Markiz sparteine was accompanied by lupanine present in small quantities, while tryptophol, lupanine and gramine were found in addition to sparteine (72%) in cv. Taper. Considerably higher concentrations of alkaloids, varying in composition, were noted in blue lupine seeds (0.026, 0.038 and 0.039 mg/100 mg DM). The predominant alkaloid lupanine, which accounted for 75.45% in cv. Sonet, 76.35% in cv. Boruta and 92.38% in cv. Elf, was accompanied by hydroxylupanine, isolupanine and angustifoline as well as low amounts of tetrahydrorombifoline and isoangustifoline.

Diets containing the seeds of three cultivars of yellow lupine and three cultivars of blue lupine, with different concentrations of alkaloids and oligosaccharides (Table 1), had an adverse effect on the growth rate of rats (Table 5). The replacement of casein with lupine seeds in rat diets limited the body weight gains of animals by around 20 g. The weight gains of rats were not affected by lupine species or cultivar. Differences in weight gain were noted despite similar feed consumption levels (417.72 vs. 419.17–424.13 g). The decrease in the growth rate of rats could be due to the presence of alkaloids in lupine seeds, although their amount did not exceed 400 g/kg BW. According to Butler et al. (1996) and Robbins et al. (1996), alkaloid concentrations up to 505 mg/kg BW do not reduce feed intake or weight gains in rats.

Experimental diets contributed to a significant increase in the weight of the cecum (0.66 g vs. 0.80–0.93 g) and cecal digesta (2.42 g vs. 3.29–4.25 g). The increase in cecal digesta weight could result from greater bacterial mass in the cecum (Wróblewska, 2003). The increase is cecal wall weight could be due to the presence of volatile fatty acids, in particular butyric acid, produced during the bacterial degradation of carbohydrates from lupine seeds. An increase in the body weights of rats following diet supplementation with lupine seeds has also been reported by Klessen et al. (2001), Juśkiewicz et al. (2003) and Brunsgaard et al. (1995), who pointed to the risk of cecal hypertrophy in rats fed diets containing non-digestible carbohydrates that undergo bacterial fermentation in the large intestine.

The parameters of bacterial fermentation in the cecum (Table 6) show that experimental diets had a significant effect on the metabolic processes in this segment of the gastrointestinal tract. The diets, particularly those based on blue lupine seeds, increased the acidity of cecal digesta, a key indicator of α-galactoside fermentation (6.40–6.60 vs. 7.03). Yellow lupine seeds had a lesser effect on the pH of cecal digesta (6.89–6.97). A decrease in the pH of cecal digesta in rats fed lupine seeds has also been noted by Wróblewska (2003) and Juśkiewicz (2003). Higher acidity levels could promote the development of beneficial microflora (Bifidobacterium, Lactobacillus) and prevent the growth of harmful bacteria (Topping and Clifon 2001).

The dry matter content of cecal digesta was lower in experimental animals, compared with the control group. Since there is a close correlation between digesta hydration and bacterial fermentation processes, it seems that non-digestible carbohydrates present in lupine seeds could contribute to the formation of water-binding bacterial biomass (Livesey, 2001). As demonstrated by Zduńczyk et al. (1998), the oligosaccharides from lupine seeds contained in diets may reduce water absorption already in the small intestine, due to an increase in digesta viscosity.

The amount of toxic ammonia produced during the bacterial degradation of protein and urea varied insignificantly, and ammonia concentrations in the cecal digesta tended to decrease in rats fed the seeds of all studied lupine cultivars (a non-significant trend). In the current study, ammonia concentrations in the cecal digesta of rats were lower than those reported by Juśkiewicz et al. (2003). According to Gdala et Buraczewska (1997), raffinose family oligosaccharides can reduce protein digestibility in the small intestine, which is why an increase in ammonia levels in the large intestine is accompanied by enhanced bacterial processes. Hambly et al. (1997) found that ammonia present in high concentrations could be a promoter of neoplastic changes in the intestines, and that ammonia production was lower in animals fed low-protein and high-fibre diets.

Experimental diets had a significant effect on the enzymatic activity of cecal microflora (Table 6). The activity of α-glucosidase increased slightly, whereas a highly significant increase was noted in the activity of β -glucosidase (0.40–0.55 vs. 0.19 U/g) capable of hydrolyzing plant glycosides and releasing harmful aglycones (Rowland, 1995). An increase was also observed in the activity levels of α- and β-galactosidases, which are involved in the degradation of structural carbohydrates (mostly cellulose and hemicelluloses) in the cecum. The end products of the process are glucose, galactose or fructose (1.37 - 2.43 vs. 0.51 and 2.49 - 4.62 vs. 1.95 U/g, respectively). The above enzymes play an important role during bacterial fermentation – they break down oligosaccharides to simple sugars by cleaving the glycosidic bonds (Gulewicz and Wardeńska, 2003). An increase in enzymatic activity in the cecal digesta of experimental group rats is indicative of rapid growth of beneficial bacteria in the cecum. According to Gibson and Wang (1994) as well as Gibson and Roberfroid (1995),

oligosaccharides are metabolized by all intestinal bacterial strains, and a particular role is played by Bifidobacterium and Lactobacillus (Gulewicz and Wardeńska, 2003)

The inclusion of lupine seeds in rat diets did not increase the activity of β-glucuronidase which may participate in the activation of procarcinogens in the cecal digesta (Topping and Clifton, 2001) and contribute to releasing toxins that had been stored in the liver. The levels of enzyme activity noted in our study point to changes in the microbial fermentation profile, which corroborates the findings of Bielecka et al. (2002) and Juśkiewicz et al. (2003). The rate of carbohydrate hydrolysis in the cecum was confirmed by an analysis of volatile fatty acids (Table 7). The total concentration of volatile fatty acids in the cecal digesta of control group animals reached 59.9 µmol/g, and it was similar to that noted in rats fed blue lupine seeds (62.02 - 68.48 µmol/g), but highly significantly lower than that determined in animals fed yellow lupine-based diets (80.26-88.25 µmol/g). Higher concentrations of oligosaccharides in yellow lupine seeds had a considerable effect on the rate of carbohydrate hydrolysis.

Acetic acid had the highest share of the total fatty acid profile. Acetic acid is the major by-product of Bifidobacterium fermentation, which modifies the cecal ecosystem (Gulewicz and Wardeńska, 2003). Once absorbed across the cecal epithelium, it is metabolized in muscle tissue. Acetic acid concentration in the cecal digesta of control group rats reached 36.70 µmol/g, and it was comparable with that noted in rats fed blue lupine seeds (33.9–44.6 µmol/g). Significantly higher acetic acid levels (51.45–58.23 µmol/g) were determined in the cecal digesta of rats receiving diets supplemented with yellow lupine seeds (higher oligosaccharide concentrations). No significant differences in propionic acid concentrations were found between groups. Propionic acid is almost entirely metabolized in the liver, and it is capable of inhibiting hepatic cholesterol synthesis from acetic acid (Martins et al., 2005). The propionic acid content of cecal digesta tended to increase with an increase in oligosaccharide concentrations in diets. The levels of butyric acid, which is an important source of energy for epithelial cells, increased after the administration of all experimental diets. According to Salminen et al. (1998), butyric acid regulates cell growth and differentiation. In our study, an increase in butyric acid concentrations was accompanied by an increase in cecal wall weight in experimental rats. The levels of isobutyric acid, isovaleric acid and valeric acid in the cecal digesta of rats varied.

An increase in fatty acid concentrations in the cecal digesta of rats fed diets containing lupine seeds with higher oligosaccharide concentrations correlates with the enhanced activities of glycolytic enzymes.

Table 1. **Composition of experimental diets** (% DM)

Item	Control	Yellow lupine *Lupinus luteus*			Blue lupine *Lupinus angustifolius*		
		Mister	Markiz	Taper	Sonet	Boruta	Elf
Casein	11.25	-	-	-	-	2	2
Lupine	-	24.30	25.00	25.40	25.50	25.10	26.50
Maize starch	71.11	61.90	61.20	60.80	60.70	59.10	57.70
Soybean oil	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Mineral premix	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Vitamin premix	2.00	2.00	2.00	2.00	2.00	2.00	2.00
DL-methionine	0.14	0.30	0.30	0.30	0.30	0.30	0.30
Cellulose	5.00	-	-	-	-	-	-
Daily intake in the diet							
Alkaloids, mg	-	7.57	8.80	7.02	37.96	37.74	26.80
Oligosaccharides, g	-	10.23	10.50	19.92	6.75	6.43	6.74

Table 2. **Chemical composition of lupine seeds** (g/kg DM)

	Yellow lupine *Lupinus luteus*			Blue lupine *Lupinus angustifolius*		
	Mister	Markiz	Taper	Sonet	Boruta	Elf
Total protein	398.5	387.5	391.9	279.9	317.9	300.7
Crude fat	34.2	36.0	34.9	41.9	34.3	32.6
N-free extractives	278.2	276.1	291.6	308.0	364.2	390.4
Crude fibre	161.3	171.0	171.3	155.1	164.9	155.6
NDF	237.9	249.4	223.1	235.6	229.4	214.6
ADF	207.2	211.1	209.8	184.2	193.0	176.8
ADL	12.9	14.5	7.7	11.2	14.7	10.6
Hemicellulose	30.7	38.3	13.3	51.4	36.5	37.8
Cellulose	194.3	196.6	202.1	173.0	178.3	166.2

Table 3. **Oligosaccharide content of lupine seeds** (g/kg DM)

Item	Yellow lupine *Lupinus luteus*			Blue lupine *Lupinus angustifolius*		
	Mister	Markiz	Taper	Sonet	Boruta	Elf
Saccharose	15.4	15.6	14.3	19.2	16.6	14.0
Raffinose	12.3	11.0	9.6	9.6	9.2	8.8
Stachyose	51.4	48.9	57.5	29.9	32.7	35.5
Verbascose	29.0	32.5	27.5	8.9	8.0	7.1
Total α–galactosides	92.7	92.4	94.6	48.4	49.9	51.4
Total oligosaccharides	108.1	108.0	108.9	67.6	66.5	65.4

Table 4. **Composition** (%) **and total content** (mg/100 mg DM) **of alkaloids in lupine seeds**

Item	Yellow lupine *Lupinus luteus*			Blue lupine *Lupinus angustifolius*		
	Mister	Markiz	Taper	Sonet	Boruta	Elf
Sparteine	100	95.58	72.03	-	-	-
Tryptophol	-	-	10.01	-	-	-
Gramine	-	-	3.38	-	-	-
Lupanine	-	4.42	14.58	75.45	76.35	92.38
Isolupanine	-	-	-	5.74	6.84	5.40
Angustifoline	-	-	-	0.96	7.35	1.11
Hydroxylupanine	-	-	-	0.74	4.19	0.44
Tetrarombifoline	-	-	-	1.85	3.59	0.67
Isoangustifoline	-	-	-	15.26	1.68	-
Total mg/100 mg DM	0.008	0.009	0.007	0.038	0.039	0.026

Table 5. **Feed intake and body weights of rats**

Item	Control	Yellow lupine *Lupinus luteus*			Blue lupine *Lupinus angustifolius*			SEM
		Mister	Markiz	Taper	Sonet	Boruta	Elf	
Initial BW, g	112.47	112.65	112.53	112.51	112.42	113.73	112.43	5.830
Final BW, g	245.17^{A}	229.91^{BC}	228.70^{BC}	223.60^{BC}	224.62^{BC}	229.58^{BC}	226.50^{BD}	2.056
BW gain, g	132.70^{Aa}	117.26^{b}	116.17^{b}	111.09^{b}	112.20^{b}	115.85^{b}	114.07^{B}	2.191
Feed intake, g/28 day	417.72	421.32	423.25	423.11	424.13	419.17	423.02	2.340

^{A,B} ≥p 0.01 ^{a,b} ≥p 0.05

Table 6. **Indices of cecal fermentation in rats**

Item	Control	Yellow lupine *Lupinus luteus*			Blue lupine *Lupinus angustifolius*			SEM
		Mister	Markiz	Taper	Sonet	Boruta	Elf	
Cecal tissue, g	0.66	0.83	0.80	0.91	0.85	0.93	0.82	0.120
Cecal digesta, g	2.64^{Ba}	3.65^{b}	3.29^{bd}	3.54^{b}	4.25^{Abc}	3.32^{bf}	4.03^{Abe}	0.120
pH	7.03^{a}	6.89^{c}	6.97^{c}	6.90^{c}	6.59^{bd}	6.40^{bd}	6.60^{bd}	0.038
DM of cecal digesta, %	23.71^{A}	20.50^{Ba}	20.66^{Bc}	20.11^{Bc}	18.36^{Bbd}	17.56^{Bbdf}	20.23^{B}	0.339
Protein, mg/100g	0.13	0.14	0.13	0.16	0.15	0.13	0.17	0.003
NH_3 mg/100g digesta	36.71	33.15	27.02	31.84	31.38	23.68	29.40	1.286
Microbial enzymes, U/g fresh cecal digesta								
α-glucosidase	0.84	0.86	0.89	1.09	0.96	1.28	1.24	0.101
β-glucosidase	0.19^{b}	0.40^{a}	0.42^{a}	0.44^{a}	0.43^{a}	0.41^{a}	0.55^{a}	0.025
α-galactosidase	0.51^{b}	1.73^{b}	2.07^{b}	2.42^{bc}	2.43^{b}	1.37^{d}	1.85^{b}	0.140
β-galactosidase	1.95^{B}	2.49^{B}	3.46	3.84^{A}	4.62^{Aa}	2.74	3.15	1.618
β-glucuronidase	0.61	0.86	0.78	0.83	0.81	0.40	0.86	0.053

^{A,B} ≥p 0.01 ^{a,b} ≥p 0.05

Table 7. **Concentrations of volatile fatty acids** (μmol/g fresh digesta) **in the cecum**

Item	Control	Yellow lupine *Lupinus luteus*			Blue lupine *Lupinus angustifolius*			SEM
		Mister	Markiz	Taper	Sonet	Boruta	Elf	
VFA content	59.90^{Bb}	88.85^{A}	86.85^{A}	80.26^{ac}	62.02^{Bd}	68.48^{B}	59.99^{Bd}	1.862
Acetate	36.70^{b}	55.08^{a}	53.89^{a}	51.45^{a}	33.90^{b}	44.60^{b}	34.40^{b}	1.348
Propionate	10.20	10.50	10.57	9.12	7.90	9.13	8.42	0.362
Isobutyrate	1.80	0.74	0.92	0.76	0.71	0.82	0.82	0.093
Butyrate	8.71^{B}	20.55^{A}	17.09^{A}	15.68^{A}	17.30^{A}	12.01^{A}	14.03^{A}	1.049
Isovalerate	1.54^{b}	1.50^{b}	3.92^{Aa}	2.81^{Aa}	0.81^{B}	0.82^{B}	0.82^{B}	0.073
Valerate	0.95	0.48	0.46	0.44	1.40	1.10	1.50	0.110

^{A,B} ≥p 0.01 ^{a,b} ≥p 0.05

Conclusions

The alkaloids contained in yellow and blue lupine seeds added to experimental diets did not reduce feed intake, but they limited the growth rate of rats, in comparison with control animals fed casein-based diets. The values of the above parameters were not affected by lupine cultivar. The amount and composition of oligosaccharides from lupine seeds had a beneficial influence on cecal function, increasing the activities of bacterial glycolytic enzymes in the cecal microflora and decreasing the pH of cecal digesta and ammonia concentrations in the cecum. Desirable changes in the concentrations and profile of volatile fatty acids in the cecal digesta resulted from the inclusion levels of oligosaccharides in rat diets.

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