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Original article

The effect of Linola and W92/72 transgenic flax seeds on the rabbit caecal fermentation – *in vitro* study

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Abstract

The effect of W92/72 transgenic flax seeds taken from a variety of Linola on the production of SCFA, ammonia and methane by bacteria inhabiting rabbit caecum was studied. The *in vitro* method was used where caecal contents from rabbits was incubated with W92/72 transgenic or Linola flax seeds, or without any additives (control samples).

The total concentration of SCFA was higher in samples with the addition of flax seeds than in the control samples. The increase in concentrations of acetic, propionic and butyric acids was the highest in samples with Linola seeds added. A higher percentage of propionic and butyric acids was observed in the contents incubated with addition of flax seeds as compared to the control samples. This increase was the result of a percentage decrease in acetic acid. No differences were observed in the concentration of ammonia between fermented samples. Moreover, the addition of flax seeds resulted in slight decrease of pH in incubated samples. In gas samples, the methane level was higher in samples with flax seeds added, although the highest level was found in samples with transgenic seeds.

In addition, gas pressure was significantly higher in samples with flax seeds added as compared to control samples, and this may indicate a higher intensity of microbiological fermentation processes. These studies suggest that neither Linola nor W92/72 flax seeds have any unfavorable effect on the caecal microflora activity of rabbits. A beneficial influence of flax seeds on the microbiological fermentation process in rabbit caecum was observed, based on an increase in percentage ratio of propionic acid in samples with flax seeds added.

Key words: caecal fermentation, rabbit, SCFA, methane, flax seeds, GMO

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Introduction

The determination of the effect of feed additives on caecal microflora is one of the most important studies concerning their application in animal nutrition. Microorganism population stability provides the correct absorption of food components and thus their usage by the organism. In recent years, much more attention has been paid to the role of microbiological fermentation in the alimentary tract, and through the analysis of products of bacterial activity, such as short chain fatty acids (SCFA), ammonia and gases, especially methane, its effect on the health of single-stomached animals has been determined. In these animals, the large intestine is a place where saprophytic bacteria occur in the highest abundance; products of their activity are at the highest concentration. The concentration of the final products of bacterial fermentation in the large intestine reflects activity of local microflora and thereby has an effect on the processes running in this particular fragment of the alimentary tract (Bergman 1990).

Many previous studies have demonstrated an interaction between the activity of microorganisms inhabiting the large intestine, the plasma cholesterol level and the risk of coronary heart diseases (Jenkins et al. 1991, Pereira et al. 2004, Wong et al. 2006). Authors of various studies suggest the interaction of microflora through SCFA as the products of their intestinal activity. It was demonstrated that propionate had an inhibiting effect on cholesterol synthesis and lowered cholesterol level in the liver and plasma (Bergreen et al. 1996, Cheng and Lai 2000, Wong and Jenkins 2007). The effect of SCFA on cholesterol levels was observed by Jenkins et al. (2002) as well. The high-fiber diet, which elevated SCFA level in the large intestine, decreased the cholesterol level, HDL, LDL:HDL ratio and B:A-I apolipoprotein level, which resulted in a decrease of risk of the occurrence of coronary heart diseases.

Seeds of oil plants, rich in unsaturated fatty acids, are one of the dietary components which have beneficial effects on lipid metabolism. Flax seeds are considered as one of the richest sources of ω3, mainly α-linolenic acid. Studies have shown the beneficial impact of flax seeds on plasma cholesterol levels (Bierenbaum et al. 1993, Pellizon et al. 2007). However, the unsaturated fatty acids contained in seeds of traditional varieties are susceptible to oxidation. A high level of flax seeds in a diet rich in α-linoleic acid may decrease the level of vitamin E, change the metabolism of prostaglandins, increase oxidation in lipids and finally cause oxidation stress (Ratnayake et al. 1992). However, the beneficial effect of using flax seeds with a decreased level of α-linoleic acid on lipid metabolism was noted (Prasad et al. 1998). Recently, a significant interest in new varieties of flax seeds with altered proportions of fatty acids was observed. These varieties are subject to genetic modifications in order to increase the pro-healthy values of their seeds. The background variety for the transgenic line used in these studies is Linola (*Linum usitassimum var. Linola*) with a relatively low level of α-linolenic acid (up to 2.45% of total fatty acid content) and a very high quantity of linoleic acid (up to 69% of total fatty acid content). The reduced content of linolenic acid resulted in an improvement of the oxidative status of this plant. A further improvement of the plant antioxidative status was achieved by transgenic plant generation. The W92/72 transgenic line was obtained from the Linola variety. This line has elevated levels of antioxidant compounds, which has been demonstrated in previous studies (Lorenc-Kukuła et al. 2005, 2007). The expression of cDNAs encoding key enzymes of polyphenols biosynthesis, caused an increase in the antioxidant capacity in these plants and their seeds, which resulted in improved antioxidant properties (Lorenc-Kukuła et al. 2007). It is supposed that the high level of natural antioxidants in flax seeds of new line will significantly decrease the degree of fatty acid oxidation and thereby have an effect on lipid metabolism in the organism of consumer.

The aim of the present study was to determine the effect of flax seeds of the new transgenic line, W92/72, as well as Linola variety on the microorganisms activity in rabbit caecum. This was based on the analysis of the main products of *in vitro* microbiological fermentation.

Materials and Methods

Animals and experimental design

Nine White Giant rabbits aged 12 weeks were used (average body weight was 4416 ± 397 g). The animals were housed in stainless steel cages with a free access to drinking water and were given *ad libitum* access to a standard pelleted diet (Table 1) according to the recommendations given for feeding standards (de Blas and Wiseman 1998). After a 3-week adaptation period to the diet, the animals were slaughtered (using xylazine and sodium pentobarbital injections) in order to collect caecal contents for analyses.

The experiment was performed after receiving approval from the Local Ethics Commission for Experiments on Animals in Wroclaw, Poland (license No. 65/2009).

Table 1. Composition and nutritional values of the basal feed mixture.

Ingredients	
Dried grass [%]	13
Dried alfalfa [%]	15
Wheat bran [%]	17
Wheat grain [%]	10
Barley grain [%]	10
Corn [%]	15
Extraction soybean meal [%]	10
Rape meal [%]	5
Rapeseed oil [%]	1
Vitamin-mineral premix[1] [%]	4
Chemical analysis	
Crude protein [%]	17.00
Crude fat [%]	3.60
Crude fibre [%]	11.80
Crude ash [%]	5.50
Nitrogen free extract [%]	47.60
Starch [%]	21.50
Soluble sugars [%]	7.40
Metabolizable energy [MJ/kg]	10.95
Linoleic acid [%]	0.46
Linolenic acid [%]	0.09
Calcium [%]	0.95
Phosphorus [%]	0.45
Natrium [%]	0.15
Chlorine [%]	0.40
Potassium [%]	1.65
Magnesium [%]	0.43

[1] Premix provided per kg of diet: vitamin A, 15000 IU; vitamin D_3, 1504.0 IU; vitamin B_1, 8.7 mg; vitamin B_2, 13.1 mg; vitamin B_6, 7.1mg; vitamin B_{12} 0.01 mg; vitamin E, 57.2 mg; vitamin C, 73 mg; vitamin K, 39.9 mg; folic acid, 1.7 mg; niacin, 63.10 mg; pantothenic acid, 30.1 mg; choline 884 mg; inositol, 1334 mg; biotin, 0.272 mg; Mn, 80 mg; Cu, 15 mg; Fe, 197 mg; Zn, 62 mg; Co 0.523 mg; iodine, 0.638 mg; Se, 0.157 mg; F, 0.032 mg.

In vitro caecal fermentation

Immediately after dissections, the caecal contents were squeezed out and mixed with a spatula and their pH were measured using a pH-meter CP-401 (ELMETRON, Zabrze, Poland) with a EPP-3 electrode and temperature sensor. The caecal contents from each rabbit were divided into four subsamples of 20 g each. Each subsample was diluted fivefold with a buffer solution at pH 7.2 (Adjiri et al. 1992) and transferred into serum bottles of capacity 125 ml (Sigma-Aldrich). One subsample was not incubated (blank) and immediately was mixed with $HgCl_2$ to stop the fermentation process. One of the other subsamples was a control and had no addition of flax seeds. To the remaining two subsamples, the ground Linola or W92/72 seeds were added, respectively, in amount of 0.6 g/10 g of the fresh caecal contents. The bottles were thoroughly flushed with CO_2 and hermetically closed by a manual crimper (Restek) using rubber stoppers and aluminum crimp seals with removable center (Sigma-Aldrich). The incubation was performed in a shaking water bath at 39°C for 12 h. At the end of incubation, the fermentation gas was sampled with a gas-tight syringe for analyses. In the remaining liquid sample, fermentation was stopped by adding $HgCl_2$.

Plant material

The Linola and W92/72 flax seeds were obtained from the Department of Genetic Biochemistry, Faculty of Biotechnology, University of Wroclaw, where they were cultivated and where the W92 plants were generated. Briefly, for transformation the binary vector containing three cDNAs from *Petunia hybrida*, encoding chalcone synthase (CHS, EMBL/GenBank database acc. No. **X04080**), chalcone isomerase (CHI, EMBL/GenBank database acc. No. **X14589**) and dihydroflavonol reductase (DFR, EMBL/GenBank database acc. No. **X15537**) in the sense orientation under the control of the 35S promoter and OCS terminator was used (Lukaszewicz et al. 2004). The transgenic plants were preselected *via* PCR using primer specific for the kanamycin resistance gene (*npt II*), and then selected by means of a northern blot analysis. The details of plant transformation, selection and transgenic plant analysis were described previously (Lorenc-Kukuła et al. 2005). Flax seeds (cv. *Linola*) from control and transgenic plants were grown on a semi-technical scale (1ha each) in a field in the vicinity of Malbork, Poland; and the seeds were harvested after 3 months of growth.

The transgenic plants have an enhanced essential flavonoid biosynthesis, through the simultaneous overexpression of three genes encoding enzymes responsible for flavonoids synthesis, such as chalcone synthase, chalcone isomerase, and dihydroflavonol reductase. All genes are under the control of the 35S promoter. As a result of such overexpression, plants demonstrate an increased level of natural antioxidants, flavonoids, such as kaempferol and quercetin, as well as secoisolariciresinol diglucoside, the main lignan occurring in flax (Lorenc-Kukuła et al. 2007). The fatty acids composition of oil from Linola and transgenic seeds W92/72 are presented in Table 2. W92/72 flax seeds have significant higher level of monounsaturated fatty acids (MUFA) as opposed to seeds from background varieties (Linola) (Table 2). The W92/72 line contains the highest amount of MUFA among all of the studied transgenic varieties. The level of polyunsaturated fatty (PUFA) acids is also higher than in Linola seeds (Żuk et al. 2011).

Table 2. Fatty acids composition of oil from Linola and transgenic seeds W92/72 expressed in µg/gFW.

	Linola	W92/72
16:0	11.3	13.8
16:1	0.16	0.26
16:2	0.12	0.18
16:3	0.10	0.12
18:0	6.38	6.49
18:1	32.6	34.7
18:2	**158.6**	**218.5**
18:3	**3.64**	**5.39**
20:0	0.26	0.18
20:1	0.19	0.29
22:0	0.12	0.13
22:1	0.10	0.15
24:0	0.03	0.05
Total	**213.6**	**280.1**
Saturated	18.1	20.6
Unsaturated	195.4	259.6
PUFA	162.4	224.2
MUFA	33.0	35.4

Technologies 7890A) with a flame ionization detector (FID) and Agilent J&W column DB-23, with helium carrier gas, in order to determine the total SCFA as well as acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic and caproic acids concentrations. The ammonia in the samples was separated by micro-diffusion in Conway units and determined with a Nessler reagent using a spectrophotometer (Bio-Rad Smart Spec 3000) at a wavelength $\lambda = 410$ nm. The pH value was measured in all samples.

Data were subjected to a one-factorial ANOVA using the STATISTICA 8.0 software package. The significance of differences was determined using the multiple comparison Tukey's test for $P < 0.05$ and $P < 0.01$.

Results

The average pH value of the caecal contents collected directly after slaughtering the animals was 5.88 ± 0.05. Because a buffer pH 7.2 was added, the diluted samples had higher pH, which ranged from 6.38 to 6.89. The pH of incubated samples was statistically lower than the pH of blank samples ($P < 0.01$, Table 3). Samples, where flax seeds were added, had a slightly lower pH than samples incubated without them.

Table 3. pH values and SCFA and ammonium concentrations of the fermented caecal samples with flax seed addition.

		Blank	Control	Linola	W92/72	SEM
pH		6.89^{A}	6.46^{B}	6.38^{B}	6.38^{B}	0.02
SCFAs	Total*	57.5^{A}	152.4^{B}	201.5^{B}	178.2^{B}	12.97
	Acetic*	28.8^{A}	84.4^{B}	109.3^{B}	94.9^{B}	8.71
	Propionic*	8.29^{A}	21.1^{Ba}	30.9^{Bb}	28.0^{B}	2.35
	Isobutyric*	1.63^{A}	4.26^{B}	5.11^{B}	4.32^{B}	0.39
	Butyric*	12.3^{A}	34.9^{B}	43.2^{B}	42.9^{B}	2.87
	Isovaleric*	1.52	1.78	2.78	3.09	0.56
	Valeric*	1.45^{A}	2.40	3.21^{B}	3.90^{B}	0.26
	Isocaproic*	0.41	0.71	0.88	0.44	0.29
	Caproic*	1.29^{a}	1.73	2.28^{b}	1.93	0.24
	Acetic (%)**	58.7	60.8	57.2	57.4	1.94
	Propionic (%)**	16.6	14.7	16.7	16.6	1.03
	Butyric (%)**	24.8	24.5	26.1	26.0	1.40
Ammonia		6.7^{a}	16.2^{b}	16.2^{b}	16.5^{b}	2.21

Explanations: "Blank" – not incubated samples, "Control" – samples incubated without additives, "Linola" – samples incubated with Linola seed addition, "W92/72" – samples incubated with W92/72 seed addition
*) mmol/kg of caecal content
**) molar proportion in the total concentration of the acetic, propionic and butyric acid
A, B – values in rows with different letters differ significantly at $P < 0.01$
a, b – values in rows with different letters differ significantly at $P < 0.05$

The total concentration of SCFA increased by about 3-times in the incubated contents after 12-hours of fermentation as compared to the blank samples ($P < 0.01$, Table 3). No statistical differences between incubated samples were observed. However, a higher concentration of SCFA was found in caecal contents with addition of flax seeds, both the Linola and W92/72 varieties, than

Analyses

Gas samples were analyzed for methane using the Natural Gas Analyzer Model Arnel 2008 (Perkin Elmer) with a back-flush system and thermal conductivity detector (TCD) and four columns: two columns packed with DC 230, a column with a molecular sieve 13X and a HST6 column. A helium was used as a carrier gas. Additionally, before the gas analysis the manometric pressure inside each bottle was measured.

Liquid samples were centrifuged (2800 g per 20 min) and analyzed on a gas chromatograph (Agilent

the control samples; whereas the highest increase of SCFA was observed in samples with addition of Linola seeds.

Individual SCFA levels increased to various degrees in incubated samples in relation to the blank samples. The highest increase was noticed for acetic acid, propionic acid and butyric acid, especially in samples with addition of Linola seeds, where their concentration increased almost 4-times ($P < 0.01$). In the case of propionic acid, the increase was significant in relation to the control samples ($P < 0.05$, Table 3). In the samples incubated with addition of W92/72, the concentrations of the above-mentioned acids were also higher than in the control samples. Similarly, the concentration of isobutyric acid was the highest in samples with Linola seeds, where a 3-fold increase was noted in comparision with the blank samples ($P < 0.01$). A slightly smaller increase was observed for isovaleric acid, valeric acid, isocaproic acid and caproic acid in incubated samples and usually the highest concentrations occurred in samples where flax seeds were added. The highest values of isovaleric and valeric acids were observed in samples with W92/72 seeds, while in the cases of isocaproic and capronic acids this increase was only observed in samples with addition of Linola seeds (Table 3).

The percentage levels of the most important SCFA (acetic, propionic and butyric acids) in their total concentration was similar in all studied samples. However, the higher level of propionic acid, as compared to control samples, and butyric acid as compared to both blank and control samples was observed in the samples incubated with addition of flax seeds. The increase in quantity of the above-mentioned acids was made at the expense of decreasing percentage level of acetic acid.

The concentration of ammonia was significantly lower in all incubated samples in comparison with blank samples (Table 3). No differences between the fermented samples were noticed.

The methane level in the gas samples collected after the end of fermentation was higher in the samples where seeds were added and the highest level of methane was observed in samples after the addition of W92/72 seeds (Table 4). Moreover, a significantly higher pressure of gas was observed in samples with both kinds of seeds added as compared to control samples ($P < 0.05$), which suggests the higher intensity of microbiological fermentation processes.

Discussion

Rabbit is often used as a model in *in vitro* experiments concerning microbial fermentation since its relatively large caecum (40% of the digestive tract), which is colonized by symbiotic bacterial populations. Caecal pH and SCFA concentrations are classical variables characterizing the extent and the pattern of caecal fermentation, and changes of these values are correlated with the health status of rabbits (Garcia et al. 2002).

The fermentation in rabbit caecum resembles changes occurring in the rumen of ruminants; however, there are some traits differentiating both processes. A characteristic trait of fermentation in the caecum of young rabbits is a hydrogen-dependent acetate formation (reductive acetogenesis), which with age is partially substituted with methanogenesis (Piattoni et al. 1996). In the rumen, reductive acetogenesis is of relatively small significance, with the exception of a short time after birth (Morvan et al. 1994). Other trait differentiating processes occurring in rabbit caecum from fermentation occurring in rumen are SCFA mutual proportions. Contrary to rumen, the concentration of butyrate is higher than propionate in the caecum of rabbit (Garcia et al. 2002, Bennegadi-Laurent et al. 2004).

The most important products of caecal fermentation are SCFA, ammonia, and gases (mainly CO_2, CH_4 and H_2). SCFA are mostly produced as a result of carbohydrate degradation, such as cellulose, hemicellulose, pectin, starch, other dextrans and soluble carbohydrates, and also through the bacterial decomposition of proteins and fat. Branched-chain fatty acids, such as isobutyric and isovaleric acids, may be formed as the result of amino acids fermentation. In the alimentary tract, acetic,

Table 4. The methane production and gas pressure of the fermented caecal samples with flax seed addition.

	Control	Linola	W92/72	SEM
Methane*	7.42	8.98	9.54	2.07
Gas pressure**	124.0^{A}	135.5^{B}	135.1^{B}	1.74

Explanations: "Control" – samples incubated without additives, "Linola" – samples incubated with Linola seed addition, "W92/72" – samples incubated with W92/72 seed addition

*) mmol/kg of caecal content

**) kPa

A, B – values in rows with different letters differ significantly at $P < 0.01$

propionic and butyric acids are produced in the highest amounts and, after the absorption, are used as a source of energy in epithelial cells of the large intestine. Propionate is a very effective substrate for glucose synthesis, and participates in glycogenesis and the formation of long-chain fatty acids in the liver. Acetate participates in lipogenesis, milk fat synthesis, cholesterogenesis and ketogenesis (Bergman 1990, Rémésy et al. 1995).

An accumulation of products of bacterial activity occurs in fermented chyme during *in vitro* fermentation (Adjiri et al. 1992). In our studies, an increase of molar concentrations and changes in the mutual rations of most analysed SCFA, was observed during *in vitro* fermentation. To facilitate the comparison with available data in literature, the percentage levels of three major acids at their total concentration were estimated (Garcia et al. 1995, Piattoni et al. 1995, Gidenne et al. 2002, Bennegadi-Laurent et al. 2004).

Present studies do not demonstrate significant effect of W92/72 or Linola seeds on SCFA profile in the caecum of the rabbit, which may suggest that there are no unfavorable effects of these seeds on intestinal microflora. Available literature is scant in information about effect of flax seeds on fermentation processes in the caecum of the rabbit. The effect of flax seeds on the profile of microbiological fermentation in the cow rumen was studied by Gonthier et al. (2004). The researchers observed decrease in the molar proportion of acetic acid and an increase of propionic acid in flaxseed-fed animals. Similarly to present studies, this situation resulted in a decrease of the acetate:propionate ratio as compared to control samples. However, no increase in the butyric acid molar proportion was observed. Different results were obtained by Beauchemin et al. (2009), who in a similar study introduced calcium salts of long-chain fatty acids as a source of fat in the diet. No differences were observed in the levels of acetate and propionate during analyses of ruminal fermentation patterns in cows; however, the molar proportion of butyrate decreased when flax seeds were added.

An increase in SCFA concentration after the flax seeds addition was also obtained during *in vitro* fermentation using human faecal microbiota. No changes were observed in the proportions of acetic, propionic and butyric acids, both in *in vitro* and *in vivo* rat models (Aura et al. 2006).

Similarly to our research, other studies have shown changes in SCFA proportions in the alimentary tract after the addition of fat. During fermentation in sheep rumen, the effect of adding *cis*-oleic and linoleic acids occurred as a decrease in the percentage of acetic acid and an increase in the percentage level of propionic acid, with small differences in percentage levels of butyric acid. However, linolenic acid had the influence on an increase of butyrate level (Zawadzki 1993).

The addition of fat also decreased the acetate:propionate ratio during *in vitro* fermentation of rabbit caecum; a similar effect was obtained using soybean oil and lard (Chen and Li 2008). In this case an increase of butyric acid concentration in caecum was also observed. The decrease of acetic:propionic acid ratio in caecum was obtained after sunflower oil feed supplementation (Falcão-e-Cunha et al. 2004). However, this addition decreased the total SCFA concentration. Although, it should be highlighted that both effects concerned rabbits that were fed by fodder consisting mostly of wheat bran; no such effect was observed after the addition of fat fodder based on dehydrated alfalfa or dehydrated beet pulp. Similar results were obtained in our previous studies on rabbits where a humic-fatty preparation containing fatty acids from rapeseed oil was used. An increase in the level of propionic acid and a decrease in the acetate:propionate ratio in caecal content was noticed during *in vitro* study (Mišta 2007).

The environment of the large intestine is considered to be strongly proteolytic thanks to the presence of the population of amino acid-fermenting bacteria. As a result of the activity of the bacteria, ammonia, CO_2 and, depending on the substrate, various VFA are created, including acetic, butyric, propionic, isobutyric, 2-methylobutyric or isovaleric acid. A series of factors influence the amino acid fermentation and hence the production of ammonia, including H_2 pressure, chyme reaction, the presence of carbohydrates (for example, glucose inhibits the activity of some proteolytic bacteria), and, under the *in vivo* conditions, a time in which the chyme remains in the intestine (Macfarlane and Gibson 1995).

According to Garcia et al. (2002), the concentration of ammonia in the rabbit caecum ranges between 1.86 and 23.9 mmol/l and our recent data are within these limits. This value depends mainly on the age and dietary components, especially fibre and protein (Garcia et al 1995, Gidenne 1997). When protein ingestion exceeds its requirements, the return of urea with blood to the caecum may increase leading to an increase of the ammonia concentration (Fraga 1998). In an experiment of Garcia et al. (1995), a decrease in the ammonia concentration in the rabbit caecum, with an increase of the natural detergent fibre content in rabbit diet, was observed. Bellier et al. (1995) found a decrease of the ammonia content with the age of an animal, as opposed to the VFA content, which grew at the same time. However, Gidenne and Bellier (1992), who used caecal cannulation in their experiment, found the highest level of ammonia in the caecum of

the adult rabbits with an empty stomach, which amounted to 24.4 mmol/l on the average, whereas the same value was half as high (11.5 mmol/l) in the case of the young rabbits. The differences decreased after the morning meal and reversed 9 hours later, and the ammonia concentration decreased by 75% at the same time.

Gonthier et al. (2004) observed small decrease in ammonia production during ruminal fermentation after the addition of flax seeds. On the other hand, a slightly higher concentration of NH_3 in cow rumen was found by Beauchemin et al. (2009) after the addition of flax seeds. It should be noted that sunflower seeds caused significantly higher production of ammonia in the above-quoted studies. In the present study, no differences were observed in ammonia production during intestinal fermentation. No significant differences in the concentration of ammonia in rabbit caecal contents after the addition of plant fats (soybean oil) were also observed by other authors (Chen and Li 2008).

In the present study, pH value of rabbit caecal contents decreased during fermentation because of the accumulation of acids produced by microflora. A slight decrease in pH values was observed after the addition of flax seeds to fermented caecal contents; similarly as in Chen and Li (2008) studies after the addition of soybean oil. A very small decrease in pH under the influence of flax seeds was observed during ruminal fermentation (Gonthier et al. 2004, Beauchemin et al. 2009). Beauchemin et al. (2009) found a slightly higher decrease of pH value after the addition of sunflower seeds.

In these studies, the methane concentration increased during fermentation with addition of flax seeds, especially the W92/72 line. This increase may be explained by the enlargement of fermentation intensity in those samples and the production of higher amount of gases, which is confirmed by their higher pressure in analysed samples (Table 3). However, a decrease of methane production after addition of flax seeds was observed during ruminal fermentation by Beauchemin et al. (2009).

In conclusion, the obtained results suggest that both: the new transgenic line W92/72 and Linola seeds did not have negative effects on the microbiological processes occurring in the ceacum of rabbit. Moreover, the increase of molar proportion of propionic acid under the influence of these flax seeds addition was observed. This is favorable because of the positive effects of propionate on lipid metabolism which was shown by other authors (Bergreen et al. 1996, Cheng and Lai 2000, Jenkins et al. 2002, Wong and Jenkins 2007).

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