Ann. Anim. Sci., Vol. 13, No. 1 (2013) 73–83, DOI: 10.2478/v10220-012-0060-0

THE EFFECT OF DIFFERENT FORMS OF SUNFLOWER OIL AND PROTEIN SOURCES IN THE DIET ON PANCREATIC JUICE SECRETION AND PANCREATIC ENZYMES ACTIVITY IN SHEEP*

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Abstract

The aim of the study was to determine the effect of various forms of sunflower oil in the diet with protein degraded at different rates in the rumen on pancreatic juice secretion and activity. The experiment was conducted on 24 adult Corriedale rams weighing about 40 ± 1.5 kg, catheterized in the pancreatic and bile ducts and fistulated in the duodenum. The animals were fed diets consisting of meadow hay, potato starch, different degradable protein (casein or maize gluten, a source of zein) and different forms of sunflower oil (calcium salts, seeds and oil). It was stated that addition of various forms of fat to the diet did not significantly influence the secretion of pancreatic juice, regardless of the source of protein. However, sunflower seeds and oil used in the diet had a significant effect on bile secretion, protein content, proteolytic activity of trypsin and plasma lipid indices. No significant differences were observed in the lipolytic activity of the pancreatic juice, although lipase activity was higher when zein was used as the main protein source. It was concluded that dietary addition of certain combinations of protected or unprotected sunflower oil and different degradable protein may improve pancreatic activity and probably affect plasma lipid indices in sheep.

Key words: sheep, sunflower oil, zein, casein, secretion of pancreatic juice, pancreatic enzymes

High-yielding ruminants require high energy and protein concentrations in the diet. To fulfil these needs, various sources of them are used including oil or full-fat seeds and proteins, the ruminal degradability of which varies significantly. The composition of a diet can influence microbial activity in the rumen and the composition of digesta flowing to the stomach and duodenum (Kowalski, 1997).

The proteolytic activity of the pancreas and its capacity for proteolytic adaptation depending on protein source and concentration in the diet is less investigated in ruminants than in monogastric animals. However, the composition of protein flowing

*Research funded by the Ministry of Science and Higher Education, Grant No. 2 P06Z 046 27.

to the duodenum is more stable due to rumen fermentation, in which the main protein flowing to the small intestine is microbial. In animals with well-developed forestomach, feed intake and digesta flowing into the duodenum are also less diet dependent than in monogastric animals, because of constant inflow of digesta (Hvelplund and Madsen, 1985; McAllan et al., 1988).

However, it is thought that an increase in lipolytic activity of the pancreas is associated with increased fat concentration in the diet, and thus also in the small intestine. In ruminants, most of fat flowing to the duodenum is in the form of saturated fatty acids, mainly palmitic and stearic fatty acids. The protection of vegetable oils against hydrogenation in the rumen can significantly influence changes in composition of fat flowing to the duodenum, in particular the concentration of triglycerides (Bauman and Griinari, 2003).

In the literature there are only a few studies about dietary compounds affecting pancreatic juice secretion and pancreatic enzyme activity. Some results of these experiments are inconsistent. It is not clear if the differences between nutrient digestion in the small intestine are caused by the differences in the extent of secretion and activity of pancreatic juice enzymes. Khorasani et al. (1990), when supplementing diets with soybean and rapeseed meal, which are known for their different digestion rates, did not observe any significant differences in trypsin and chymotrypsin activity in pancreatic juice, as well as in its secretion. However, opposite findings were noted after casein infusion to the duodenum (Ben-Ghedalia et al., 1982). In some experiments carried out on sheep, rapeseed oil in comparison with protected oil in the form of salts was found to have an effect on pancreatic juice and bile secretion (Rawa et al., 2007). However, Johnson et al. (1974) noted a decrease in secretion of pancreatic juice and lipase activity during saffron or coconut oil infusion to the duodenum.

The effect of the diet with different forms of sunflower oil, protected or unprotected against bacterial hydrolysis in the rumen on intestinal digestion has received less study. Knowledge of the correlations between amount or forms of fat, different digestion rates of dietary protein flowing to the small intestine and also the activity of pancreatic enzymes could show ways of improving nutrient digestion by ruminants. Different degradable protein (casein or zein) and different forms of sunflower oil with a high concentration of linoleic acid may change the content of duodenal digesta, which probably affects the secretory activity of the pancreas.

The aim of the study was to determine the influence of different forms of sunflower oil (SFO) and protein sources in the diets for sheep on pancreatic juice secretion and activity of pancreatic enzymes. Sheep were used as a model for ruminants.

Material and methods

The experiment was carried out on 24 adult Corriedale rams weighing about 40 ± 1.5 kg, fitted with catheters in the pancreatic and bile ducts (Kato et al., 1999; Pierzynowski, 1983). The animals were also fistulated with modified duodenal can-

nulas, which allowed pancreatic and biliary juice to return to the duodenum after the samples were taken (Rawa et al., 2008). The procedures were accepted by the Local Ethics Committee for animal experiments.

The animals were fed twice a day at 8 a.m. and 4 p.m. with two isoprotein diets (17% of crude protein in DM) consisting of meadow hay (600 g), potato starch (500 g), casein (140 g) – which contained 120 g of easily degradable protein in the rumen, or maize gluten (200 g) containing 120 g of zein, which is slowly degraded in the rumen. Moreover, diets were enriched with unprotected and protected forms of fat against bacterial hydrolysis in the rumen – sunflower oil (5% in DM) or its forms in amounts which allowed reaching 5% oil in DM, i.e. calcium salts of sunflower fatty acids oil or whole dehulled sunflower seeds. The control diet was not supplemented with fat. For animals from the groups which received no sunflower seeds in the diet, the protein level was compensated by sunflower oil meal addition. Total protein level in the diet met lamb requirements according to IZ-INRA standards (1993). The level of energy content varies because of oil supplementation.

After a two-week adaptation and feeding period the animals were moved to metabolism cages, where the pancreatic juice was collected for 8 hours during three days. The pancreatic juice and bile were collected into beakers placed in a cold bath after feeding time, which were weighed after every 30 minutes. After 5% of pancreatic juice was collected, taken and cooled, the remainder was mixed with bile and pumped back into the duodenum by a peristaltic pump.

After the end of collection, the animals returned to the rearing pens and a new diet with another protein source was given. The same procedure was repeated with all forms of fat and different sources of protein (Table 1).

Table 1. Experimental design

Protein in basal diet		Form of fat added to the basal diet (C)
Casein	Zein	
C	C	Without fat addition (control)
Ca-S	Ca-S	Calcium salts of sunflower oil
S	S	Sunflower seeds
O	O	Sunflower oil

The blood plasma collected from the jugular vein 2 h after feeding was analysed for total protein, lipase and cholesterol. The experimental samples of lipolytic and proteolytic enzymes were stored at –80°C. The protein content was analysed by the method of Lowry et al. (1951), trypsin and chymotrypsin activity according to Hummell (1959), and lipase by SIGMA Diagnostics LIPASE-PS, Procedure No. 805-B. The total protein level in blood plasma was analysed using the method described by Lowry et al. (1951), and lipase activity and cholesterol concentration using a VITROS DT 60 II analyser. The results obtained were subjected to one-way analysis of variance for every experimental factor (forms of fat or different degradable protein) with Tukey test (Statgraphics Plus 7.0). Significance was declared at $P \leq 0.05$ and $P \leq 0.01$.

Results

Different forms of sunflower oil in the diet did not significantly influence the pancreatic juice secretion, but the impact of protein on this index was noted (Table 2). The secretion of pancreatic and pancreatico-biliary juice in sheep fed diets with sunflower seeds (S) and maize gluten, containing protein with low rate of ruminal degradation (zein), was significantly higher in comparison to the animals receiving casein (P≤0.05).

However, a substantial decrease was observed in pancreatic and pancreatico-biliary juice secretion in group O receiving dietary zein (P≤0.05).

Bile secretion was affected partly by both experimental factors. In the group of animals receiving sunflower oil with dietary casein, an increase in bile and pancreatico-biliary juice secretion was noted in comparison to groups C and Ca-S (P≤0.01). A similar situation was observed for sheep fed diets with sunflower seeds and casein (P≤0.05). The source of protein strongly affected bile secretion in group S supplemented with zein, where a significant increase was observed (P≤0.05).

Table 2. Secretion of pancreatic juice, bile and pancreatico-biliary juice (ml/h)

Groups	Casein	Zein	SEM, for the row
Secretion of pancreatic juice			
C	16.7	17.5	0.90 NS
Ca-S	16.8	17.5	0.54 NS
S	15.0	17.9	0.63*
O	17.5	15.0	0.52*
SEM, for the column	0.42 NS	0.47 NS	
Secretion of bile			
C	61.2 Aa	67.2 a	3.42 NS
Ca-S	60.1 Aa	68.2 a	3.46 NS
S	69.8 ABa	88.4 b	3.47*
O	89.3 Bb	73.2 ab	3.97 NS
SEM, for the column	2.69 0.01	2.77 0.05	
Secretion of pancreatico-biliary juice			
C	77.9 A	84.7 a	3.53 NS
Ca-S	76.9 A	85.7 a	3.70 NS
S	83.8 A	106.3 b	4.16*
O	106.8 B	88.1 a	4.12*
SEM, for the column	2.77 0.01	3.02 0.05	

C – control, Ca-S – calcium salts of sunflower oil, S – sunflower seeds, O – sunflower oil.
SEM, for the row * – P≤0.05; NS – non-significant.
SEM, for the column a, b – P≤0.05; A,B – P≤0.01.

The protein content in pancreatic juice differed for both experimental factors (Table 3). The addition of sunflower oil to the diet increased protein content in pancreatic juice, when zein was used as protein (P≤0.05). In turn, the lowest concentration of protein was observed after full-fat seeds were supplemented (P≤0.05). The opposite results were stated for animals receiving casein in the diet (P≤0.01).

Moreover, casein increased the protein content of pancreatic juice in all feeding groups except for the O group (P≤0.01).

Table 3. Protein content in pancreatic juice

Groups	Protein content in pancreatic juice (mg/ml)		SEM, for the row
	Casein	Zein	
C	79.6 AB	71.7 ab	3.57 NS
Ca-S	90.4 B	79.0 a	4.62 NS
S	89.9 B	61.5 b	3.69**
O	67.2 A	81.3 a	3.44*
SEM, for the column	2.70 0.01	2.73 0.05	-

C – control, Ca-S – calcium salts of sunflower oil, S – sunflower seeds, O – sunflower oil.
SEM, for the row * P≤0.05; ** P≤0.01; NS – non-significant.
SEM, for the column a, b – P≤0.05; A,B – P≤0.01.

Table 4. Trypsin, chymotrypsin and lipase activity in pancreatic juice

Groups	Casein	Zein	SEM, for the row
Trypsin activity (U/ml)			
C	70.3 ABa	65.3 AB	3.31 NS
Ca-S	89.1 Bb	90.5 C	3.65 NS
S	72.5 ABa	52.3 A	3.44**
O	58.4 Aa	76.9 BC	3.11*
SEM, for the column	2.39 0.01	2.50 0.01	-
Chymotrypsin activity (U/ml)			
C	19.7 Aa	29.0 AB	1.09**
Ca-S	41.2 Cc	35.5 B	2.08 NS
S	34.5 BCd	23.4 A	1.77**
O	26.6 ABb	45.8 C	1.91**
SEM, for the column	1.16 0.01	1.28 0.01	-
Lipase activity (U/L)			
C	837	948	36.9 NS
Ca-S	841	935	45.6 NS
S	869	932	35.6 NS
O	910	938	40.8 NS
SEM, for the column	28.6 NS	28.4 NS	-

C – control, Ca-S – calcium salts of sunflower oil, S – sunflower seeds, O – sunflower oil.
SEM, for the row * P≤0.05; ** P≤0.01; NS – non-significant.
SEM, for the column a, b – P≤0.05; A,B – P≤0.01.

It was noted that source of protein and forms of fat used in the diet had an influence on proteolytic activity in pancreatic juice (Table 4).

Taking into account the forms of fat, the highest trypsin activity was observed in Ca-S groups, irrespective of protein source (P≤0.01). However, the lowest concentration for this enzyme was stated in animals which received combinations of sunflower oil and casein, and sunflower seeds and zein, respectively (P≤0.01).

On the other hand, when we consider the source of protein added to the diet, the highest trypsin activity was found in group Ca-S. However, significant differences were observed for S and O groups between casein and zein groups.

Similar to trypsin activity, the diet with casein and calcium salts of sunflower oil offered to the animals contributed to the highest activity of chymotrypsin in comparison to groups C and O (P≤0.01). However, the lowest concentrations of this enzyme were obtained in group C (P≤0.01).

Furthermore, where zein and sunflower seeds were added to the diet, the values of chymotrypsin were significantly reduced, while sunflower oil addition improved this parameter. Source of protein significantly affected chymotrypsin activity in all oil groups except for zein and oil supplementation.

Both experimental factors did not significantly influence lipase activity in pancreatic juice, although there was increased lipolytic activity in animals receiving dietary zein, regardless of the form of fat used.

In lambs fed diets with sunflower oil, a significant increase in plasma lipase activity in comparison to other experimental groups was observed (P≤0.05) (Table 5). The addition of calcium salts of sunflower oil with zein substantially decreased total protein concentration in blood plasma when compared to other experimental groups (P≤0.01). No significant influence of different forms of fat on total cholesterol level was stated, although elevated total cholesterol concentrations as a result of sunflower oil and sunflower seeds supplementation were observed for both protein sources.

Table 5. Lipid and protein indices in blood plasma of sheep fed protein of different origin and different forms of SFO

Indices	Total protein (g/L)	Lipase (U/L)	Total cholesterol (mmol/L)
Control group – casein	80	164 b	0.31
+ calcium salts of sunflower oil	78	181 b	0.29
+ sunflower seeds	78	154 b	0.37
+ sunflower oil	79	264 a	0.47
Control group – zein	81 A	197 b	0.39
+ calcium salts of sunflower oil	76 B	221 b	0.38
+ sunflower seeds	82 A	198 b	0.48
+ sunflower oil	81 A	308 a	0.46

a, b – values in columns with different letters differ significantly (P≤0.05); A, B – as above for P≤0.01.

Discussion

Knowing the action of the pancreas on specific diets may show the possibilities of improving digestion in ruminants. We suppose that supplementing protein with different rates of degradation (casein or zein) and different forms of sunflower oil, which are not a natural feed for ruminants, will modify the secretory activity of the pancreas, leading to better utilization of fatty acids in the carcass. The addition of unprotected forms of fat to sheep diets may also increase plasma concentrations of lipase and total cholesterol.

The literature reveals enormous variation in the amount of pancreatic juice secretion in sheep and in other species. These discrepancies can also be caused by pancreatico-biliary secretion and its relations, or by pancreatic juice alone.

In the present study the total pancreatic juice and bile secretion ranged from 76.9 to 106.8 ml/h depending on the diet. However, Johnson et al. (1974) reported significantly lower secretion values (from 57.5 to 69.5 ml/h). On the other hand, Kowalik et al. (2001) noted increased secretion of biliary-pancreatic juice (from about 83 to 174 ml/h) in sheep fed diets with starch. Therefore, differences in the amount of juice obtained during collection depend mainly on animal breed, feeding (nutrients), body weight, as well as method of catheter insertion (Harmon, 1992; Kato et al., 1999; Kowalik et al., 2001; Żebrowska et al., 2001; Šileikienė et al., 2004).

The results of our research showed that the use of easily degradable protein in the rumen (casein) and different forms of sunflower oil had no significant effect on pancreatic juice secretion. The decrease in pancreatic juice secretion in rams fed diets with casein and sunflower seeds and with zein and sunflower oil is due to different rates of energy and protein utilization in the diet. Diets supplemented with casein and sunflower seeds reduced the energy utilization from seeds, which probably flowed to rumen less degraded. Thus, the energy found in seeds could be available only in the small intestine. On the other hand, in the diet with zein, the energy supplied with sunflower oil was not utilized in the rumen, and, what is more, zein as a protein resistant to rumen degradation was available only in the small intestine. Richards et al. (2003), who postruminally infused casein and corn starch into the abomasum of steers, did not observe any changes in pancreatic juice secretion. The amounts of casein used in the diet (0, 60, 120, or 180 g/d) also did not affect trypsin and chymotrypsin concentration. Furthermore, Wang and Taniguchi (1998) did not show any differences in pancreatic juice secretion after casein addition to the diet, compared to the unsupplemented group. Brannon (1990) observed that changes in the proteolytic activity of pancreas were associated with changes in the amount and content of protein flowing to the small intestine. Żebrowska et al. (2001) confirmed these results when feeding sheep with 200 g of total protein in dry matter, which increased the secretion of pancreatico-biliary juice (1398 g) and chymotrypsin activity, when compared to animals receiving smaller amounts of protein (130 g; 1160 g) ($P<0.01$). When the dietary protein content was lower, endogenous nitrogen was observed to decrease from 3.4 to 2.7 g N/24 h in secreted juice ($P\leq0.05$). However, the activity of trypsin and amino acids content in pancreatico-biliary juice were similar for all diets.

In the present experiment it was observed that protein concentration in pancreatic juice was significantly higher in animals receiving casein and sunflower seeds in comparison to the group receiving zein. Protein readily degraded in the rumen and partially protected fat may affect microbial protein synthesis by utilizing products of protein degradation. Furthermore, this process was not interfered by unsaturated fatty acids, because sunflower seeds did not directly act on rumen microflora, in comparison to natural oil. The only exception were sheep fed oil-supplemented diets in which zein, as a protein resistant to rumen degradation, caused a marked increase in protein content of juice. However, when abomasally infusing coconut oil with similar fatty acid profile to sunflower oil, Johnson et al. (1974) did not observe any significant effect of these fats on either the protein content of pancreatic juice or its lipolytic activity.

In the present study trypsin and chymotrypsin activity was modified by different protein source and form of fat added to the diet, which supports our research hypothesis. Some literature data concerning the proteolytic enzyme activity of the pancreas suggests that, depending on protein concentration in the diet, this activity can be modified by protein rate and products of protein digestion in the small intestine. In ruminants, most protein flowing to the intestine after digestion is of microbial origin. Because the concentration of microorganisms protein is constant, it cannot affect the proteolytic enzyme activity (Harmon, 1992). However, when slowly degraded protein is fed to animals, the amount of "feed" protein flowing to the duodenum can be greater, although Richards et al. (1998) noted no significant effects of casein on the trypsin activity. Also Swanson et al. (2002) did not observe any changes in the proteolytic activity between control group and animals receiving casein infusion to the abomasum. Khorasani et al. (1990) did not show any differences in trypsin activity when animals were fed diets with various sources of protein from soybean and canola meal with the exception of rumen. In turn, Swanson et al. (2008) observed a positive influence of crude protein in the diet on trypsin activity as well as noting that gradually increasing crude protein concentration from 8.5 to 14.5% could increase proteolytic trypsin activity. However, Richards et al. (2003) did not observe any differences in trypsin and chymotrypsin activity when feeding different casein levels to cows.

On the other hand, the strong effect of fat added to the diet on pancreatic proteolytic activity in our experiment can be supported with the manner of trypsin and chymotrypsin activation. Linoleic acid, which is the main compound of sunflower oil, takes part in activation of these enzymes. This acid can be converted to arachidonic acid (AA), which is known as one of eicosanoid precursors. Intestinal juice contains enterokinase, which is necessary for activation of trypsinogen to trypsin, which in turn activates chymotrypsinogen. Thus, such a process is necessary for a proteolytic activation of pancreatic juice. A physiological correlation between fatty acid profile in the diet and proteolytic enzyme activity was stated in several studies (Jelińska 2005; Sommer et al., 2002).

In the present study, the lipolytic activity of pancreatic juice was not strongly dependent on diet composition, probably due to differences within groups. Our findings disagree with the study assumptions. Nonetheless, the increase in bile secretion contributed to a numerical increase in lipase activity in the group receiving dietary zein. This upward trend could depend on the specific interaction between lipase and biliary salts (Konturek et al., 2004; Maldonado-Valderrama et al., 2011). Furthermore, the increase in lipase activity in the group supplemented with casein and sunflower oil could also be considered to result from the highest bile secretion. These observations are in agreement with Arienti et al. (1974), who suggested that bile, functioning as a buffer, might have a major influence on lipase protection from inactivation caused by duodenal digesta. Wang and Taniguchi (1998) observed a significant influence of casein on lipase activity, and suggested that increasing protein content in the abomasum could affect pancreatic lipase and amylase secretion. However, when examining pancreatic secretion in lambs fed different diets, Pierzynowski (1986) noted very high stability of pancreatic lipase activity. The activity of this

enzyme increased by a factor of two when fasted animals were fed a diet containing easily degradable compounds.

Unprotected vegetable oils may influence physiological blood indices (Huard et al., 1998). Already in 1978, Nestle et al. suggested that supplementing sunflower oil to ruminant diet increased plasma cholesterol concentrations from about 1.9 to 2.5 mmol/L. Moreover, elevated levels of this index were also observed by supplementing sunflower seeds (Zhang et al., 2006; Borowiec et al., 2008). In the present study, feeding sheep diets with zein and protected form of fat significantly reduced the plasma concentration of total protein due to slow degradation of zein in the rumen, where less ammonia was absorbed. According to the research hypothesis, sunflower oil addition to the diet significantly increased lipase activity, probably because of a greater absorption of medium-chained fatty acids in the rumen and free fatty acids in the small intestine in the blood. Likewise, the increased contribution of unprotected forms of fat (sunflower oil and sunflower seeds) to the diet for ruminants may increase the total cholesterol level. However, the reference values were not exceeded, which can be explained by the natural ability of healthy organisms to regulate the ratio of cholesterol intake to cholesterol synthesis (Wong et al., 1993). Sunflower oil contains a large proportion of unsaturated fatty acids, which are known as cholesterol suppressors. Therefore, the increasing concentration of cholesterol in blood plasma after fat supplementation is derived from specific digestion processes of fats in the rumen, where unsaturated fatty acids undergo biohydrogenation by bacteria, thereby increasing the saturated fatty acid pool.

To summarize, feeding animals diets with proper combinations of protected or unprotected forms of sunflower oil and otherwise digested protein can modify mainly proteolytic (and to a lesser extent lipolytic) activities of pancreas and probably affect plasma lipid indices in sheep. Further experiments should be conducted to evaluate more precisely the impact of diet composition on ileal digestibility of nutrients.

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Accepted for printing 5 IX 2012

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Wpływ formy oleju słonecznikowego oraz źródła białka w dawce na sekrecję soku trzustkowego oraz aktywność enzymów trzustkowych u owiec

STRESZCZENIE

Celem podjętych badań było określenie wpływu różnych form oleju słonecznikowego podawanych z białkiem o różnym tempie degradacji w żwaczu na sekrecję i aktywność soku trzustkowego. Doświadczenie przeprowadzono na 24 dorosłych tryczkach Corriedale o masie ciała około 40 kg z założonymi kateterami do przewodu trzustkowego i żółciowego oraz dwunastnicy. Zwierzęta żywiono sianem łąkowym i skrobią ziemniaczaną z dodatkiem kazeiny (szybki rozkład w żwaczu) lub glutenu kukurydzianego, jako źródła zeiny (wolno rozkładanej w żwaczu) oraz różnymi formami oleju słonecznikowego (sole wapniowe kwasów tłuszczowych, nasiona i olej). Wykazano, że rodzaj tłuszczu w diecie nie wpłynął znacząco na sekrecję soku trzustkowego, natomiast obecność różnych form tłuszczu (nasiona słonecznika lub olej słonecznikowy) istotnie wpłynęła na sekrecję żółci, zawartość białka w soku, aktywność proteolityczną trypsyny oraz wskaźniki lipidowe w osoczu. Nie zaobserwowano istotnych różnic w aktywności lipolitycznej soku trzustkowego, natomiast aktywność lipazy była wyższa przy zastosowaniu zeiny jako źródła białka.

Dodatek chronionych lub niechronionych form oleju słonecznikowego oraz białka o różnym stopniu rozkładu w żwaczu do diety owiec może kształtować aktywność trzustki i prawdopodobnie wpływać na wskaźniki lipidowe w osoczu. Jednak potrzeba dalszych badań by dokładniej określić wpływ składu diety na jelitową strawność składników pokarmowych.