Rumen degradability and whole tract digestibility of flavonolignans from milk thistle (*Silybum marianum*) fruit expeller in dairy cows

L. Křížová[1], J. Watzková[1], J. Třináctý[1], M. Richter[1], M. Buchta[2]

[1]Department of Animal Nutrition and Quality of Livestock Products, Agriresearch Rapotín Ltd., Pohořelice, Czech Republic

[2]Moravol, spol. s r.o., Kramolín, Czech Republic

ABSTRACT: The objective of this study was to determine rumen degradability and total digestibility of flavonolignans from a milk thistle fruit expeller in dairy cows considering milk production and changes in plasma flavonolignans. The experiment was carried out on three lactating Holstein cows and was divided into three periods as follows: preliminary period (Pr, 3 days) was used for the diet stabilization followed by the adaptation period (A, 6 days) in which the treatment was applied and by the balance period (B, 4 days). Cows were fed individually twice a day (6:40 and 16:40 h) *ad libitum* the diet based on maize silage, lucerne hay and supplemental mixture. In the periods A and B the diet was supplemented with 150 g/day of milk thistle fruit expeller applied in two equal portions at each feeding. Average daily intake of dry matter and basic nutrients was similar in all periods ($P > 0.05$). Milk yield and composition were not affected by the treatment ($P > 0.05$). The milk thistle fruit expeller used in this experiment contained 4.10 ± 0.10 mass percentage of the silymarin complex. Digestibility of silybin A and silybin B was 40.0 and 45.5%, respectively. Digestibility of other components of the silymarin complex was 100%. The highest value of the effective degradation was found for taxifolin (59.11%), while the effective degradation of the other flavonolignans ranged from 23.28 to 35.19%. Animals receiving the milk thistle fruit expeller had a higher content of plasma conjugated silybin ($P < 0.001$) than those without its supplementation.

Keywords: silymarin complex; *in sacco* technique; digestibility; plasma

Fruits of milk thistle (*Silybum marianum* /L./ Gaertner, Asteraceae) have been used for more than 2000 years to treat liver and gallbladder disorders, including hepatitis, cirrhosis and jaundice, and to protect the liver against poisoning with chemical and environmental toxins (Křen and Walterová, 2005). Its active compound – the silymarin complex – is found primarily in fruits (Rainone, 2005). The fruits consist of approximately 70–80% of silymarin flavonolignans and approximately 20 to 30% of chemically undefined fractions, comprising mostly polymeric and oxidized polyphenolic compounds (Křen and Walterová, 2005). Further, the fruits also contain betaine, trimethylglycine, and essential fatty acids that may contribute to the hepatoprotective and anti-inflammatory effects of silymarin (Luper, 1998; Saller et al., 2001). The most prevalent component of the silymarin complex is silybin (SB, 50–60% of silymarin), a mixture of two diastereomers A (SB-A) and B (SB-B) at the 1:1 ratio. Furthermore, silymarin contains also considerable amounts of other flavonolignans, such as silychristin (SC, 20%), silydianin (SD, 10%), isosilybin (ISB, 5%), dehydrosilybin, and a few flavonoids, mainly taxifolin (TF; Šimánek et al., 2000).

These compounds are of considerable pharmacological interest owing to their strong hepatoprotective and anticholesterolaemic activity (Valenzuela

Supported by Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 2678846201).

et al., 1986; Krečman et al., 1998). Although SB is thought as the main component of silymarin, both quantitatively (Quercia et al., 1980) and therapeutically (Morazzoni and Bombardelli, 1995), SC and SD also display an antioxidant activity (Morazzoni and Bombardelli, 1995), and the results of Krečman et al. (1998) suggested that SB was even more effective when associated with other constituents, probably because the availability of the former compound was lower when used as a single compound compared to its use as a part of the silymarin complex. Although the therapeutic potential of silymarin seems to be high, its bioavailability is poor due to its low solubility in water (Blumenthal et al., 2000), enteral absorption (Giacomelli et al., 2002; Mayer et al., 2005) and degradation by gastric fluid (Blumenthal et al., 2000).

Due to its hepatoprotective effects, silymarin was tested as a natural treatment in some metabolic disorders in dairy cows. Vojtíšek et al. (1991) used a milk thistle fruit meal in the diet of ketotic cows, Tedesco et al. (2003, 2004a, b) used a silymarin extract in the form of oral drench in lactating dairy cows exposed to dietary aflatoxin contamination or in periparturient cows subjected to subclinical fatty liver. Results of these studies are inconsistent. As suggested by Tedesco et al. (2003), the efficiency of silymarin could be influenced by the form of this substance and by the level of ruminal fermentation. Thus, the objective of this study was to determine the rumen degradability and total digestibility of flavonolignans from a milk thistle fruit expeller in dairy cows considering milk production and changes in plasma flavonolignans.

MATERIAL AND METHODS

Animals and diets

The experiment was carried out on three high-yielding lactating Holstein cows (lactation 2, week of lactation 29–32) of the average weight 551.3 kg (SEM = 15.7). The experiment consisted of 3 periods. The 3-day preliminary period (Pr, 3 days) was used for the diet stabilization followed by the adaptation period (A, 6 days) in which the treatment was applied and by the balance period (B, 4 days).

Cows were fed individually twice a day (6:40 and 16:40 h) *ad libitum* the diet based on maize silage, lucerne hay and supplemental mixture (Table 1). In the periods A and B the diet was supplemented with 150 g/day of milk thistle fruit expeller (Moravol, spol. s r.o., Kramolin, Czech Republic) and was applied in two equal portions at each feeding. Feed intake, lactation performance and health condition of cows were monitored during the experiment.

Sampling and analyses

Samples of feed were taken twice in each period, refusals were monitored daily, an aliquot of them was analysed. Faeces were collected by grab sampling during 4 days of period B and preserved by chloroform. After the end of period B the faeces were homogenized and a representative sample was taken for each animal. Samples of feed, feed refusals and faeces were dried at 55°C for 2 days, ground (1 mm) and then stored until they were analysed

Table 1. Composition of the diet (in g/kg of dry matter) of dairy cows fed basal diet (Pr) supplemented with milk thistle fruit expeller (A) and balance experiment (B)

Component	Pr	A	B
Maize silage	558.3	553.2	553.2
Lucerne hay	100.2	99.5	99.5
Supplemental mixture[1]	341.5	338.7	338.7
Milk thistle fruit expeller	–	8.6	8.6

Pr = preliminary period (3 days), control diet without milk thistle fruit expeller; A = adaptation period (6 days), control diet supplemented with 150 g/day of milk thistle fruit expeller; B = balance period (4 days), control diet supplemented with 150 g/d of milk thistle fruit expeller

[1]composition of supplemental mixture (in g/kg of dry matter): wheat (250), barley (200), soybean meal (125), sunflower meal (125), corn (100), sunflower expellers (50), malt sprouts (50), calcium salt of fatty acids (30), linseed (20), limestone ($CaCO_3$, 20), dicalcium phosphate (DCP, 20), sodium chloride (NaCl, 5), magnesium phosphate ($Mg_3(PO_4)_2$, 5); vitamine A (12 000 m.j./kg), vitamine D_3 (2000 m.j./kg), vitamine E (50 mg/kg), copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$, 27 mg/kg)

for the content of dry matter (DM), crude protein, crude fibre, fat and ash according to AOAC (1984). The content of neutral detergent fibre (NDF, with α-amylase) was determined according to Van Soest et al. (1991). The content of PDIN, PDIE (digestible protein in the intestine when rumen fermentable N or energy supply are limiting, respectively) and NEL (net energy of lactation) was calculated according to Sommer (1994). Samples of feed, feed refusals and faeces were further used for determination of the silymarin complex according to methods described below.

Cows were milked twice daily at 6:45 h and 16:45 h and milk yield was recorded at each milking during the experiment. On the last day of periods Pr and A, samples of milk were taken from morning and evening milking, preserved by 2-bromo-2-nitropropane-1.3-diol (Bronopol, D & F Control Systems, Inc., San Ramon, USA) and analysed for basal components with an infrared analyser (Bentley Instruments Inc., Chaska, USA).

At the end of periods Pr and the B blood samples were taken from the jugular vein 3 times a day (7:00, 10:00 and 13:00 h) into heparinized tubes. After blood collection, the samples were immediately centrifuged at 1500 g for 15 min and stored at –20°C for subsequent analyses of plasma metabolites and flavonolignan content. The plasma metabolites were determined by spectrophotometric methods using a Cobas Mira automatic analyser (Roche diagnostics, Basel, Switzerland) and kits for standard enzymatic methods (Biovendor, Randox, Lachema, Czech Republic).

Degradability of flavonolignans from milk thistle was determined in the 4-day experimental period using an *in sacco* technique. Each bag (5 × 14 cm) was made of nylon (Uhelon T, Hedva, Moravská Třebová, Czech Republic; 42-μm pore size) and filled with 1 g of air-dried and ground (1 mm) sample of the milk thistle expeller. The nylon bags were inserted into the rumen after feeding, withdrawn after 0, 2, 4, 8, 16, 24, and 48 h, rinsed in running cold tap water for 1 minute, washed in a water bath for 10 min, and dried at 60°C according to Třináctý et al. (1996). Samples of the milk thistle fruit expeller after incubation in the rumen were further used for determination of milk thistle components.

Analyses of flavonolignans

Plasma

An SB standard (Ivax-CR a.s., Opava, Czech Republic) was subjected to high-performance liquid chromatography (HPLC) analysis for the comparison of its retention times with those of SB contained in the tested plasma. All other chemicals used in the analysis were of high purity (≥ 99%). Plasma samples were deproteinized by acetonitrile and centrifuged at 5000 rpm for 10 min. Consequently, supernatants were decanted and evaporated under a gentle flow of nitrogen and in the next step dissolved in 200 μl of the mobile phase (29/3/68 acetonitrile, methanol, 0.1% formic acid).

The separation of SB diastereomers was performed with Shimadzu Class VP system with UV detection (289 nm) using a Merck RP-18e (5 μm) LiChrospher 100 column (250 mm × 4.6 mm *i.d.*) at a mobile phase flow rate of 1 ml/min. Time of analysis was 15 min. HPLC analysis was based on the method of Gunaratna and Zhang (2003).

After HPLC analysis, potential conjugates of SB were submitted to enzyme hydrolysis by β-glucuro-

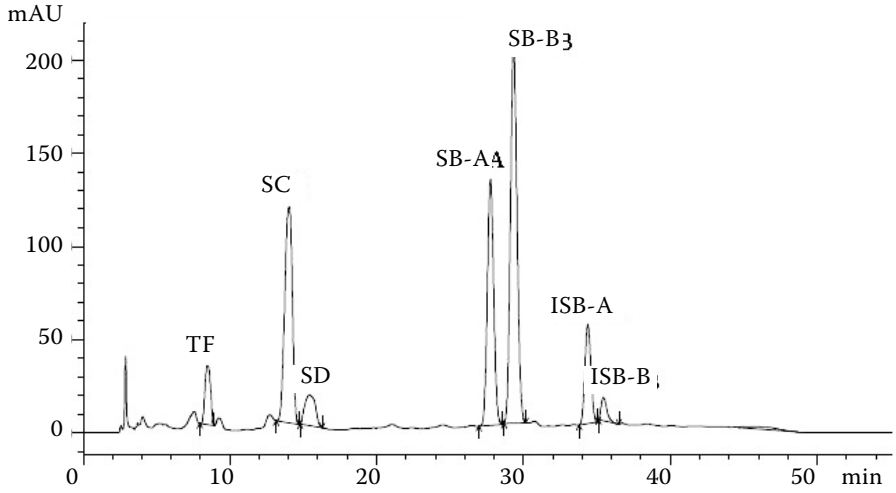

Figure 1. High performance liquid chromatogram of the milk thistle fruit expeller (288 nm)

TF = taxifolin, SC = silychristin, SD = silydianin, SB-A = silybin A, SB-B = silybin B, ISB-A = isosilybin A, ISB-B = isosilybin B

Table 2. Daily intake of nutrients, yield and composition of milk of dairy cows fed basal diet (Pr) supplemented with milk thistle fruit expeller (A) and balance experiment (B)

Nutrients	Pr	A	B	SEM	*P*
Dry matter (kg/day)	15.83	16.22	16.09	0.155	0.297
Organic matter (kg/day)	14.70	15.08	14.96	0.144	0.284
Crude protein (kg/day)	2.41	2.49	2.48	0.026	0.051
Crude fiber (kg/day)	2.50	2.63	2.60	0.033	0.082
Fat (kg/day)	0.52	0.56	0.55	0.016	0.101
NDF(kg/day)	5.05	5.22	5.17	0.057	0.195
PDIN(kg/day)	1.52	1.57	1.56	0.017	0.151
PDIE (kg/day)	1.39	1.41	1.40	0.011	0.557
NEL (MJ/day)	93.67	94.88	94.22	0.781	0.587
Milk yield (kg/day)	16.48	17.14	16.60	0.281	0.321
Fat (g/day)	42.07	48.10		0.343	0.281
Protein (g/day)	34.73	35.67		0.065	0.368
Lactose (g/day)	45.5	47.97		0.152	0.315
Urea (mg/100 ml)	25.33	26.57		3.818	0.833

Pr = preliminary period (3 days), control diet without milk thistle fruit expeller; A = adaptation period (6 daye), control diet supplemented with 150 g/day of milk thistle fruit expeller; B = balance period (4 days), control diet supplemented with 150 g/day of milk thistle fruit expeller, milk was not sampled in this period

NDF = neutral detergent fiber with α-amylase; PDIN, PDIE = digestible protein in the intestine when rumen fermentable N supply or energy supply are limiting, respectively; NEL = net energy of lactation

nidase/arylsulphatase. 100 µl of sample was incubated (37°C, 1 h) with 150 µl acetate buffer (0.1M, pH 4) and 6 µl β-glucuronidase/arylsulphatase at 4 IU/ml. After incubation, ice methanol was added to the sample and the mixture was centrifuged at 5000 rpm for 5 min. The supernatant was evaporated, dissolved in the mobile phase and injected into the HPLC system for reanalysis.

Feed, feed refusals, faeces, degradability

Silymarin [a mixture of SC, SD, SB-A, SB-B, ISB (diastereomers A and B, ISB-A and ISB-B)] in the quality of CRL pharmacopoeial standard (EDQM, Strasbourg, France); TF – standard for the substance identity with an assay min. 90% (Sigma-Aldrich, Prague, Czech Republic); methanol and acetonitrile – HPLC grade (Lach Ner, Brno, Czech Republic) were used in this experiment. All other used chemicals were of the quality according to Ph. Eur. 6.0 (2008) gift from Lach Ner (Brno, Czech Republic).

Samples of feed, feed refusals, faeces, and samples from the *in sacco* technique were homogenised and ground to particles below 0.5 mm in size (IKA A11, IKA WERKE, Germany). 4 ml of distilled water was added to the accurately weighed amount of a sample (PURELAB Maxima systems, ELGA, Bucks, UK) and the sample was let stand for one hour. Then 46 ml of dissolving mixture (26 ml of CH_3CN and 20 ml CH_3OH) was added and the mixture was homogenized using an ultrasonic bath (RK 514 BH, Bandelin, Badelin, Germany) for one hour and the storage of the mixture at 4°C for 12 h without mixing followed. After tempering to an ambient temperature 5 ml of the solution was pipetted through the cotton plug, evaporated at 42°C under reduced pressure and redissolved in CH_3OH. This solution was diluted and filtered through a 0.45 µm PTFE filter (PALL, Somersby, Austria) before HPLC analysis.

The HPLC separation was performed using a Luna C18 (250 × 4.6 mm, 5 µm) column (Phenomenex, Torrance, USA) at a mobile phase flow rate of 0.9 ml/min with a Shimadzu LC 20 apparatus equipped with SPD-M20A diode-array detector (UV detection at 288 nm, Figure 1). The

Table 3. Basic plasma parameters of dairy cows in preliminary (Pr) and balance period (B)

Component	Pr	B	SEM	*P*
Total protein (g/l)	82.87	81.70	3.986	0.848
Albumine (g/l)	33.80	33.97	0.364	0.765
Glucose (mmol/l)	4.24	4.29	0.237	0.890
NEFA (mmol/l)	0.26	0.34	0.158	0.731
BHB (mmol/l)	0.49	0.59	0.174	0.700
Urea (mmol/l)	5.61	4.69	0.582	0.323
Bilirubine (mmol/l)	6.13	6.23	0.813	0.936
AST (μkat/l)	1.15[a]	1.50[b]	0.087	0.046
GMT (μkat/l)	0.73	0.73	0.056	1.000
LDH (μkat/l)	34.53	34.88	0.860	0.789

Pr = preliminary period (3 days), control diet without milk thistle fruit expeller; B = balance period (4 days), control diet supplemented with 150 g/day of milk thistle fruit expeller

NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; AST = aspartate aminotransferase; GMT = glutamate transferase; LDH = lactate dehydrogenase

[a,b]means within the same row without a common superscript differ ($P < 0.05$)

mobile phases were composed of (A): CH_3OH/H_2O/85% H_3PO_4 (34.8/64.7/0.5 vol.) and (B): CH_3OH/H_2O/85% H_3PO_4 (49.75/49.75/0.5 vol.). The gradient elution was performed as follows: 0 min (A/B, 100/0); 5 min (100/0); 33 min (0/100); 43 min (0/100); 45 min (100/0) and 55 min (100/0). The run time of analysis was 50 minutes. The HPLC method was based on the determination of flavonolignans by an assay described in the Ph. Eur. 6.0 (2008, article 2071).

Statistical analysis

Means of nutrient intake, milk yield, basic plasma parameters and content of conjugated SB in plasma were compared using two-way ANOVA of Statgraphics 7.0 package (Manugistics Inc., and Statistical Graphics Corporation, Rockville, USA).

RESULTS

Daily intake, milk yield and plasma parameters

Nutrient intake is presented in Table 2. Daily intake of DM and other nutrients did not differ significantly in the particular periods ($P > 0.05$). Similarly, milk yield or composition was not affected by the treatment ($P > 0.05$). Concentrations of

Table 4. Content of conjugated silybin (SB) in plasma of dairy cows fed basal diet (Pr) and balance experiment (B) at individual time intervals

	Time of sampling (h)						SEM	*P*		
	7:00		10:00		13:00					
	Pr	B	Pr	B	Pr	B		treat.	time	treat.× time
Conjugated SB (ng/ml)	0.00	317.07	0.00	285.30	0.00	455.87	27.581	***	*	*

Pr = preliminary period (3 days), control diet without milk thistle fruit expeller; B = balance period (4 days), control diet supplemented with 150 g/day of milk thistle fruit expeller

*$P < 0.05$, ***$P < 0.001$

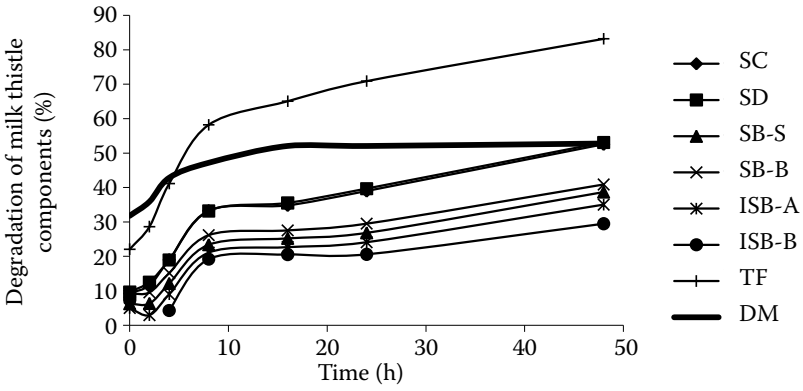

Figure 2. Degradation of dry matter (DM) and flavonolignans

SC = silychristin, SD = silydianin, SB-A = silybin A, SB-B = silybin B, ISB-A = isosilybin A, ISB-B = isosilybin B, TF = taxifolin) determined *in sacco* technique after 0, 2, 4, 8, 16, 24, and 48 h incubation in rumen

plasma metabolites are shown in Table 3. No effect of the period on plasma metabolite concentrations was observed, with the exception of AST (aspartate aminotransferase) that was significantly higher in period B in comparison with period Pr ($P < 0.05$). The inclusion of the milk thistle fruit expeller in diet resulted in significant differences in plasma conjugated SB between Pr and B diets ($P < 0.001$) and time intervals ($P < 0.05$, Table 4).

Rumen degradability and the whole tract digestibility of flavonolignans

The milk thistle fruit expeller used in this experiment contained 4.10 ± 0.10 mass percentage of the silymarin complex and 0.24 ± 0.01 mass percentage of TF. Contents of individual flavonolignans in the expeller were as follows: SC = 10.45 ± 0.35 g/kg, SD = 1.51 ± 0.06 g/kg, SB-A= 9.24 ± 0.28 g/kg, SB-B = 15.1 ± 0.50 g/kg, ISB-A = 3.48 ± 0.15 g/kg, ISB-B = 1.11 ± 0.10 g/kg, and TF = 2.39 ± 0.05 g/kg.

Parameters and pattern of rumen degradation of DM and individual components of the silymarin complex are given in Table 5 and Figure 2. Parameter a (the soluble rapidly degradable fraction) of each flavonolignan with the exception of TF was lower than for DM. The highest value of effective degradation (ED) was found out for TF (59.11%), while the ED of the other flavonolignans ranged from 23.28 to 35.19%. Similar rates of degradation were observed for ISB-A, ISB-B, SB-A and SB-B.

The values of the whole tract digestibility of individual nutrients and flavonolignans are documented in Table 6. Digestibility of SB-A and SB-B was similar, 40.0% and 45.5%, respectively. Contents of ISB-A, ISB-B, SD, SC and TF in faeces were below the detection limit, thus their digestibility was considered to be 100%.

DISCUSSION

In this study, no significant differences in milk yield among the experimental periods were observed. Similar findings were reported by Tedesco et al. (2003). On the other hand, in their later study Tedesco et al. (2004b) reported higher milk yield at the beginning of lactation in cows receiving 10 g

Table 5. Ruminal degradability parameters of dry matter (DM) and the silymarin complex of milk thistle fruit expeller

Parameters	DM	SB-A	SB-B	ISB-A	ISB-B	SD	SC	TF
a (%)	31.17	9.67	12.91	8.41	11.04	14.37	13.45	24.27
b (%)	21.79	31.94	30.94	30.26	25.45	41.19	40.98	57.26
c (h^{-1})	0.17	0.06	0.06	0.06	0.06	0.06	0.06	0.09
ED (%)	47.20	25.06	27.85	23.28	23.58	35.19	34.39	59.11

a = soluble rapidly degradable fraction; b = potentially degradable fraction; c = fractional rate of degradation; ED = effective degradability

SB-A = silybin A, SB-B = silybin B, ISB-A = isosilybin A, ISB-B = isosilybin B, SD = silydianin, SC = silychristin, TF = taxifolin

Table 6. Whole tract digestibility of nutrients and the silymarin complex of milk thistle fruit expeller

	Nutrients digestibility (%)						
	dry matter	organic matter	NDF	crude protein	fat		
Mean	72.9	74.4	57.4	69.3	87.9		
SEM	2.3	1.8	3.8	1.4	0.9		
	Silymarin complex digestibility (%)						
	SB-A	SB-B	ISB-A	ISB-B	SD	SC	TF
Mean	40.0	45.5	100.0[1]	100.0[1]	100.0[1]	100.0[1]	100.0[1]
SEM	11.1	11.0	0.0	0.0	0.0	0.0	0.0

NDF = neutral detergent fiber with α-amylase; SB-A = silybin A; SB-B = silybin B; ISB-A = isosilybin A; ISB-B = isosilybin B; SD = silydianin; SC = silychristin; TF = taxifolin

[1]content of flavonolignans in faeces under detection limit

of silymarin daily during the periparturient period. Vojtíšek et al. (1991) also noted higher milk yield in ketotic cows in early lactation treated with 300 g/day of milk thistle meal. Although all abovementioned trials, including our experiment, were the short-term ones (9–25 days), it was possible to expect changes in milk yield in high-yielding cows at the beginning of lactation when the cows experienced a negative energy balance resulting in fatty liver (Tedesco et al., 2004b) or showed signs of clinical ketosis (Vojtíšek et al., 1991). No effect of silymarin on the milk composition was observed in our study (data not presented). This is in agreement with findings of Tedesco et al. (2002, 2003, 2004b).

Changes in plasma parameters after silymarin supplementation were inconsistent. Tedesco et al. (2004b) did not find any differences in plasma constituents with the exception of a higher level of non-esterified fatty acids (NEFA) 7 days prepartum in silymarin-treated cows. They suggested that an increase in NEFA occurred predominantly during the prepartum period and appeared as a unique effect of silymarin because no changes in plasma NEFA were observed in other parts of the periparturn period. A positive effect of milk thistle fruits on plasma ketones and β-hydroxybutyrate (BHB) level in ketotic cows was reported by Vojtíšek et al. (1991). As suggested by Enjalbert et al. (2001), it was possible to expect changes in plasma BHB when the cows experienced negative energy balance, fatty liver or ketosis. Plasma BHB levels ranging from 1.2 mmol/l (Enjalbert et al., 2001) to 1.4 mmol/l (Geishauser et al., 2000) were used to define subclinical ketosis. As evident from our results (BHB values being 0.49 and 0.59 mmol/l in period Pr and B, respectively), the cows in our experiment were not in a risk of ketosis. No significant changes in plasma metabolites were determined in our study with the exception of the AST level that was higher in period B in comparison with period Pr ($P < 0.05$). Studies dealing with the effect of the silymarin complex on plasma AST in cows are scarce. According to Vojtíšek et al. (1991) plasma AST in ketotic cows was not affected by dietary supplementation of the milk thistle fruit meal. On the other hand, according to Wang et al. (1996), silymarin significantly lowered the levels of serum γ-glutamyl transpeptidase, alanine transaminase and AST in rats. Similarly, in long-term human studies, AST levels were significantly lower after silymarin treatment (Magliulo et al., 1978; Pares et al., 1998). But in short-term human studies no effect of silymarin on AST was observed (Buzzelli et al., 1993, 1994; Šimánek et al., 2001). The higher content of AST in period B determined in our study resulted rather from the experiment-related stress, mainly increased manipulation with animals during balance and digestibility studies, than from the effect of silymarin supplementation.

As reported by Rickling et al. (1995) or Wen et al. (2008), silymarin flavonolignans after oral administration were quickly metabolized to their conjugates, primarily forming glucuronides, and the conjugates were primary components present in human plasma. Wen et al. (2008) further demonstrated that all six silymarin flavonolignans were rapidly eliminated, and the conjugated silymarin flavonolignans had relatively longer half-lives than their free forms. In our study, plasma conjugated

SB was determined at all sampling times in period B with the highest plasma concentration at the last sampling time (13:00 h), it was approximately 6 h after feeding. This is in agreement with Dixit et al. (2007), who reported that the peak plasma concentration of silymarin conjugates was reached in 6–8 hours.

Although silymarin shows a high potential as a natural hepatoprotective and immunomodulative agent, its bioavailability is low. A number of studies were carried out to enhance the bioavailability of silymarin such as cyclodextrin complexation (e. g. Arcari et al., 1992), incorporation in solid dispersion (e. g. Chen et al., 2005), preparation of silymarin in the form of salts of polyhydroxyphenylchromanones (Madaus et al., 1976) and other more soluble derivatives (Giorgi et al., 1989) or phospholipid complexation (e.g. Škottová et al., 2000).

In the previous studies on cows, silymarin was used in the form of milk thistle meal (Vojtíšek et al., 1991, 1993) or silymarin extract (Tedesco et al., 2003, 2004a,b). There is only one study (Tedesco et al., 2003) in which silymarin was used in the form of silymarin Phytosome, a complex of silymarin and soy phospholipids at a 1:2 molar ratio. Based on the *in vitro* and *in vivo* monogastric studies (Livio et al., 1990; Morazzoni et al., 1992) this form should have the higher bioavailability of active compounds. However, using such a form in dairy cows (Tedesco et al., 2003) failed to show higher bioavailability. The authors suggested that this failure may be a result of different behaviour of this substance in the rumen. Thus, similarly to human or monogastric studies, the effectiveness of silymarin in ruminants could probably be influenced by its solubility, degradation in the rumen and digestibility. To our knowledge, no study has been published to study the rumen degradability or digestibility of the silymarin complex in cows. Although digestibility of ISB-A, ISB-B, SD, SC and TF reached up to 100%, digestibility of SB-A and SB-B was 40.0% and 45.5%, respectively. These findings are close to general values reported by Blumenthal et al. (2000) or Dixit et al. (2007).

Acknowledgements

Special thanks go to the company Moravol, spol. s r.o. (Miroslav, Czech Republic) for supplying the milk thistle fruit expeller and for its support mainly in determination of flavonolignan content. The authors express their thanks further to Mgr. Petra Jančová and Prof. MUDr. Vilím Šimánek, DrSc. (Palacky University Olomouc, Faculty of Medicine and Dentistry, Department of Medical Chemistry and Biochemistry, Czech Republic), who determined the content of flavonolignans in plasma.

REFERENCES

AOAC (1984): Official Methods of Analysis. 14th ed. Association of Official Analytical Chemists, Arlington, USA, 1984, 1141 pp.

Arcari M., Brambilla A., Brandt A., Caponi R., Corsi G., Di Rella M., Solinas F., Wachter W.P. (1992): A new inclusion complex of silibinin and beta-cyclodextrins: *in vitro* dissolution kinetics and *in vivo* absorption in comparison with traditional formulations. Bollettino Chimico Farmaceutico, 131, 205–209.

Blumenthal M., Goldberg A., Brinkmann J. (2000): Herbal Medicine. Integrative Medicine Communications: Newton.

Buzzelli G., Moscarella S., Giusti A., Duchini A., Marena C., Lampertico M. (1993): A pilot study on the liver protective effect of silybin-phosphatidylcholine complex (IdB1016) in chronic active hepatitis. International Journal of Clinical Pharmacology, Therapy and Toxicology, 31, 456–460.

Buzzelli G., Moscarella S., Barbagli S., Marena C., Gentilini P. (1994): Therapeutic effect of silipide in patients with chronic hepatitis C non-responders (NTs) to interferon (IFN) treatment. Journal of Hepatology, 21 (Suppl. 1), S100.

Chen W., Xia H., Wu W. (2005): Optimized preparation of silymarin dripping pills by a central composite design response surface method. Chinese Traditional and Herbal Drugs, 36, 679–683.

Dixit N., Baboota S., Kohli K., Ahmad S., Ali J. (2007): Silymarin: A review of pharmacological aspects and bioavailability enhancement approaches. Indian Journal of Pharmacology, 39, 172–179.

Enjalbert F., Nicot M.C., Bayourthe C., Moncoulon R. (2001): Ketone bodies in milk and blood of dairy cows: Relationship between concentrations and utilization for detection of subclinical ketosis. Journal of Dairy Science, 84, 583–589.

Geishauser T., Leslie K., Tenhagi J., Bashiri A. (2000): Evaluation of eight cow-side ketone testes in milk for detection of subclinical ketosis in dairy cows. Journal of Dairy Science, 83, 296–299.

Giacomelli S., Gallo D., Apollonio P., Ferlini C., Distefano M., Morazzoni P., Riva A., Bombardelli E., Mancuso S., Scambia G. (2002): Silybin and its bioavailable phospholipid complex (IdB 1016) potentiate *in vitro* and *in vivo* activity of cisplatin. Life Sciences, 70, 1447–1459.

Giorgi R., Conti M., Pifferi G. (1989): Soluble derivative of silybin a method of preparing them and pharmaceutical composition containing them. US patent 4886791.

Gunaratna C., Zhang T. (2003): Application of liquid chromatography-electrospray ionization-ion trap mass spectrometry to investigate the metabolism of silibinin in human liver microsomes. Journal of Chromatography B, 794, 303–310.

Krečman V., Škottová N., Walterová D., Ulrichová J., Šimánek V. (1998): Silymarin inhibits the development of diet-induced hypercholesterolemia in rats. Planta Medica, 64, 138–142.

Křen V., Walterová D. (2005): Silybin and silymarin – new effects and applications. Biomedicine Papers, 149, 29–41.

Livio S., Seghizzi R., Pifferi G. (1990): Diffusion behaviour of IdB 1016 across artificial and biological membranes. In: 4th Eur. Congr. Biopharmaceutics and Pharmacokinetics, Geneva, April 17–19, 1990. European Journal of Drug Metabolism Pharmacokinetics, Special Issue 1990.

Luper S. (1998): A review of plants used in the treatment of liver disease: Part 1. Alternative Medicine Review, 3, 410–421.

Madaus R., Halbach G., Trost W. (1976): Salt of silymarin group with aminopolyhydroxy alcohols. U.S. patent 3994925.

Magliulo E., Gagliardi B., Fiori GP. (1978): Zur Wirkung von Silymarin bei der Behandlung der akuten Virushepatitis. Medizinische Klinik, 73, 1060–1065.

Mayer K.E., Myers R.P., Lee S.S. (2005): Silymarin treatment of viral hepatitis: a systematic review. Journal of Viral Hepatitis, 12, 559–567.

Morazzoni P., Bombardelli E. (1995): Silybum marianum (*Carduus marianus*). Fitoterapia, 66, 3–42.

Morazzoni P., Magistretti M.J., Giochetti C., Zanolo G. (1992): Comparative bioavailability of Silipide, a new flavonolignan complex, in rats. European Journal of Drug Metabolism and Pharmacokinetics, 17, 39–44.

Pares A., Planas R., Torres M., Caballeria J., Viver J.M., Acero D., Panes J., Rigau J., Santos J., Rodes J. (1998): Effects of silymarin in alcoholic patients with cirrhosis of the liver: results of a controlled, double-blind, randomized and multicenter trial. Journal of Hepatology, 28, 615–621.

Quercia V., Pierini N., Incarnato G.P., Papetti P., Gambero P. (1980): HPLC evaluation of the ratio between the antihepatotoxic constituents of *Silybum marianum*. Fitoterapia, 51, 297–301.

Rainone F. (2005): Milk Thistle. American Family Physician, 72, 1285–1288.

Rickling B., Hans B., Kramarczyk R., Krumbiegel G., Weyhenmeyer R. (1995): Two high-performance liquid chromatographic assays for the determination of free and total silibinin diastereoisomers in plasma using column switching with electrochemical detection and reverse-phase chromatography with ultraviolet detection. Journal of Chromatography B, 670, 267–277.

Saller R., Meier R., Brignoli R. (2001): The use of silymarin in the treatment of liver diseases. Drugs, 61, 2035–2063.

Šimánek V., Křen V., Ulrichová J., Vicar J., Cvak L. (2000): Silymarin: What is the name…? An appeal for the change of editorial policy. Hepatology, 32, 442–443.

Šimánek V., Škottová N., Bartek J., Psotová J., Kosina P., Balejová L., Ulrichová J. (2001): Extract from Silybum marianum as nutraceutical: a double blind placebo-controlled study in healthy young men. Czech Journal of Food Sciences, 19, 106–110.

Škottová N., Krečman V., Váňa P., Chmela Z., Ulrichová J., Šimánek V. (2000): Effect of silymarin and silibinin–phosphatidylcholine complex on plasma and lipoprotein cholesterol, and oxidation of LDL in rats fed on high cholesterol diet supplemented with currant oil. Acta Universitatis Palackianae Olomucensis, Faculty of Medicine, 144, 55–58.

Sommer A. (ed.). (1994): Nutrient Requirement and Tables of Nutritional Values of Feedstuffs for Ruminants. Research Institute of Animal Nutrition, Pohorelice, 198 pp. (in Czech)

Tedesco D., Tava A., Domeneghini C., Costa A., Sciannimanico D., Tameni M. (2002): Dietary inclusion of silymarin in periparturum dairy cows: Effects on milk productivity, plasma metabolites and liver tissue. Journal of Dairy Science, 80 (Suppl. 1), 244 (Abstr.).

Tedesco D., Tameni M., Steidler S., Galletti S., Di Pierro F. (2003): Effect of silymarin and its phospholipid complex against AFM1 excretion in an organic dairy herd. Milchwissenschaft, 58, 416–419.

Tedesco D., Domeneghini C., Sciannimanico D., Tameni M., Steidler S., Galletti S. (2004a): Silymarin, a possible heopatoprotector in dairy cows: biochemical and histological observations. Journal of Veterinary Medicine A-Physiology, Pathology, Clinical Medicine, 51, 85–89.

Tedesco D., Tava A., Galletti S., Tameni M., Varisco G., Costa A., Steidler S. (2004b): Effects of silymarin, a natural hepatoprotector, in periparturient dairy cows. Journal of Dairy Science, 87, 2239–2247.

Třináctý J., Šimek M., Komprda T. (1996): The influence of a nylon bag carrier on alfalfa crude protein degradability. Animal Feed Science Technology, 57, 129–137.

Valenzuela A., Guerra R., Videla L.A. (1986): Antioxidant properties of the flavonoids silybin and (+)-cyanidanol-3: comparison with butylated hydroxyanisole and butylated hydroxytoluene. Planta Medica, 52, 438–440.

Van Soest P.J., Robertson J.B., Lewis B.A. (1991): Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583–3597.

Vojtíšek B., Hronová B., Hamřík J., Janková B. (1991): Milk thistle (*Silybum marianum*, L., GAERTN.) in feed rations administered to ketotic cows. Veterinární Medicina, 36, 321–330. (in Czech)

Vojtíšek B., Hronová B., Hamřík J., Janková B. (1993): Dietary silymarin in cows with various planes of nutrition in puerperal period. Živočišná výroba, 38, 981–1001. (in Czech)

Wang M., Grange L., Tao J., Reyes E. (1996): Hepatoprotective properties of *Silybum marianum* herbal preparation on ethanolinduced liver damage. Fitoterapia, 67, 166–171.

Wen Z., Dumas T.E., Schrieber S.J., Hawke R.L., Fried M.W., Smith P.C. (2008): Pharmacokinetics and metabolic profile of free, conjugated and total silymarin flavonolignans in human plasma after oral administration of milk thistle extract. Drug Metabolism and Disposition, 36, 65–72.

Received: 2010–06–08
Accepted after corrections: 2010–12–08

Corresponding Author

Mgr. Ing. Ludmila Křížová, Ph.D., Agriresearch Rapotin Ltd., Department of Animal Nutrition and Quality of Livestock Products, Vídeňská 699, 691 23 Pohořelice, Czech Republic
Tel. +420 739 251 453, e-mail: ludmila.s@seznam.cz