Rumen *in vitro* Gas Production of Combinations Between Slowly and Rapidly Fermentable Fibre Sources

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Summary

An *in vitro* rumen fermentation was performed, where two sources of slowly fermentable fibre (milk thistle, MT; pure cellulose, CE) and three of rapidly fermentable fibre (tomato peels without seeds, TP; citrus pulp, CI; pectin, PE) were incubated alone or as 75:25 or 25:75 mixtures (MT:TP, MT:CI, MT:PE, CE:TP, CE:CI, CE:PE), to detect associative effects among substrates. Substrates or mixtures (0.5 g) were incubated with 75 ml of buffered rumen fluid into individual bottles at 39°C for 96 h. Differences among measured and expected GP were analysed by ANOVA. In mixtures containing MT, positive associative effects were detected within first 24 h. Only the combination of 75% MT and 25% TP showed negative interactions. In the mixtures of MT:CI and MT:PE positive associative effects were detected respectively at 24 and 12 h ($P<0.01$). Mixtures containing CE showed positive interactions only at later phases (from 48 h) of incubation. When 75% CE was added to 25% TP and CI, associative effects were greater compared to mixtures containing a lower proportion of CE (25%). In conclusion, three sources of rapidly fermentable fibre enhanced GP provided by MT and CE, even if magnitude of these effects differed among mixtures.

Key words

gas production, fermentable fibre, associative effects, rumen fermentation

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Aim

The nutritional value of low-quality forages could be improved by a suitable combination in the diet with highly fermentable substrates. Niderkorn and Baumont (2009) reported that positive associative effects can occur when slowly and rapidly fermentable forages are combined. This experiment was conducted to detect associative effects among three substrates largely diffused and available in the Mediterranean region: two industrial by-products (tomato peels and citrus pulp) as sources of rapidly fermentable fibre, and a spontaneous weed (milk thistle; *Sylibum marianum*) as source of slowly fermentable fibre. The use of milk thistle in ruminant feeding could represent an effective strategy to valorize this spontaneous weed. However, great fibre content of milk thistle suggests the need to associate this roughage with highly fermentable ingredients.

For a better characterization of the three vegetable sources above mentioned (milk thistle, tomato peels, citrus pulp), two purified products, slowly (cellulose) and rapidly fermentable (pectin), were included as standard. A fully automated *in vitro* gas production (GP) system was used to evaluate rumen fermentation of these substrates, when incubated alone or in different combinations.

Material and methods

A single incubation (96 h) was performed, where two sources of slowly fermentable fibre (milk thistle, MT; pure cellulose, CE) and other three of rapidly fermentable fibre (tomato peels, TP; citrus pulp, CI; pectin, PE), previously milled at 1 mm, were incubated alone or as the following 75:25 or 25:75 mixtures: MT:TP, MT:CI, MT:PE, CE:TP, CE:CI, and CE:PE. Tomato peels contained only peels and pectin was a commercial product (Pectin esterified from citrus fruit, P9561, Sigma). Prior to the grinding, TP and CI were dried. The five substrates and the twelve mixtures were incubated into individual glass bottles in three replicates, plus 2 blanks (bottles without feed sample), for a total of 53 bottles incubated. Each bottle (310 ml) was filled with 0.500±0.0010 g of feed sample or mixture, 50 ml of buffer and 25 ml of rumen fluid (headspace=235 ml) and placed into a ventilated incubator at 39±0.5°C. Buffer solution was prepared according to Menke and Steingass (1988). Rumen fluid was collected by an esophageal probe 2 h before morning feeding from 3 dry Holstein-Friesian cows (fed hay *ad libitum* and 2 kg/d of concentrates), stored into two thermal flasks preheated to 39±0.5°C, and transferred to the laboratory (Tagliapietra et al., 2012). Then, rumen fluid was filtered through 3 layers of cheesecloth, in order to eliminate feed particles and mixed with buffer in a 1 to 2 ratio (Menke and Steingass, 1988). All these operations required less than 30 min and they were conducted under anaerobic conditions by continuous flushing with CO_2. A fully automated *in vitro* GP system (Ankom^{RF}; Ankom Technology®, NY, USA) was used to monitor GP over the whole incubation (96 h). This system is made up of 53 bottles equipped with a pressure detector and with an electro-mechanical valve for gas venting when the pressure in the headspace of bottles reaches 3.4 kPa (Tagliapietra et al., 2010). Pressure values were converted in volume terms (ml) using the ideal gas law. For the twelve mixtures the "expected GP" was calculated as weighed mean of GP provided by substrates when they were incubated singularly (assuming the absence of associative effects between substrates). The five substrates were analyzed in triplicate for dry matter (DM), crude protein (CP), ether extract (EE) and ash (AOAC, 2003). Neutral detergent fiber content (aNDF) of substrates was determined with α-amylase and sodium sulphite (Mertens, 2002), using the Ankom^{220} Fiber Analyzer (Ankom Technology®, NY, USA). Acid detergent fibre (ADF), expressed as inclusive of residual ash, and sulphuric acid lignin (ADL) contents were analyzed sequentially after aNDF (Robertson and Van Soest, 1981). Pectin content was determined according to Bailoni et al. (2004). Measured GP values provided by the five substrates were subjected to ANOVA considering only feed as source of variation. For the twelve mixtures differences between measured and expected GP were computed and analysed by ANOVA, at various incubation times (6, 12, 24, 48, 72, and 96 h), to test if they differed significantly from zero.

Results and discussion

As expected, milk thistle contained a high level of fibrous fractions (688 and 86 g/kg DM of aNDF and ADL, respectively) and a poor CP content (43 g/kg DM) (Table 1). In agreement with literature (Ventura et al., 2009), tomato peels showed a high ADF content (300 g/kg DM), a notable ADL content (98 g/kg DM), and a fair amount of CP (121 g/kg DM). Citrus pulp showed a great content of pectin (250 g/kg DM), according to literature (Srivastava and Malviya, 2011). Two purified products (CE and PE) contained 1000 g/kg DM of cellulose and 900 g/kg DM of pectin, respectively. As expected, MT and CE showed always the lowest ($P<0.01$) GP values (Figure 1). Interestingly, fermentation of CE started only after 24 h and did not reach the plateau of GP after 96 h of incubation, compared to other substrates. Within rapidly fermentable sources, CI showed the highest ($P<0.01$) GP values at 6 and 12 h, whereas PE produced more gas ($P<0.01$)

Table 1. Proximate composition of the five substrates/products (n=3 per substrate; milled at 1 mm)

	DM g/kg	aNDF g/kg DM	ADF g/kg DM	ADL g/kg DM	PECTIN g/kg DM	CP g/kg DM	EE g/kg DM	Ash g/kg DM
Milk thistle, MT	881	688	524	86	5	43	6	130
Cellulose purified, CE	970	1000	1000	-[a]	-[a]	-[a]	-[a]	-[a]
Tomato peels, TP	73	331	300	98	76	121	17	118
Citrus pulp, CI	211	257	117	-[a]	250	57	6	26
Pectin purified, PE	970	-[a]	-[a]	-[a]	900	-[a]	-[a]	100

[a] amount not measurable

Table 2. Differences between measured and expected gas production (GP, ml/g OM) of the twelve different combinations at various incubation times

	Incubation times (h)					
	6	12	24	48	72	96
MT:TP 75:25	-13.0**	-12.7**	-21.4**	-22.7**	-26.1	-28.9
MT:TP 25:75	-5.3	+7.2	+17.7**	+9.2	+8.6	+7.7
MT:CI 75:25	-0.7	+3.6	+17.3**	+16.0	+10.6	+9.3
MT:CI 25:75	+3.2	+7.9	+23.4**	+18.3	+16.0	+15.9
MT:PE 75:25	+4.8	+39.7**	-11.0	+5.4	+2.6	-2.9
MT:PE 25:75	+3.8	+86.5**	+0.6	-1.4	+3.9	+8.1
CE:TP 75:25	-7.4**	-11.1**	-13.8**	+90.8**	+174.0**	+146.4**
CE:TP 25:75	-8.1**	-12.9**	-4.9	+52.9**	+61.2**	+48.7**
CE:CI 75:25	-11.3**	-13.8**	-12.8**	+93.5**	+153.5**	+120.6**
CE:CI 25:75	-9.1**	-5.5	+15.6**	+66.6**	+71.2**	+57.2**
CE:PE 75:25	-1.4	+5.9	-12.2**	+8.6	+61.7**	+82.5**
CE:PE 25:75	-3.1	+4.7	-15.6**	+30.3**	+53.8**	+40.4**
SEM	2.7	3.4	4.4	6.2	9.8	9.2

** differences between measured and expected values significantly different from zero (P<0.01)

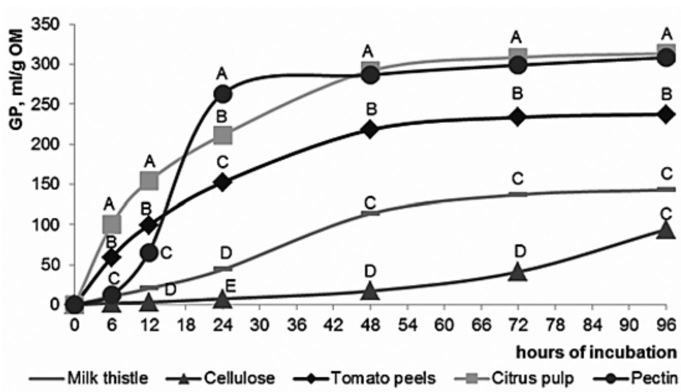

Figure 1. Cumulative GP (ml/g OM) of the five substrates/ products (n=3 per feed). Within each incubation time, dots with different letters are significantly different (P<0.01).

at 24 h. Compared to CI and PE, TP produced less gas (P<0.01) at 96 h of incubation, likely because of great ADL content. At 6 h, PE showed GP values comparable (P>0.01) to those provided by two sources of slowly fermentable fibre (MT and CE). Even if rumen fermentation of pectin is considered to be rapid (Van Soest, 1982), the Cornell Net Carbohydrate and Protein System (Lanzas et al., 2007) attributed to pectin a smaller degradability rate compared to sugars. As reported in Table 2, MT and CE showed different patterns of GP when combined with three rapidly fermentable sources (TP, CI and PE). For MT, positive associative effects were detected particularly within first 24 h of incubation. Differently, positive interactions occurred at later phases (from 48 to 96 h) in the case of CE, and were more pronounced compared to those observed for MT. When combined with TP, MT showed less pronounced associative effects compared to the mixtures with CI and PE. In particular, in the mixture composed by 75% MT and 25% TP, negative associative effects were detected until to 48 h (P<0.01). However, when TP repre-

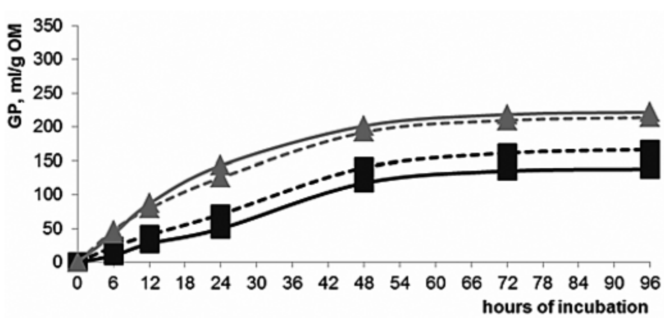

Figure 2. Cumulative expected (dashed line) and measured (continuous line) gas production (GP, ml/g OM) of two combinations (75:25, ■ and 25:75, ▲) between milk thistle (MT) and tomato peels (TP)

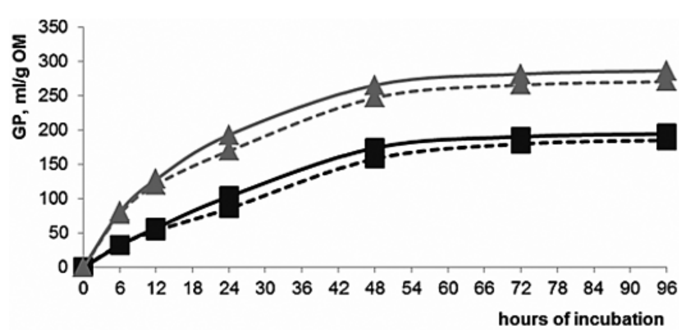

Figure 3. Cumulative expected (dashed line) and measured (continuous line) gas production (GP, ml/g OM) of two combinations (75:25, ■ and 25:75, ▲) between milk thistle (MT) and citrus pulp (CI)

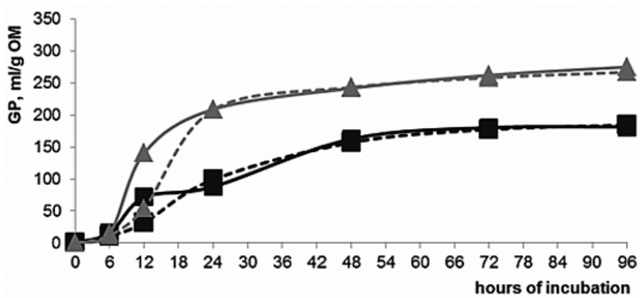

Figure 4. Cumulative expected (dashed line) and measured (continuous line) gas production (GP, ml/g OM) of two combinations (75:25, ■ and 25:75, ▲) between milk thistle (MT) and pectin (PE)

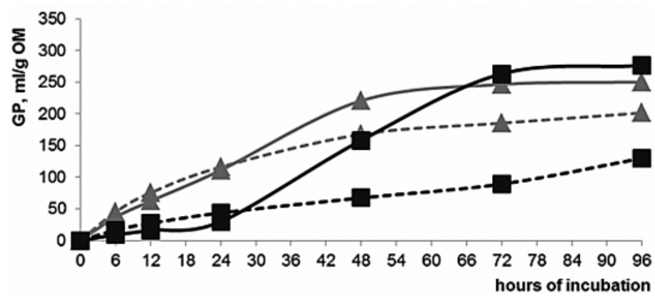

Figure 5. Cumulative expected (dashed line) and measured (continuous line) gas production (GP, ml/g OM) of two combinations (75:25, ■ and 25:75, ▲) between cellulose (CE) and tomato peels (TP)

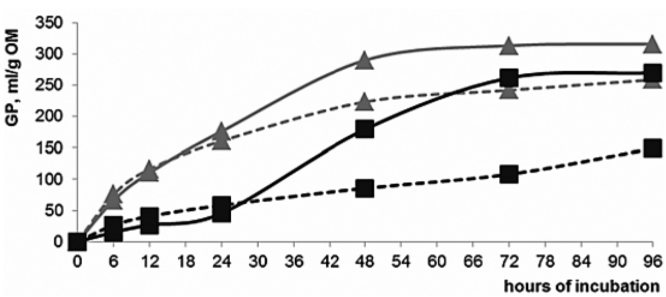

Figure 6. Cumulative expected (dashed line) and measured (continuous line) gas production (GP, ml/g OM) of two combinations (75:25, ■ and 25:75, ▲) between cellulose (CE) and citrus pulp (CI)

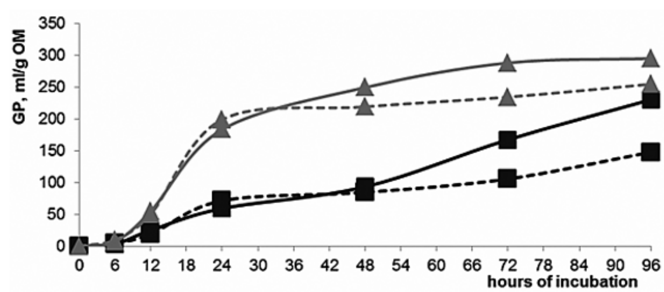

Figure 7. Cumulative expected (dashed line) and measured (continuous line) gas production (GP, ml/g OM) of two combinations (75:25, ■ and 25:75, ▲) between cellulose (CE) and pectin (PE)

sented 75% of the mixture, positive interactions were observed at 24 h ($P<0.01$; Table 2 and Figure 2). When combined with CI, MT evidenced positive associative effects at 24 h ($P<0.01$; Table 2 and Figure 3). When MT was combined with PE, associative effects were anticipated at 12 h ($P<0.01$; Table 2 and Figure 4). Almost all mixtures containing CE showed negative associative effects within 24 h of incubation, and highly positive interactions started from 48 h. More in detail, combinations between CE and TP evidenced negative associative effects ($P<0.01$) at early phases of incubation. On the opposite, strongly positive interactions were observed from 48 to 96 h, in particular when CE was 75% of the mixture (Table 2 and Figure 5). Similar patterns were found when CE was combined with CI (Table 2 and Figure 6) and PE (Table 2 and Figure 7), even if in the latter case positive interactions tended to be less pronounced. Combination of 75% CE and 25% PE showed highly positive effects at later phases of incubation ($P<0.01$). When proportion of PE was increased up to 75%, associative effects were anticipated at 48 h ($P<0.01$; Table 2 and Figure 7). In the current experiment, significant associative effects were found in the first part of incubation (<24 h) when the fermentable sources were incubated with MT. These results are in agreement with the findings obtained by other authors (Liu et al, 2002; Robinson et al., 2009). On the contrary, positive associative effects between CE and fermentable sources were more pronounced from 48 h to the end of incubation, probably because of low fermentability of CE.

Conclusions

Results of the current experiment indicated that three sources of rapidly fermentable fibre were able to enhance gas production provided by two sources of slowly fermentable fibre (milk thistle and cellulose). However, magnitude of positive effects was more pronounced for pectin and citrus pulp compared to tomato peels and especially when high levels of inclusion (75%) were used. From the practical point of view, two industrial by-products (citrus pulp and tomato peels), that are widely available in the Mediterranean region, could be exploited to improve rumen fermentation of low quality forage as milk thistle, largely diffused in same geographical area.

References

AOAC (2003). Official Methods of Analysis of the Association of Official Agricultural Chemists, 17th ed. (2nd revision). AOAC International, Gaithersburg, MD, USA.

Bailoni L., Schiavon S., Pagnin G., Tagliapietra F., Bonsembiante M. (2004). Quanti-qualitative evaluation of pectins in dietary fibre of 24 foods. Ital. J. Anim. Sci. 4: 49-58.

Lanzas C., Sniffen C. J., Seo S., Tedeschi L. O., Fox D.G. (2007) A revised CNCPS feed carbohydrate fractionation scheme for formulating rations for ruminants. Anim. Feed Sci. Technol. 136: 167-190.

Liu J.X., Susenbeth A., Sudekum K.H. (2002). *In vitro* gas production measurements to evaluate interactions between untreated and chemically treated rice straws, grass hay, and mulberry leaves. J. Anim. Sci. 80, 517-524.

Menke K. H., Steingass H. (1988). Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. Anim. Res. Dev. 28: 7-55.

Mertens D. R. (2002). Gravimetric determination of amylase-treated neutral detergent fiber in substrates with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85: 1217-1240.

Niderkorn V., Baumont R. (2009). Associative effects between forages on feed intake and digestion in ruminants. Animal 3: 951-960.

Robertson J. B., Van Soest P. J. (1981). The detergent system of analysis and its application to human foods. In: The Analysis of Dietary Fiber in Food (WPT James, O Theander, eds), Marcel Dekker Inc., NY, USA, 123-158.

Robinson P.H., Getachew G., Cone J.W.(2009). Evaluation of the extent of associative effects of two groups of four feeds using in vitro gas production technique. Anim. Feed Sci. Technol. 150, 9-17.

Srivastava P., Malviya R. (2011). Sources of pectin, extraction and its applications in pharmaceutical industry – An overview. Ind. J. Nat. Prod. Res. 2: 10-18.

Tagliapietra F., Cattani M., Bailoni L., Schiavon S. (2010). *In vitro* rumen fermentation: effect of headspace pressure on the gas production kinetics of corn meal and meadow hay. Anim. Feed Sci. Technol. 158: 197-201.

Tagliapietra F., Cattani M., Hindrichsen I. K., Hansen H. H., Colombini S., Bailoni L., Schiavon S. (2012). True dry matter digestibility of substrates evaluated *in situ* with different bags and *in vitro* using rumen fluid collected from intact donor cows. Anim. Prod. Sci. 52: 338-346.

Van Soest P. J. (1982). Nutritional Ecology of the Ruminant. O&B Books, Cornallis, OR.

Ventura M. R., Pieltain M. C., Castanon J. I. R. (2009). Evaluation of tomato crop by-products as feed for goats. Anim. Feed Sci. Technol. 154: 271-275.

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