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In situ and in vitro ruminal starch degradation of untreated and formaldehyde-treated wheat and maize

B Michalet-Doreau [1]*, C Philippeau [1], M Doreau [2]

[1] *Station de recherches sur la nutrition des herbivores ;*
[2] *Laboratoire sous-nutrition des ruminants, Inra-Theix*
63122 Saint-Genès Champanelle, France

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Summary — Ruminal starch degradation of untreated and formaldehyde-treated wheat and maize was measured in situ (trial 1) and in vitro (trial 2). The in situ starch degradability was higher for wheat than for maize (82.1 vs 52.3%), for untreated cereals than for cereals treated with 1% formaldehyde (77.3 vs 67.0%) and for cereals treated with 1% formaldehyde than those treated with 5% formaldehyde (67.0 vs 57.2%). The in vitro results were similar. The treatment of cereals by formaldehyde decreased starch degradability more for wheat than for maize, suggesting that the treatment was more efficient when cereal starch and/or nitrogen was highly degradable. Formadehyde treatment of wheat was more effective at decreasing the rate of wheat protein degradability than starch degradability. The difference of response to treatment between the two cereals may be due to differences in properties of the protein matrix of these two cereals.

starch / formaldehyde treatment / ruminal degradation

Résumé — Effet du traitement au formol sur la dégradation in situ et in vitro du blé et du maïs. La dégradation in situ (essai 1) et in vitro (essai 2) de deux céréales (blé et maïs) traitées avec 0, 1 ou 5 % de formol (350 g/L) a été mesurée. La dégradabilité in situ de l'amidon était plus élevée pour le blé (82,1 %) que pour le maïs (52,3 %), et plus élevée pour les céréales non traitées (77,3 %) que pour les céréales traitées avec 1 % de formol (67,0 %) ou 5 % de formol (57,2 %). Des résultats identiques ont été obtenus in vitro. La diminution de dégradation avec le traitement au formol était plus marquée avec le blé qu'avec le maïs, et sur les protéines des céréales que sur leur amidon. La différence de réponse au traitement entre céréales rapidement dégradables (blé) et lentement dégradables (maïs) serait à mettre en relation avec les différences de texture des endospermes de ces deux céréales.

amidon / traitement au formol / dégradation ruminale

* Correspondence and reprints
Tel: (33) 05 73 62 40 00; fax: (33) 05 73 62 42 73.

INTRODUCTION

Cereal grain is subject to extensive fermentation in the rumen, resulting in the production of volatile fatty acids and microbial cells. The proportion of starch digested in the rumen can range from 50 to 95% depending on the nature of the cereal grain and the type of processing (Nocek and Tamminga, 1991). Starch which escapes digestion in the rumen is then subject to digestion in the small intestine by the host enzymes, resulting in the formation of glucose. Different factors suggest that increasing the ruminal bypass of starch involving a shift of the propionate production towards supplying glucose could be interesting for the following reasons: 1) reduced risk of acidosis or lower milk butterfat when large amounts of cereals are fed; 2) better metabolic efficiency of glucose compared to propionate; 3) interest of glucose as a specific nutrient for gut or fetus. The amount of starch escaping from the rumen may be increased by using maize or sorghum as starch sources instead of barley or wheat, or when there is a high rate of passage of particles towards the duodenum, or by grain processing or chemical treatment (Chase, 1993; Poncet et al, 1995). Among the various chemical treatments tested by Fluharty and Loerch (1989), glyoxal, masonex, propionaldehyde and tannic acid were not efficient, and only the treatment with formaldehyde increased starch ruminal bypass by reducing the in vitro dry matter (DM) degradation of ground maize. It had no effect, however, on the ruminal starch degradation of wheat grain (Van Ramshorst and Thomas, 1988). Did these differences come either from an effective interaction between treatment and nature of the cereal, or from the experimental conditions which differed between the two trials?

To clarify this question two trials were carried out. The aim was to compare the effect of a formaldehyde treatment of two cereals, wheat and maize, on ruminal starch in situ degradation (trial 1) and in vitro digestion (trial 2).

MATERIALS AND METHODS

Experimental feeds

Native wheat (*Triticum sativum*, cv Soissons) and maize (*Zea mays*, cv Banguy) were used in this experiment. The crude protein and starch contents of the two cereals were determined respectively by Kjeldahl (AOAC, 1990) and by an enzymatic method (Thivend et al, 1965). After a hot water treatment and autoclaving, the starch was hydrolysed to glucose with a glucoamylase of fungal origin. The glucose concentration was then determined by colorimetry. Crude protein and starch contents were 10.4 and 65.6% DM for the wheat, 8.6 and 72.6% for the maize. The cereals were coarsely ground, then treated with 0%, 1% or 5% formaldehyde solution (350 g/L) by spraying. The same feeds were used in both the in situ and in vitro experiments.

Trial 1

The objective was to compare the in situ degradation of wheat and maize untreated or treated with 1 or 5% formaldehyde. Three dry Holstein cows, fitted with a ruminal cannula, were used in this experiment. They received a diet of 66% grass hay and 34% concentrates. Composition of the concentrate was 43% barley, 40% beet pulp, 10% soybean meal, 5% beet molasses and 2% mineral-vitamin premix. The daily ration was 7 kg DM, given in two equal parts at 0800 and 1600 hours. The cows were adapted to their diet for 4 weeks before measurements.

Treated and untreated cereals were compared in this trial, ie, six feeds (wheat and maize, untreated and treated with 1 and 5% formaldehyde). Approximately 3 g of feed ground through a 0.8 mm screen was put into dacron bags (Ankom Co, Fairport, NY, USA; pore size: 53 μm; internal dimensions: 5 × 10 cm) and introduced into the rumen of each of the three animals before the morning meal. Two replicates were performed for each time point, each feed and each animal. The bags were removed after 2, 4, 6, 8, 14, 24, 48 and 96 h of incubation, and were then washed in a washing machine for

6 min with three successive 2-min washings, dried at 80 °C for 48 h and weighed. The residues of the same feed and the same incubation time were pooled before starch and protein analysis by the previously stated methods.

DM, starch and protein degradation data were fitted to a single exponential equation (Orskov and McDonald, 1979):

$$D(t) = a + b\,(1 - e^{-ct})$$

where $D(t)$ is the amount degraded at time t, a is the rapidly degradable fraction, b is the slowly and potentially degradable fraction and c is the rate constant of degradation of fraction b. Modelling of the degradation curves was performed using the NLIN procedure of SAS (1988). Degradability (D) was calculated as:

$$D = a + (b \times c)/(c + k)$$

k being the particle passage rate out of the rumen, taken as 0.06 h^{-1} (Vérité et al, 1987).

Statistical analysis was performed by analysis of variance with the GLM procedure of SAS (1988), according to the model:

$$Yijkl = \mu + G_i + T_j + A_k + GT_{ij} + e_{ijkl}$$

where μ is the overall mean, G the grain source (wheat or maize), T the formaldehyde treatment (0, 1 or 5%), A the animal, GT the interaction between grain source and treatment, and e the error. Differences between treatments were tested by the Duncan t-test.

Trial 2

The objective was to compare the effects of grain source (wheat or maize) and of formaldehyde treatment on in vitro gas and VFA production, and fermented organic matter (FOM). The fermenter was a closed system described by Jouany and Thivend (1986) and detailed by Ottou and Doreau (1996). Six flasks containing the same carbohydrate sources as in trial 1 together with the fermentation medium were introduced in the fermenter. The fermentation medium in each flask was made up of 200 g rumen contents sampled from the same cows as in the previous trial, homogenised with 200 mL buffer[1] saturated with CO_2, and a N source [5 mL of 17.6% $(NH_4)_2SO_4$ solution]. The amounts of wheat and maize added to the fermenter were calculated from their starch content so that all fermenters contained 13 g starch. The cereals were ground through a 0.5 mm screen. The flasks were maintained in a lateral shaking water bath kept at 39 °C. The entire measurement procedure was repeated for 4 consecutive days.

The volume of gas arising from the fermentation was measured after 15 min, 2, 4 and 6 h of fermentation. The mean composition was analysed by gas liquid chromatography according to Jouany and Senaud (1978). The concentration and composition of the VFA produced during the fermentation (difference between sampling after incubation and initial sampling) were analysed by gas-liquid chromatography (Jouany, 1982). The quantity of FOM (in g) was calculated from the amount of the different VFA (in mol) produced in the fermenter over 6 h of incubation according to the stoichiometric equation of Demeyer and Van Nevel (1975):

$$\text{FOM} = 162\,(0.5\ \text{acetate} + 0.5\ \text{propionate} + \text{butyrate} + \text{valerate})$$

Statistical analysis was performed by analysis of variance with the GLM procedure of SAS (1988), according to the model:

$$Yijkl = \mu + G_i + T_j + D_k + GT_{ij} + e_{ijkl}$$

where μ is the overall mean, G the grain source (wheat or maize), T the formaldehyde treatment (0, 1 or 5%), D the day of measurement, GT the interaction between grain source and treatment, and e the error. The differences between treatments were tested by the Duncan t-test.

RESULTS

Trial 1

The DM degradability (table I) was significantly ($P < 0.001$) higher for wheat than

[1] NaCl = 2.35 g; KCl = 2.25 g; $MgCl_2$, 6 H_2O = 0.50 g; $CaCl_2$, 2 H_2O = 0.36 g; $NaHCO_3$ = 92.0 g; Na_2HPO_4, 12 H_2O = 71.24 g dissolved with water to a total volume of 5 L.

Table I. Influence of formaldehyde treatment on in situ degradation of cereals (trial 1).

Cereal	*Wheat*			*Maize*				*Effect of*		
% of formaldehyde	0	1	5	0	1	5	*SE*	*Nature of cereal*	*Treatment*	*Interaction*
Dry matter										
a	72.9	63.4	44.0	37.4	34.0	34.9	1.5	***	***	***
b	22.2	31.9	50.2	60.0	61.9	60.3	1.8	***	***	***
c	0.168	0.068	0.042	0.043	0.038	0.037	0.015	***	***	***
Degradability	89.0	80.2	64.7	62.2	57.7	57.6	1.0	***	***	
Starch										
a	84.2	64.1	32.5	25.7	18.3	12.8	2.4	***	***	***
b	16.3	34.4	69.2	80.0	88.1	91.8	2.3	***	***	***
c	0.430	0.055	0.054	0.037	0.036	0.039	0.048	**	**	**
Degradability	98.5	82.5	65.3	56.2	51.5	49.2	0.6	***	***	***
Nitrogen										
Degradability	80.3	63.8	49.4	52.0	42.0	41.1	1.4	***	***	***

**: $P < 0.01$; ***: $P < 0.001$.

for maize. The rapidly degradable fraction was higher, the slowly degradable fraction was lower for wheat than for maize, and this was degraded more rapidly for wheat than for maize. The undegradable fraction was similar for the two cereals, 4.9 and 2.6 respectively for wheat and maize. The DM degradability was lower with 1% formaldehyde than without treatment and lower with 5% formaldehyde than with 1% formaldehyde ($P < 0.001$). This treatment effect was greater for wheat than for maize (significant interaction, $P < 0.001$). When observed, the decrease in DM degradability with the formaldehyde treatment was due to a decrease in the degradation rate constant for the two cereals and for wheat, a decrease in the rapidly degradable fraction with a comparable increase in the slowly degradable fraction.

Starch degradation was more rapid for untreated wheat and slower for maize treated with 5% formaldehyde. In all cases, the degradation was complete after 48 h of incubation. Starch degradability (table I) was always higher for: wheat than for maize ($P < 0.001$), untreated cereals than for cereals treated with 1% formaldehyde, and cereals treated with 1% than with 5% formaldehyde ($P < 0.001$). In comparison to treatment with 1% formaldehyde, 5% formaldehyde decreased the starch degradability by 33.2 percentage units with wheat and 7.0 percentage units with maize.

The nitrogen degradation stopped almost completely after 2 h of incubation for maize treated with 1% and 5% formaldehyde and for wheat treated with 5% formaldehyde. For this reason, it was not possible to adjust the data of the two feeds to any model. The degradability calculated by a stepwise method (Kristensen et al, 1982) (table I) was higher for: wheat than for maize ($P < 0.001$), and for the untreated cereals than

for the treated ones ($P < 0.001$). Although no correction was made for microbial contamination, it can be reasonably assumed that the differences in degradability due to formaldehyde treatment are effective. Indeed microbial contamination mainly depends on the N content of the feed (Michalet-Doreau and Ould Bah, 1992). Nitrogen content is not modified by formaldehyde treatment.

Trial 2

Total gas production in the fermenters (fig 1) was higher for wheat than for maize ($P < 0.001$) between 1 to 6 h of incubation. Gas production was not modified with 1% formaldehyde, but was rapidly stopped with 5% (table II). The production of methane, carbon dioxide and VFA's as well as the amount of fermented organic matter for 6 h incubation were significantly higher for wheat than for maize ($P < 0.001$) (table II).

The 1% formaldehyde treatment caused a decrease in the production of all VFA except butyrate and fermented organic matter ($P < 0.001$). The 5% level essentially stopped fermentation and the production of methane, carbon dioxide and VFA.

DISCUSSION

The in situ starch degradability of untreated maize grains was low (56%) and of the same magnitude (62%, 58 and 57%, respectively), as reported by Herrera-Saldana et al (1990), Cerneau and Michalet-Doreau (1991) and Grings et al (1992), as maize ground to similar particle size. The in situ starch degradability of untreated wheat grains was very high (98.5%). When the cereal was ground through the same size screen, Herrera-Saldana et al (1990) and Walhain et al (1992) found starch degradabilities of 95 and 93%, respectively. Our in vitro results led to sim-

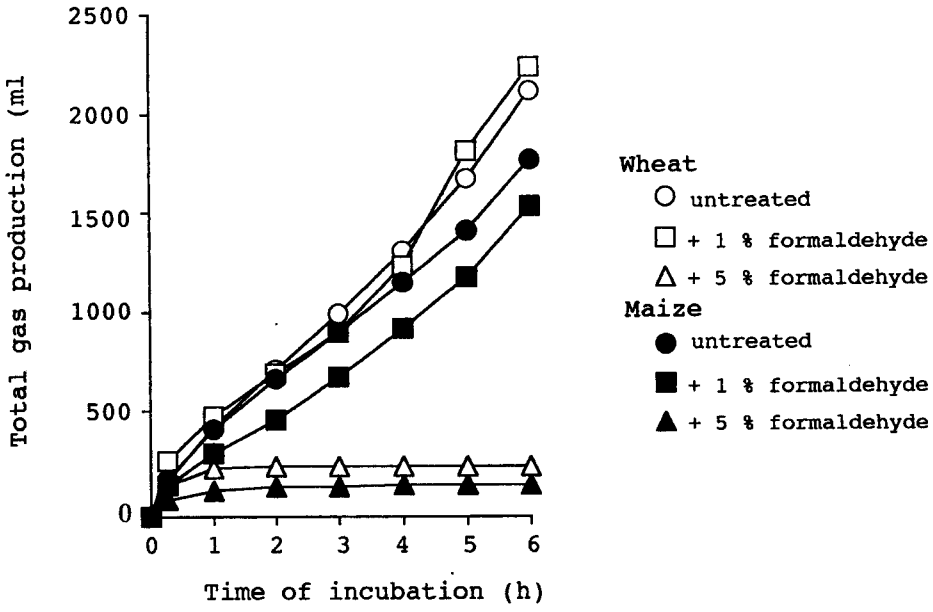

Fig 1. Total production of gas in the fermenters during 6 h of incubation.

Table II. Influence of formaldehyde treatment on in vitro 6-h fermentation of cereals (trial 2).

Cereal	*Wheat*			*Maize*				*Effect of*		
% of formaldehyde	0	1	5	0	1	5	*SE*	*Nature of cereal*	*Treatment*	*Interaction*
Gas production (mL)	2137	2262	253	1788	1559	160	73	***	***	***
Methane (mL)	297	259	2	225	163	1	15	***	***	***
Carbon dioxide (mL)	1515	1596	68	1213	948	56	47	***	***	**
VFA total production (m*M*)	130.5	86.5	4.9	102.1	78.2	1.1	3.7	***	***	*
Acetate (m*M*)	84.5	50.3	3.6	66.4	47.8	0.8	2.6	***	***	NS
Propionate (m*M*)	25.0	19.4	0.7	20.0	16.0	0.2	0.8	***	***	NS
Butyrate (m*M*)	14.4	14.1	0.4	12.7	11.7	0.2	0.8	***	***	*
Fermented organic matter (g)	4.60	3.22	0.16	3.65	2.87	0.04	0.13	***	***	*

NS = non significant; *: $P < 0.05$; **: $P < 0.01$; $P < 0.001$.

ilar conclusions: a faster starch fermentation for wheat than for maize grains. However, the wheat starch degradability may be overestimated because the proportion of undegraded DM which is lost through bag pores is much higher for wheat than for maize, and is mainly made up of starch (Michalet-Doreau, 1990). In cereals, the extent of starch digestion in the rumen is dictated by the nature of surrounding materials, protein matrix and endosperm cell walls (Kotarski et al, 1992). Differences in starch degradation rate between cereals might be partly explained by differences in the degradation rate of the protein matrix which surrounds the starch granules (McAllister et al, 1993).

In our study, the treatment of wheat by formaldehyde (1%) decreased the in situ and in vitro starch degradation. This effect of formaldehyde treatment on barley, another rapidly degradable cereal, was reported in vitro by McAllister et al (1990a) and in numerous in situ studies (Kassem et al, 1987; Morgan et al, 1989; McAllister et al, 1990b; Ortega-Cerrilla and Finlayson, 1991, 1994). The extent of this decrease varied between 6 and 40%, with levels of formaldehyde from 0.5 to 2%. This extent of the decrease did not only depend on the level of formaldehyde. The decrease in starch ruminal degradability did not vary when the quantity of formaldehyde added to the cereal was multiplied by 2 (Kassem et al, 1987; Ravelo et al, 1988; Morgan et al, 1989; McAllister et al, 1990b); or when the amount of formaldehyde added to the cereal was multiplied by 3 (Morgan et al, 1989) or by 5 as in our study, starch degradability decreased with increasing level of formaldehyde. The variability in the response of the barley starch degradability could be partly due to the level of formaldehyde, and so to the process of formaldehyde incorporation.

In our study, the treatment of cereals by formaldehyde decreased the in situ starch degradability more for wheat than for maize, suggesting that the treatment is more efficient when the cereal starch and/or nitrogen is highly degradable. Moreover formalde-

hyde treatment of wheat was more effective at decreasing the rate of wheat protein degradability than starch degradability. Similar results were found concerning the effect of formaldehyde treatment on starch and nitrogen degradability of barley grains (Kassem et al, 1987, McAllister et al, 1990b). Treatment with formaldehyde would increase the resistance of the protein matrix to microbial attack and thereby would shelter starch granules from microbial digestion (McAllister et al, 1990b). This difference in formaldehyde response between cereal grains was observed in vitro by Oke and Loerch (1991), but no direct comparisons between rapidly and slowly degradable starch grains have been made in situ.

According to Sauvant et al (1994), the in situ results amplify the in vivo differences of ruminal starch degradation between feeds. Using the prediction equation proposed by these authors and our in situ results, the in vivo starch ruminal degradation of wheat grains would decrease from 93 to 85% and 77% after formaldehyde treatment at 1 and 5% respectively, and that of maize grains would be modified to even lower levels. Curiously only the in vivo experiment using maize showed a significant effect of formaldehyde treatment (Oke et al, 1991) whereas, in most experiments using wheat or barley, (a trial by Van Ramshorst and Thomas, 1988, being the exception), no significant effect was found (Morgan et al, 1989; McAllister et al, 1992, Ortega-Cerrilla and Finlayson, 1991, 1994). This apparent contradiction between in situ and in vivo results can be explained by the null or low variation due to treatment when measured simultaneously in situ and in vivo (Morgan et al, 1989; Ortega-Cerrilla and Finlayson, 1991, 1994). For trials on ruminants, the efficiency of formaldehyde treatment of large batches might be lower than treatment of small quantities necessary for in situ or in vitro trials. The variability in in situ, in vitro and in vivo results concerning the effect of cereals treatment might be due to process of formaldehyde incorporation.

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