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Effect of site of starch digestion on portal nutrient net fluxes in steers

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Processing of maize grain is known to modulate the site of starch digestion, thus the nature and amount of nutrients delivered for absorption. We assessed the effect of site of starch digestion on nutrient net fluxes across portal-drained viscera (PDV). Three steers, fitted with permanent digestive cannulas and blood catheters, successively received two diets containing 35 % starch as dent maize grain. Diets differed according to maize presentation: dry and cracked (by-pass, BP) *v.* wet and ground (control, C). Ruminal physicochemical parameters were not significantly affected. Between C and BP, the decrease in ruminal starch digestion was compensated by an increase in starch digestion in the small intestine. The amount of glucose and soluble α-glucoside reaching the ileum was not affected. The amount of glucose disappearing in the small intestine increased from 238 to 531 g/d between C and BP, but portal net flux of glucose remained unchanged (−97 g/d). The portal O_2 consumption and net energy release were not significantly affected, averaging 16 % and 57 % of metabolizable energy intake, respectively. The whole-body glucose appearance rate, measured by jugular infusion of [6,6-$^2H_2$]glucose, averaged 916 g/d. The present study shows that the increase in the amount of glucose disappearing in the small intestine of conventionally fed cattle at a moderate intake level induces no change in portal net flux of glucose, reflecting an increase in glucose utilization by PDV. That could contribute to the low response of whole-body glucose appearance rate observed at this moderate level of intestinal glucose supply.

Cattle: Digestion: Absorption: Starch: Glucose: Portal-drained viscera

High amounts of cereals incorporated in the diet of high-producing ruminants can induce digestive disorders related to an excessive fermentation rate of starch in the rumen. A shift in site of starch digestion from rumen to intestines could prevent these disorders, but this change influences ruminal fermentation products and microbial protein synthesis, and also the nature of nutrients derived from starch digestion, i.e. volatile fatty acids or glucose, which differ in their energetic efficiency (Owens *et al.* 1986). The nutritional factors affecting ruminal starch digestion in cattle are well known: nature of cereal, genotype, maturity and grain processing are the main factors controlling the amount of starch escaping ruminal digestion (see reviews by Poncet *et al.* 1995; Huntington, 1997; Michalet-Doreau & Doreau, 1999). Up to 35 % of ingested starch has been shown to be digested in the small intestine in steers (Axe *et al.* 1987; Stock *et al.* 1987) and cows (Rémond *et al.* 2004), but the extent of starch digestion in the small intestine can be limited by time or surface exposure of starch to enzymes in this compartment, or by a limited capacity of starch-hydrolysing enzymes, principally pancreatic α-amylase (Walker & Harmon, 1995; Swanson *et al.* 2002) or brush border α-glucosidase (Kreikemeier & Harmon, 1995). Also, the density of glucose transporters decreases markedly between the proximal and the distal portion of the intestine (Ferraris *et al.* 1989). Thus, a non-negligible amount of starch (Knowlton *et al.* 1998; Philippeau *et al.* 1999*b*; Rémond *et al.* 2004), glucose and soluble α-glucoside (Kreikemeier *et al.* 1991; Kreikemeier & Harmon, 1995) can reach the large intestine for subsequent fermentation in both steers and cows. Assessing the metabolism of absorbed glucose by portal-drained viscera (PDV) requires simultaneous measurements of both small intestinal glucose disappearance and net portal flux, which has been attempted using abomasal infusions (Kreikemeier *et al.* 1991; Kreikemeier & Harmon, 1995; Taniguchi *et al.* 1995; Krehbiel *et al.* 1996; Harmon *et al.* 2000) but not in conventionally fed animals. The main objective of this study was to assess digestive and portal net nutrient fluxes in steers receiving two diets differing in the partition of starch digestion between rumen and small intestine. In addition, whole-body glucose appearance rate was monitored. We attempted to achieve the shift in starch digestion between rumen and small intestine by modulating DM content and processing a dent maize genotype, as suggested by previous results from cows (Rémond *et al.* 2004).

Material and methods

Animals, experimental design and diets

Three steers of Salers breed (296, 325, and 340 kg body weight (BW); 11, 13 and 12 months at the beginning of measurements) were used. Animals were castrated and surgically fitted with a

Abbreviations: ADF, acid detergent fibre; BM, body weight; BP, by-pass; C, control; MDV, mesenteric-drained viscera; ME, metabolizable energy; OM, organic matter; PDV, portal-drained viscera; VFA, volatile fatty acid; WBGRa, whole-body glucose appearance rate.

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permanent ruminal cannula (internal diameter 60 mm) made of polyamide and polyvinyl chloride (Synthesia, Nogent-sur-Marne, France). Three weeks later, they were surgically fitted with T-shaped cannulas (internal diameter 17 mm) made from plastisol (Synthesia) with a gutter-type base placed at the proximal duodenum and the terminal ileum, as well as catheters in the portal and mesenteric veins and mesenteric artery. The surgery was conducted aseptically under general anaesthesia using isoflurane. Surgical procedures and post-surgical care were conducted under conditions approved by the national legislation on the care and use of laboratory animals (statutory order no. 87–848, 19 October 1987, *Journal Officiel*, France). Catheter patency was maintained by flushing the catheter with physiological saline and twice-weekly filling with heparinized saline. The animals were housed individually and were allowed 6 weeks to recover from surgery, during which period they were adapted to the experimental diet.

Animals received two diets consisting of 36 % hay (first-cut of natural grassland from the Auvergne region, harvested at early ear emergence), 53 % maize grain (dent genotype) and 11 % soyabean meal, on a DM basis. The organic matter (OM) digestibility and metabolizable energy (ME) content of the diets, estimated on the basis of tabulated feed values (INRA, 1989), were 82 % and 12·1 MJ/kg DM, respectively. The same maize grain was incorporated in the two diets but differed in its presentation: wet and ground (control, C) *v*. dry and cracked (by-pass, BP). DM content (103°C, 24 h) and mean particle size measured by sieving (Waldo *et al.* 1971) were, respectively, 71·5 % and 1·21 mm for the C grain and 86·9 % and 3·07 mm for the BP grain. Wet maize was conserved whole in anaerobic bags and ground before distribution. Due to the necessity of using wet grain quickly after opening the anaerobic bag, animals successively received the BP diet (period 1) then the C diet (period 2). They also received 100 g/d mineral supplement (Ca–P–Mg–Na, 20:10:5:5 %) containing vitamins and microelements and 100 g/d sodium bicarbonate, and had free access to water and block salt. Diets were offered every 4 h in six equal meals per day (3, 7, 11, 15, 19 and 23 h) via an automatic feeder. The intake level was fixed at 5 kg DM, i.e. 1·58 times maintenance requirements for each animal and diet. Each experimental period consisted of 2 weeks of adaptation to diet and 2 weeks of measurements.

Measurements

Intake, total tract digestibility and nutrient flow. Daily amounts of feed offered and orts were weighed and recorded for individual steers. The DM content (24 h at 103°C) was determined weekly for feeds and daily for orts. Composite samples of fresh feeds and orts were pooled for each steer and period for subsequent analysis. Total tract digestibility was determined on 6 consecutive days by total daily faecal collection during the first week of measurements. Faeces were weighed and mixed before sampling. A representative sample (500 g) was dried (24 h at 103°C) for DM content determination. Another representative sample (5 % of daily excretion) was pooled for each steer and period and frozen for subsequent analysis. Duodenal and ileal nutrient flows were determined for the digestibility period using $YbCl_3$ as a marker (Siddons *et al.* 1985). The Yb solution (1 g Yb dissolved in 1·2 litres of water) was infused continuously (50 ml/h) into the rumen, following a priming infusion (1200 ml). Infusions began 5 d before the beginning of sampling digesta, and continued until the end of the digestibility measurement period. Samples of duodenal (200 g) and ileal (200 g) contents were collected four times daily every 3 h on 2 consecutive days in the middle of the digestibility period, providing representative samples of duodenal and ileal contents representing 1·5 h intervals over 12 h, i.e. three feeding cycles. Samples were weighed and then frozen for subsequent analysis. The amount of duodenal and ileal contents collected over the digestibility period were taken into account in the calculation of faecal digestibility. Fresh feeds, orts, and duodenal, ileal and faecal samples were analysed for ash (550°C, 6 h) and crude protein (Association of Official Analytical Chemists, 1990). Starch (Faisant *et al.* 1995) and acid detergent fiber (ADF), using α-amylase (Van Soest & Robertson, 1980) were analysed on fresh feeds and orts, and on lyophilized duodenal, ileal and faecal samples. Yb (Siddons *et al.* 1985) was analysed on lyophilized duodenal, ileal and faecal samples. Duodenal, ileal and faecal samples withdrawn before infusions were used as blank for Yb determination. Following an extraction procedure derived from Besle *et al.* (1981), glucose and soluble α-glucoside (maltose, maltotriose, maltotetraose, maltopentaose and maltoheptaose) on lyophilized ileal samples were analysed by HPLC (Bio Rad column, HPX 87P) as described by Kaar *et al.* (1991).

Ruminal microbial synthesis. Ruminal microbial synthesis was determined on 2 d per animal per period. Two days before the beginning of the digestibility period, samples (1 litre) of ruminal contents were withdrawn at 08.00, 10.40 and 13.20 hours by aspiration via the ruminal cannula using a tube placed in the ventral sac. After sieving (4 mm), the ruminal liquor was centrifuged (10 min, 4°C, 800 ***g***). Liquid-associated bacteria were isolated from supernatant by centrifugation (20 min, 4°C, 27 000 ***g***). Nucleic acid bases were determined on lyophilized duodenal samples and ruminal bacteria (Lassalas *et al.* 1993), and N was determined on lyophilized ruminal bacteria.

Ruminal physicochemical parameters. Ruminal physicochemical parameters were determined on 1 d per animal per period. At the end of the digestibility period, samples (50 ml) of ruminal liquor were withdrawn at 08.00, 10.40 and 13.20 hours by aspiration via the ruminal cannula using a tube placed in the ventral sac. The pH was immediately measured using a combination electrode. After filtration (100 μm), samples for ammonia and volatile fatty acid (VFA) determination were preserved by adding 0·1 volume orthophosphoric acid at 5 % (v/v) or 4 volumes NaCl at 20 % (w/v), respectively, and then frozen (− 15°C) before analysis. Ammonia was determined colorimetrically by the phenol-hypochlorite automated method, and VFA was determined by GC using 4-methylvaleric acid as an internal standard, as previously described (Nozière *et al.* 2000).

Portal net nutrient fluxes. Measurements of portal net fluxes were performed on 1 d per animal per period. One day before or after duodenal and ileal content were sampled, a physiological sterile saline solution (pH 7.4) containing *p*-aminohippuric acid (10 % w/v) was continuously infused into the distal mesenteric vein catheter (60 ml/h) following a prime injection (20 ml) at 06.45 hours to allow determination of portal blood flow by downstream dilution. Blood samples were withdrawn simultaneously from the artery and portal veins eight times daily at 60 min intervals, i.e. over two successive feeding cycles. Sampling started at 07.30 hours (i.e. 30 min after a meal). Samples were slowly drawn into blood syringes containing EDTA-K as anticoagulant for determination of *p*-aminohippuric acid, ammonia and glucose (60 min intervals), other metabolites and packed cell volume (120 min intervals). Samples were drawn into heparinized

gas-tight syringes for O_2 determination (120 min intervals). Immediately after sampling, the heparinized gas-tight syringes were sealed and kept on ice, and blood O_2 concentration was measured with an oxygen meter (Strathkelvin Instruments, Glasgow, UK) as described by Tucker (1967). Packed cell volume was determined by centrifuging blood in capillary tubes. Blood ammonia and *p*-aminohippuric acid were determined immediately on whole blood by the phenol-hypochlorite and the *N*-α-naphthyl ethylene diamine dichlorhydrate methods, respectively, using a continuous Autoanalyser (Alliance, Méry-sur-Oise, France), as previously described (Nozière *et al.* 2000).

After adding 0·1 volume of norleucine (1·25 mM) as an internal standard, blood was deproteinized with 0·1 volume of sulphosalicylic acid (40 %, w/v), then kept at −15°C for subsequent determination of amino acids and urea by ion-exchange chromatography on the physiological column of a Beckman Autoanalyser (model 6300, Beckman Instruments, Palo Alto, CA, USA). Plasma was kept at −15°C for subsequent enzymatic determination of β-hydroxybutyrate, glucose and lactate, using a multianalyser (Elan, Merck-Clevenot, Nogent-sur-Marne, France) as previously described (Han *et al.* 2002). Whole blood was kept at −80°C for subsequent determination of VFA by GC with 2-ethylbutyric acid as internal standard, after deproteinization with metaphosphoric acid, as described by Nozière *et al.* (2000). Blood flow through PDV was calculated as described by Katz & Bergman (1969). Plasma flow was calculated from blood flow and packed cell volume as described by Nozière *et al.* (2000). Net fluxes of metabolites and O_2 across PDV were calculated as described by Katz & Bergman (1969). Positive net fluxes represented net release in the vein, whereas negative net fluxes represented net uptake by tissues. Totalling of net release of energy was based on heats of combustion of 876 (acetate), 1528 (propionate), 2310 (four-carbon VFA and β-hydroxybutyrate), 2838 (five-carbon VFA), 1368 (lactate) and 2001 (amino acids) kJ ME per mol.

Whole-body glucose appearance rate. On the day portal net fluxes were measured, a physiological sterile saline solution (pH 7·4) containing [6,6-$^2H_2$]glucose (70 g/l) was continuously infused (1·65 g/h) following a priming infusion (1·32 g) at 12.50 hours, via a catheter placed the day before into the jugular vein. Arterial blood (7·5 ml) was sampled in tubes containing EDTA-K 90, 105 and 120 min after the beginning of infusion. Jugular blood withdrawn before injection was used as blank for [6,6-$^2H_2$]-glucose determination. After deproteinization with 1 volume of 1·2M-$HClO_4$, plasma was kept at −80°C before glucose (Kone Instruments Corporation, Espoo, Finland) and [6,6-$^2H_2$]glucose enrichment by GC–MS (GC 8060 chromatograph coupled to a VG Platform II, Fisons Instruments, Altrincham, UK) determination, as described by Lemosquet *et al.* (2004*b*). The whole-body glucose appearance rate (WBGRa, g/h), accounting for gluconeogenesis plus glycogenolysis plus intestinal absorption of glucose, was determined according to the following equation:

$$\text{WBGRa} = F \times (IE_{\text{inf}}/IE_{\text{p}} - 1)$$

where F is [6,6-$^2H_2$]glucose infusion rate (g/h), IE_{p} (mol% excess) is plasma [6,6-$^2H_2$]glucose enrichment and IE_{inf} is isotopic enrichment of the infusate (i.e. 98 mol% excess).

In situ *starch degradability*. *In situ* ruminal degradation was measured for both C and BP maize grains. Grains (3 g DM) were placed in 5 × 10 cm nylon bags (pore size 53 μm, Ankom Co., Fairport, NY, USA), with presentation similar to that fed to steers for duodenal fluxes measurement. Incubations were performed in duplicate during 3, 6, 9, 15, 24 and 48 h in the rumen of fistulated cows fed a whole-plant maize silage-based diet. Starch was analysed in feed and *in situ* residues (Faisant *et al.* 1995). The theoretical degradability of starch was calculated by the step-by-step method (Kristensen *et al.* 1982) with a fixed particle passage rate of 0·04 per h. Since fitting to first-order kinetic model using the non-linear regression procedure failed to converge, the fractional degradation rate was determined by logarithmic transformation followed by linear regression (Mertens, 1993).

Statistical analyses

Due to experimental constraints, treatment and period effects were confounded in this trial. Data were analysed by ANOVA using the MIXED procedure of Statistical Analysis Systems (1996) with treatment as fixed effect and animal as random effect. Significance was declared at $P<0{\cdot}05$, and $P<0{\cdot}10$ was considered a trend.

Results

Digestion

DM, organic matter and starch digestion. DM and OM intake were similar between treatments, averaging 4815 and 4625 g/d, respectively (Table 1). The total tract digestibility of OM decreased from 80 to 74 % of intake between C and BP. Although it did not reach statistical significance, this was mainly related to a decrease in OM digestion in the rumen from 42 to 34 %. The amount of starch intake was comparable for both C and BP, averaging 1757 g/d. The total tract digestibility of starch tended to decrease from 96 to 91 % of starch intake between C and BP. Although it did not reach statistical significance, this was related to a decrease in starch digestion in the rumen (74 to 55 %) that was highly compensated by an increase in starch digestion in the small intestine (14 to 30 %). The amount of starch apparently digested in the small intestine changed from 238 to 531 g/d between C and BP. The amount of glucose and soluble α-glucoside reaching the ileum was similar between treatments, averaging 79 and 100 g/d for C and BP respectively. It corresponded to glucose, maltose, maltotriose, maltotetraose, maltopentaose and maltoheptaose with respective molar percentages of 38, 44, 16, 1·8, 0·3 and 0·3 (Fig. 1).

ADF and nitrogen digestion. The amounts of ADF intake were similar between treatments, averaging 718 g/d (Table 2). The total tract digestibility of ADF changed from 65 to 50 % of intake between C and BP. Although it did not reach statistical significance, this was mainly related to a decrease in ADF digestion in the intestines from 18 to 8 %. The amount of N intake was similar between treatments, averaging 106 g N/d. Duodenal NH_3 and non-NH_3 N flows were similar between treatments, averaging 2·8 and 114·8 g/d, respectively. Microbial contribution to duodenal non-NH_3 N flow, as measured from liquid-associated bacteria composition, did not differ significantly between treatments, averaging 58 and 47 % for C and BP, respectively. Assuming liquid-associated bacteria underestimated microbial N flow to the duodenum by 20 % (Yang & Poncet, 1993), and an endogenous flow of 6 g N/d, the ruminal degradability of dietary N was estimated to be 73 and 58 % for C and BP, respectively. Ileal

Table 1. Intake and digestion of organic matter (OM) and starch in three steers fed diets differing by presentation of maize grain: wet and ground (control) *v.* dry and cracked (by-pass)

(Values are means with their standard errors)

	Treatment			
	Control	By-pass	SE	*P*
DM intake (g/d)	4806	4824	27	0·69
OM				
intake (g/d)	4619	4631	25	0·77
Apparently digested in the rumen				
g/d	1920	1582	123	0·17
% intake	41·6	34·2	2·7	0·16
Apparently digested in the small intestine				
g/d	1076	1443	326	0·48
% intake	23·3	31·1	7·0	0·49
Apparently digested in the large intestine				
g/d	693	396	191	0·39
% intake	15·0	8·6	4·1	0·39
Apparently digested in the total tract				
g/d	3689	3421	81	0·05
% intake	79·9	73·9	1·6	0·02
Starch				
Intake (g/d)	1736	1778	11	0·12
Apparently digested in the rumen				
g/d	1277	969	107	0·18
% intake	73·5	54·5	6·0	0·16
Apparently digested in the small intestine				
g/d	238	531	89	0·15
% intake	13·7	29·9	5·0	0·15
Apparently digested in the large intestine				
g/d	150	114	40	0·60
% intake	8·6	6·5	2·3	0·59
Apparently digested in the total tract				
g/d	1664	1615	21	0·23
% intake	95·8	90·8	0·8	0·05
Ileal glucose and soluble α-glucoside				
g/d	79	100	10	0·27

NH_3 and non-NH_3 N flows and faecal N excretion were similar between treatments, averaging 1·7, 44·6 and 35·4 g N/d, respectively.

Ruminal parameters. Ruminal parameters were not significantly affected by treatments, ranging from 6·28 to 6·54 for pH, from 7·17 to 5·64 mM for ammonia, and from 104·2 to 97·2 mM for total VFA between C and BP, respectively (Table 3). The

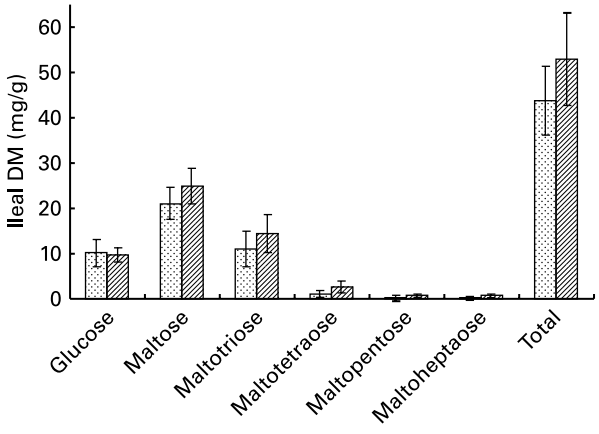

Fig. 1. Concentrations of glucose and soluble α-glucoside in ileal content of three steers fed diets differing by presentation of maize grain: wet and ground (control, ▨) *v.* dry and cracked (by-pass, ▨). Values are means with their standard errors represented by vertical bars.

Table 2. Intake and digestion of acid detergent fibre (ADF) and nitrogen in three steers fed diets differing by presentation of maize grain: wet and ground (control) *v.* dry and cracked (by-pass)

(Values are means with their standard errors)

	Treatment			
	Control	By-pass	SE	*P*
ADF				
Intake (g/d)	735	701	34	0·34
Apparently digested in the rumen				
g/d	340	292	58	0·62
% intake	46·6	41·3	7·9	0·68
Apparently digested in the intestines				
g/d	134	56	36	0·21
% intake	18·4	8·3	5·3	0·20
Apparently digested in the total tract				
g/d	474	348	33	0·11
% intake	65·0	49·6	5·3	0·18
Nitrogen				
Intake (g/d)	106	106	1	0·48
Duodenal N flow (g/d)	115	120	20	0·87
NH_3-N (g/d)	2·3	3·2	0·4	0·26
Non-NH_3-N (g/d)	113	117	20	0·89
Microbial N (g/d)	65·3	54·9	7·9	0·45
Non-microbial N (g/d)	47·2	62·2	16·6	0·55
Ileal N flow (g/d)	47·6	45·0	10·5	0·88
NH_3-N (g/d)	2·1	1·3	0·4	0·31
Non-NH_3-N (g/d)	45·5	43·7	10·1	0·91
Faecal N excretion (g/d)	33·3	37·5	3·5	0·31

ADF, acid detergent fibre

VFA molar percentages were similar between treatments, averaging 63, 20, 1·4, 13, 1·9 and 1·1 for acetate, propionate, isobutyrate, butyrate, isovalerate and valerate, respectively.

In situ *starch degradability*. *In situ* starch degradability and fractional degradation rate decreased from 51·0 to 29·6 % ($P<0·001$) and from 3·7 to 2·2 %/h ($P<0·08$) in the C and the BP maize grain, respectively (Fig. 2).

Net fluxes of nutrients across portal-drained viscera

Arterial concentrations. Arterial concentrations of individual and total VFA, ammonia, urea, essential and total amino acids in blood, and β-hydroxybutyrate and glucose in plasma were similar between treatments (Table 4). The arterial concentration decreased for lactate and O_2, and tended to decrease for non-essential and glycogenic

Table 3. Ruminal pH, volatile fatty acids (VFA) and ammonia concentrations in three steers fed diets differing by presentation of maize grain: wet and ground (control) *v.* dry and cracked (by-pass)

(Values are means with their standard errors)

	Treatment			
	Control	By-pass	SE	*P*
pH	6·28	6·54	0·16	0·37
Ammonia (mM)	7·17	5·64	0·64	0·23
Total VFA (mM)	104·2	97·2	4·3	0·37
Acetate (%)	63·3	62·7	1·6	0·82
Propionate (%)	19·5	19·7	1·7	0·94
Isobutyrate (%)	1·4	1·3	0·1	0·40
Butyrate (%)	12·3	13·1	1·0	0·55
Isovalerate (%)	1·9	1·9	0·3	0·94
Valerate (%)	1·1	1·1	0·1	0·63

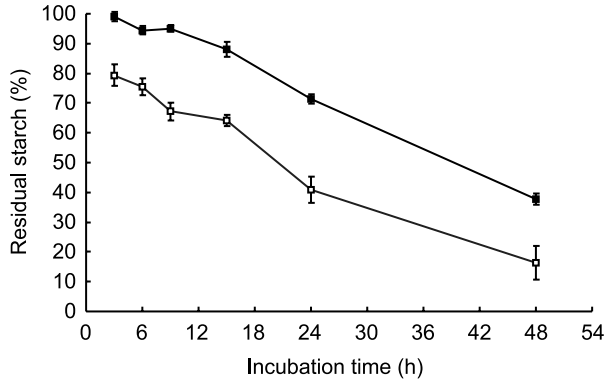

Fig. 2. *In situ* degradation of maize grain differing by presentation, wet and ground (control, □) *v.* dry and cracked (by-pass, ■), in the rumen of three cows fed a whole-plant maize silage-based diet. Values are means with their standard errors represented by vertical bars.

amino acids, from 613 to 419 μM, from 6574 to 5498 μM, from 1351 to 1200 μM and from 952 to 795 μM, respectively, between C and BP. The decrease in both non-essential and glycogenic amino acids appeared mainly related to glutamine (from 269 to 152 μM) although it did not reach statistical significance.

Portal net fluxes. Portal blood and plasma flows tended to decrease between C and BP (Table 5). Portal net appearance of individual and total VFA, β-hydroxybutyrate, lactate, essential, non-essential, glycogenic and total amino acids, portal net release of energy, and portal net uptake of glucose, urea and O_2 were similar between treatments. Portal net appearance of ammonia tended to decrease from 108 to 73 mmol/h between C and BP.

Whole-body glucose appearance rate

The low variation coefficient of plasma glucose concentration (1·1–6·3 %) and ^2H enrichment (0·1–2·1 %) indicated that the plateau for ^2H enrichment in plasma was achieved 90 min after the beginning of infusion (Lemosquet *et al.* 2004*a*,*b*). The WBGRa was measured on the three steers with the BP treatment, and averaged 932 (SE 21) g/d. Since blood ran out slowly from the catheters during the second period for two animals, the WBGRa was measured on only one steer receiving the control treatment, and was 869 g/d. This value was lower than the 951 g/d measured on the same steer with the BP treatment.

Table 4. Arterial concentrations (μM) of nutrients in three steers fed diets differing by presentation of maize grain: wet and ground (control) *v.* dry and cracked (by-pass)

(Values are means with their standard errors)

	Treatment			
	Control	By-pass	SE	*P*
Plasma				
β-Hydroxybutyrate	477	531	75	0·66
Lactate	613	419	30	0·01
Glucose	4239	4376	225	0·61
Blood				
Acetate	838	950	202	0·73
Propionate	28·3	25·6	9·0	0·85
Isobutyrate	2·10	1·63	0·62	0·65
Butyrate	10·2	13·6	4·13	0·61
Isovalerate	1·70	2·30	0·77	0·64
Valerate	0·033	0·100	0·047	0·42
Ammonia	58·0	65·0	36·0	0·61
Urea	1588	1813	248	0·59
Essential amino acids	647	709	55	0·40
Non-essential amino acids	1351	1200	79	0·07
Glycogenic amino acids	952	795	38	0·05
Total amino acids	1998	1908	97	0·25
O_2	6574	5498	452	0·03

Table 5. Portal net fluxes of nutrients in three steers fed diets differing by presentation of maize grain: wet and ground (control) *v.* dry and cracked (by-pass)

(Values are means with their standard errors)

	Treatment			
	Control	By-pass	SE	*P*
Portal blood flow (l/h)	659	550	52	0·10
Portal plasma flow (l/h)	466	407	35	0·11
Portal net fluxes (mmol/h)				
Acetate	695	602	59	0·38
Propionate	187	176	12	0·22
Isobutyrate	8·14	7·38	1·24	0·66
Butyrate	37·5	38·8	9·3	0·92
Isovalerate	9·41	9·35	1·94	0·98
Valerate	2·76	2·88	0·66	0·87
Total volatile fatty acids	940	836	72	0·41
β-Hydroxybutyrate	71·5	81·3	16·6	0·72
Lactate	36·1	26·4	27·6	0·74
Glucose	−22·4	−22·7	29·5	0·98
Ammonia	108·0	73·0	9·1	0·10
Urea	−80·1	−64·2	36·0	0·17
Essential amino acids	34·8	26·0	14·0	0·69
Non-essential amino acids	9·7	16·2	20·5	0·81
Glycogenic amino acids	3·1	13·0	14·5	0·62
Total amino acids	44·4	42·2	33·0	0·96
O_2	−892	−712	89	0·20
Portal net release energy (kJ/h)	1363	1252	114	0·48

Discussion

Due to the necessity of using wet grain quickly after opening the anaerobic bag, dietary treatments and period effects were statistically confounded. Many factors concur to attribute differences between C and BP to dietary treatments: the short duration of the experiment (4 weeks between the first and the second period), the stability in body weight and intake between the two periods and the expected differences observed between C and BP. However, this limitation in the experimental design has been considered in the discussion of results.

Digestibility and ruminal digestion of organic matter, starch and cell walls

Compared with other studies on steers fed maize providing starch at 35–50 % of DM intake (Cole *et al.* 1976; Lee *et al.* 1982; Kung *et al.* 1992; Philippeau *et al.* 1999*b*), the total tract digestibility of OM, starch and ADF was high in our trial: 74–80 *v.* 64–6 % for OM; 91–96 *v.* 76–99 % for starch; 50–65 % *v.* 44 % for ADF. No major effect of feeding frequency on total-tract OM digestion has been reported in steers at restricted intake (review by Galyean & Owens, 1991). This high digestibility could be due to the nature of the feedstuffs, but also to the low intake level in the present study (14 g OM/kg BW) compared to others (13–25 g OM/kg BW). In cattle fed between 20 and 60 % concentrate, Doreau

et al. (2000) reviewed that an increase in intake of 10 g/kg BW induces an average decrease in digestibility of 2·0 g/100 g for OM and 5·2 g/100 g for ADF. The moderate intake level in the present study, corresponding to 90 % *ad libitum*, illustrates the difficulty of reaching a high intake when animals are fitted with both catheters and intestinal cannulas.

The contribution of the rumen to digestion of OM (52–46 %) and starch (77–60 %) was consistent with the values previously reported in steers fed maize providing 35–50 % of DM intake: 40–77 % for OM (Kung *et al.* 1992; Philippeau *et al.* 1999*b*) and 43–96 % for starch (Boss & Bowman, 1996; Philippeau *et al.* 1999*b*). The decrease in OM ruminal digestion between C and BP was related to a decrease in starch digestion (−19 g/100 g), whereas cell wall digestion was affected little (−5 g/100 g). This reflects that no large modifications in cellulolytic microbial activity occurred, which is consistent with the physicochemical ruminal parameters that remained comparable between treatments. Also, the portal net appearance of VFA was not significantly affected. The decrease in ruminal digestion of starch between C and BP may be attributed to a decreased accessibility of starch granules. This is consistent with the decreased degradability of dietary N between C and BP, since ruminal starch degradation is closely related to protein degradation in the grain endosperm (McAllister *et al.* 1993). It may be emphasized that dietary N ruminal degradability with diet BP (58 %) was consistent with the value of 56 % given by the French nitrogen feeding system (INRA, 1989).

As intended, ruminal starch digestion was lower with the wet and ground grain (BP) than with the dry and cracked grain (C). Increasing DM content (Knowlton *et al.* 1998) and/or mean particle size (Galyean *et al.* 1979; Rémond *et al.* 2004) has already been shown to decrease ruminal starch digestion of maize grain. The 55 % ruminal starch digestibility with the BP treatment is consistent with previous results obtained with dry rolled grain of dent maize genotype with a comparable particle size for both steers (61 %; Philippeau *et al.* 1999*b*) and cows (54 %; Rémond *et al.* 2004). With the C grain, 20 % starch disappeared in the first 3 h. With grains exhibiting a rapidly degradable fraction of starch of less than 20 %, starch washed out of the bag at time zero accounted for less than 8 % of incubated starch (Philippeau *et al.* 1999*a*). Particulate losses may thus only partly explain the decrease in starch *in situ* degradability between C and BP grain, that was also related to a decrease in fractional degradation rate, consistently with previous results (Lykos & Varga, 1995; Rémond *et al.* 2004). The extent of the decrease in starch *in situ* degradability between C and BP grain reached 21 g/100 g, which was close to the nineteen-point decrease in *in vivo* ruminal starch digestion. *In situ* and *in vivo* variation of ruminal starch digestion induced by the particle size of maize grain has been reported previously to be quantitatively similar (Rémond *et al.* 2004). *In situ* starch degradability was lower than *in vivo* ruminal starch digestion. This is in line with the fact that *in situ* underestimates ruminal digestion for slowly degradable starch (Nocek & Tamminga, 1991; Offner & Sauvant, 2004). This may be related to a lower accessibility of starch to microbes, thus a lower expression of amylase activity in the bags (Nozière & Michalet-Doreau, 1997), where grains are not submitted to mastication. Nevertheless, the extent of the difference between *in situ* and *in vivo* measurements (24 g/100 g in the present study) is surprisingly high, but remains consistent with results previously obtained with a dent variety (Nozière *et al.* 2003).

The amount of starch escaping ruminal digestion ranged from 460 to 804 g/d between C and BP, and intestinal digestion of BP starch took place almost exclusively in the small intestine. Limits to starch digestion in the small intestine may be related to time and surface exposure of starch to enzymes in this compartment, or to a limited capacity of starch-hydrolysing enzymes (Owens *et al.* 1986). In the present study, the limited level of intake favoured a high exposure time in the intestines. It may also have been expected that starch reaching the duodenum was mainly distributed in the small particles, as observed by Philippeau *et al.* (1999*b*), favouring a high surface exposure of starch to enzymes in the intestines. No plateau in the amount of starch digested in the small intestine was observed when the amount of starch entering the duodenum increased to 1500 g/d in steers (Kreikemeier *et al.* 1991). This suggests that the capacity of starch-hydrolysing enzymes was not a limiting factor in our study, although the increase in the amount of starch reaching the duodenum between C and BP may have decreased pancreatic α-amylase secretion (Swanson *et al.* 2002).

The amount of total glucose and soluble α-glucoside accounted for 36 % (w/w) of starch apparently reaching the ileum (222 and 277 g/d with C and BP, respectively), which is consistent with the value of 28 % reported by Kreikemeier & Harmon (1995) in steers receiving abomasal infusions of maize starch. Also, the contribution of glucose to total soluble α-glucoside reaching the ileum was 18–23 % (w/w) in our study, which is also consistent with the value of 18 % reported by Kreikemeier & Harmon (1995). This indicates that glucose production exceeded glucose disappearance capacity, which may be related to the decreasing transport activity between the proximal and the distal regions of the intestine (Ferraris *et al.* 1989), and to a low contribution of passive diffusion in the glucose absorption process (Krehbiel *et al.* 1996). In our study, the limitation in glucose disappearance had no major quantitative impact, since the luminal concentration of glucose at the ileum remained low (< 6 mM). This is in good agreement with the simulation of glucose concentration at the distal small intestine proposed by Huntington (1997), with a limitation of transport by sodium–glucose transporters and a supply of duodenal glucose close to that measured in the present study (500–1000 g/d).

Portal net flux and whole-body appearance rate of glucose

Portal blood flow averaged 187 litres/d per kg $BW^{0.75}$ for BP and C, which is consistent with the estimation derived from ME intake (194 litres/d per kg $BW^{0.75}$) in cattle (Huntington, 1984). Also, the small decrease in portal blood flow between C and BP may be attributed to the low decrease in OM digestibility.

Since starch determination in digestive contents accounted for both glucose and soluble and insoluble α-glucoside, the amount of glucose that disappeared in the small intestine corresponded to the amount of starch apparently digested in this compartment. Although it increased from 238 to 531 g/d between C and BP, the portal net flux of glucose remained unchanged and negative (−97 g/d). Net uptake of arterial glucose by PDV has been clearly demonstrated in forage-fed ruminants (Huntington, 1984). Portal net appearance has been observed when large amounts of starch (8·4–13·3 g/d per kg $BW^{0.75}$) were infused into the duodenum in cows (Huntington & Reynolds, 1986) and steers (Taniguchi *et al.* 1995; Harmon *et al.* 2000), and when large amounts of infused starch (5–11·3 g/d per kg $BW^{0.75}$) were apparently digested in the small intestine in steers (Kreikemeier *et al.*

1991; Kreikemeier & Harmon, 1995). In contrast, with a lower amount of starch infused to the duodenum (4·9 g/d per kg $BW^{0.75}$), portal net flux of glucose remained negative (net uptake) and unchanged in cows (Huntington & Reynolds, 1986), as observed in the present study where the amount of starch apparently digested in the small intestine was moderate (2·9–6·8 g/d per kg $BW^{0.75}$). Also, in sheep, a duodenal infusion of glucose reaching 7·4 g/d per kg $BW^{0.75}$ was not sufficient to shift portal net flux of glucose from net uptake to net appearance (Piccioli Cappelli *et al.* 1997), and in steers an increase in maize starch intake from 15·3 to 30·5 g starch/d per kg $BW^{0.75}$ decreased portal net uptake of glucose from −138 to −52 g/d, without shifting to net appearance (Huntington *et al.* 1996). The portal net uptake of glucose measured in the present study (−97 g/d with a maize starch intake of 22·7 g/d per kg $BW^{0.75}$) is in good agreement with these results.

Our results suggest that PDV glucose use was increased between C and BP. Previous studies reported that utilization of glucose in portal tissues increased significantly (Balcells *et al.* 1995) or not (Piccioli Cappelli *et al.* 1997) as a result of duodenal glucose infusion in sheep. Also, Harmon *et al.* (2000) reported that shifting the site of starch infusion from rumen to small intestine increased glucose utilization by PDV tissues in steers. Janes *et al.* (1985) found in sheep that use of arterial glucose by mesenteric-drained viscera (MDV) was similar for forage and concentrate diets, and that nearly 100 % of small intestinal starch disappearance was accounted for as net glucose appearance across MDV with the concentrate diet. Reynolds & Huntington (1988) and Huntington *et al.* (1996) reported a net glucose appearance across MDV in concentrate-fed steers whereas net glucose flux across PDV was negative. These results suggest that the increase in PDV glucose use between C and BP may be attributed to non-MDV tissues. In forage-fed animals, the contribution of non-MDV tissues to PDV use of arterial glucose has been shown to reach 19–42 % (Reynolds & Huntington, 1988), or more than 50 % (Seal *et al.* 1992; Seal & Parker, 1994) in steers, and 58–72 % (Han *et al.* 2002) in sheep. When MDV measurements were performed in the cranial mesenteric vein (draining 85 % of the whole small intestine), the contribution of non-MDV reached 84 % in sheep (Rémond *et al.* 2003).

In our trial, portal net flux of glucose remained unchanged, suggesting that WBGRa probably increased little, and only increased when endogeneous glucose production increased and/or glycogenolysis decreased. Similarly, when duodenal glucose infusion in lactating dairy cows was increased from 2·9 to 6·8 g/d per kg $BW^{0.75}$, WBGRa did not change (Lemosquet *et al.* 2004*a*). Unfortunately, we did not measure WBGRa in both treatments, except on one steer, for which the increase in the amount of starch digested in the small intestine of 258 g/d (from 210 to 468 g/d) was accompanied by a limited increase in WBGRa of 82 g/d (from 869 to 951 g/d). However, the mean WBGRa value (11·8 (SE 0·5) g/d per kg $BW^{0.75}$) obtained with our four data (three BP plus one C) is in the range of total glucose splanchnic production measured on steers fed concentrate diets, i.e. 7–16 g/d per kg $BW^{0.75}$ (Reynolds *et al.* 1991; Eisemann *et al.* 1996; Huntington *et al.* 1996).

Nitrogen balance

Compared to total N apparently digested in the digestive tract (72·3 and 68·9g N/d for C and BP, respectively), portal net fluxes of amino acids (17·7 and 19·7g N/d) were consistent with the values (20·4–25·2 g α-amino N/d) reported in steers with 87–115 g N apparently digested in the digestive tract (Taniguchi *et al.* 1995). Portal net appearance of ammonia was lower in our experiment (36·3 and 24·5 g N/d) than in the Taniguchi *et al.* (1995) experiment (66·7–98·9 g N/d), due to a lower ruminal NH_3 concentration (5·6–7·2 mM *v.* 12·8–21 mM). The decrease in portal net appearance of ammonia N between C and BP was consistent with the decreased ruminal degradability of dietary N. Portal net uptake of urea N averaged −48 g N/d, which remains high compared to predictive models from dietary concentrate (Huntington *et al.* 1996) or body weight (Eisemann *et al.* 1996), both predicting −28 g N/d. This discrepancy was due to values for one animal, as portal net uptake of urea N averaged −25 g N/d for the other two animals. Assuming a portal net flux of urea N of −25 g N/d, the totalling of portal amino acid N, ammonia N and urea N net fluxes indicates that 46 g of N apparently digested was not taken into account in this balance between digestive and portal net fluxes of N. Similarly, Taniguchi *et al.* (1995) observed that up to 34 g N/d apparently digested was not recovered in the totalling of α-amino N, ammonia N or urea N net fluxes across PDV in steers. A portion of ammonia reaching peripheral blood through peritoneal fluid without traversing the liver (Chalmers *et al.* 1971) may partly account for the discrepancy in the balance between digestive and portal net fluxes of N. The contribution of amino acid peptides to portal net release of total amino acid has been shown to reach 67 % in young forage-fed steers (130 kg BW; Seal & Parker, 1996). With a similar methodology, this contribution was lower (32 %) in older steers (260 kg BW) fed 50 % concentrate diets and was not affected by dietary protein degradability (Han *et al.* 2001). Assuming a contribution of 35 % in the present experiment, the portal net release of N from peptides could not exceed 10 g N/d. The contribution of salivary, biliary and pancreatic N secreted in the gut, together with portal net release of N from proteins and nucleic bases, is probably not sufficient to explain the discrepancy between apparent digestibility and portal net appearance of N, which could reflect N deposition and/or oxidation in the PDV.

Energy balance

Oxygen consumption by PDV and the totalling of energy released in the portal vein accounted for 16 and 57 % of ME intake, respectively. These results are consistent with other results from steers fed at a comparable energy intake to the present study: 13–23 % of ME intake for PDV O_2 consumption (Huntington *et al.* 1988; Reynolds *et al.* 1991) and 46–71 % of ME intake for portal net energy release (Reynolds & Huntington, 1988; Reynolds *et al.* 1992). Totalling of PDV O_2 net uptake and energy release as VFA, β-hydroxybutyrate, lactate and amino acid reached 40·3 MJ/d, i.e. 74 % of ME intake. There are few reports of simultaneous measurements of PDV O_2 uptake and energy release. In line with our results, total recovery has been reported to vary from 65 to 93 % of ME intake in cattle (Reynolds & Huntington, 1988; Eisemann *et al.* 1996). Sources of energy not taken into account in these recoveries include heat of fermentation, lipids, α-keto acids, nucleic acids and peptides. Total VFA, β-hydroxybutyrate, lactate and amino acid released in the portal vein accounted for 43, 8, 2 and 4 % of ME intake, respectively. This was in line with previous studies on steers (Huntington

et al. 1988; Reynolds & Huntington, 1988; Eisemann *et al.* 1996) for VFA (35–52 %), β-hydroxybutyrate (7–9 %) and lactate (2–5 %), but our results were lower for amino acids (5–10 %). This may be related to N deposition in the PDV, as discussed earlier.

Since ruminal concentrations do not reflect ruminal production or absorption rates, the comparison between ruminal concentrations and portal net appearance is tenuous, but Huntington (1999) suggested the possibility of using ruminal concentrations to indicate VFA proportions available for liver metabolism. In the present study, the molar contribution of acetate, propionate and butyrate to total VFA averaged 73, 20 and 4 %, respectively, for portal net appearance, whereas it averaged 63, 20 and 13 %, respectively, for ruminal concentrations. The ratio between portal net appearance and ruminal concentrations was thus the highest for acetate and the lowest for butyrate, which is consistent with many studies (see review by Huntington, 1999) and in line with a decrease in portal recovery with increasing VFA chain length (see review by Rémond *et al.* 1995). Assuming a portal recovery of 72 % for arterial acetate, and 71, 69 and 25 % for acetate, propionate and butyrate produced in the lumen (Nozière & Hoch, 2005), the production of VFA (acetate + propionate + butyrate) with C and BP may account for 76 % of ME intake, i.e 10·5 mol/kg OM digested, which is consistent with other estimations in steers fed hay/concentrate diets (Sharp *et al.* 1982; Siciliano-Jones & Murphy, 1989). Assuming a portal recovery of 87 % of arterial β-hydroxybutyrate (Kristensen *et al.* 2000), the production of β-hydroxybutyrate by PDV tissues may account for 95 % of butyrate not recovered in the portal vein. Although β-hydroxybutyrate released in the portal vein can also derive from acetate, this suggests that butyrate was not extensively oxidized to CO_2 by PDV, as has been suggested by Kristensen *et al.* (2000) in sheep. This also suggests that limitation of ketogenesis was not achieved in this trial. This is in good agreement with the observations of Krehbiel *et al.* (1992) in steers receiving a ruminal 150 mmol/h infusion of butyrate, comparable to the production estimated in the present study.

Conclusion

To our knowledge, this work constitutes the first attempt to compare both digestive and portal fluxes of nutrients in conventionally fed cattle. The main objective was to assess the role of BP starch on portal absorption of nutrients. With a moderate intake level, no significant changes in the amount and nature of energy reaching the liver via the portal vein were observed. The increase in the amount of glucose disappearing in the small intestine induced no change in portal net flux of glucose, reflecting an increased glucose utilization by PDV. This could contribute to the low response of whole-body glucose appearance rate at this moderate level of intestinal glucose supply observed in the literature data.

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