PRELIMINARY STUDIES ON THE MODULATION OF RAPESEED MEAL TOXICITY IN CONVENTIONAL RATS BY POORLY DIGESTIBLE CARBOHYDRATES

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INTRODUCTION

Greer and Deeney (1959) were the first to hint that glucosinolate hydrolysis could be the consequence of intestinal microflora metabolism. Experiments using germ-free and conventional rats and chickens fed rapeseed meal based-diets definitively gave evidence of the responsibility of the intestinal microflora in the release of toxic glucosinolate derivatives in vivo. Furthermore each whole microflora had specific effects as determined using gnotobiotic rats harboring a whole chicken flora and conversely (Nugon-Baudon et al. 1988).

The aim of the present study was to investigate whether the "myrosinase-like" activity of intestinal bacteria could be countered by changes in the diet components.

Indeed, it is well established that metabolic activity of the intestinal microflora is influenced by dietary factors (Rowland 1988 ; Savage 1977). In particular, carbohydrates that are resistant to intestinal enzymes and thus are poorly absorbed in the upper regions of the gut gain access to the large intestine where they interact with the bacterial populations (Rowland 1988 ; Mallett et al. 1988). These poorly digestible materials may bring about changes in the concentration or relative species diversity of cecal bacteria (Wyatt et al. 1988 ; Andrieux et al. 1989). Alternatively, the cecal pH changes induced by the microbial fermentation of these polysaccharides may directly influence some bacterial enzymic activities (ß-glucosidase, ß-glucuronidase, nitroreductase,...) (Rowland 1988 ; Mallett et al. 1988).

MATERIALS AND METHODS

Diets

Six pelleted semi-synthetic diets were used (Table 1). All of them contained 39% dehulled DARMOR 00 rapeseed meal (37.3 µmol glucosinolates/g dry matter) kindly supplied by the Centre Technique Interprofessionnel des Oléagineux Métropolitains (CETIOM) as sole source of protein.

The control diet (diet 1) contained 53% maize starch as carbohydrate fraction. In the experimental diets various amounts of poorly digestible carbohydrates were substituted for maize starch : amylomaize starch (Eurylon, Roquette frères, Lestrem, France) provided 50% and 75% of the carbohydrate content in diets 2 and 3 respectively ; cooked potato starch (Vico, Montigny Lenvrain, Vic-sur-Aisne, France) and raw potato starch (Doitteau, Corbeil, France) provided 50% of the carbohydrate fraction in diets 4 and 5 respectively ;

in diet 6, 10% of the maize starch was replaced by inulin (ARD, Paris, France).

The glucosinolate content of the rapeseed meal was analysed following a gas-liquid chromatography method (CETIOM AFNOR 1982) (Table 2).

Animals

Twenty-four Fischer 344 male rats, weighing about 85 g at the beginning of trials, were randomly separated in 6 groups of 4 animals each. Each group received one of the diets. Room temperature was 21°C and lights were on a 12h:12h light-dark cycle. Food and tap water were given ad libitum for 6 weeks. Feed intake of each group and individual body-weight gain were recorded once a week.

Sample Collection

Rats were sacrificed with a lethal dose of chloroform and weighed. The abdomen was opened and the cecum was quickly ligatured and removed. Glucosinolate target organs (liver, kidneys, thyroid glands) were removed afterwards and weighed after removal of surrounding fat.

The pH of the cecal contents was measured using a micro pH-electrode (Ingold, Paris, France). Samples of cecal content were deep frozen in liquid nitrogen with saturated mercuric chloride solution (10%, v/v), and stored at -20°C until short chain fatty acids (SCFA) analysis.

SCFA Analysis

SCFA were determined using a gas-liquid chromatography method (Ottenstein et al. 1971). Samples were centrifuged and supernatants were deproteinized with phosphotungstic acid (0.4 ml of saturated solution per g of content) for 16 hours at 0°C, then centrifuged prior to analysis.

Statistical Analysis

Each experimental group was compared to the control one using a multiple comparison *t* procedure (Dunnet 1964).

RESULTS

Weight gain was improved in the group fed on diet 2 (+17%) and decreased in the groups fed either on diet 4 (-15%) or on diet 6 (-21%) (Table 3). These effects were not correlated with major feed intake changes : 1.8 kg, 1.9 kg, 1.9 kg and 1.7 kg.

Liver weights were similar whatever the diet. On the contrary, kidneys hypertrophy was significantly reduced in the group fed on diet 2 (-10%) and goiter was diminished in both groups fed on diets 2 and 3 (-23% and -15%) although these improvements were not strong enough to be significant (Table 3).

The pH of the cecal contents was decreased by diets 2 and 3 (-0.59 and -0.42 pH unit) but this modification was significant with diet 2 only (Table 3).

On the whole, some adverse effects of rapeseed meal were significantly reduced in the group fed on diet 2. These

improvements occurred together with a lowering in the cecal pH.

SCFA analysis, performed for both groups fed on diets 1 and 2, showed that total concentrations of SCFA were similar whatever the diet : 56.4 ± 2.2 versus 58.1 ± 7.6 µmol/g of cecal content (mean ± SEM) for diets 1 and 2 respectively.

Nevertheless a major re-organization of the SCFA profile occurred in the cecal content from rats fed on diet 2 (Fig. 1). The relative amount of acetate was not modified. On the contrary, propionic acid concentration was increased whereas butyric acid concentration was strongly decreased. Minor branched-chain SCFA concentrations were also reduced even below detection limits, i.e. 0.2 µmol/g of cecal content (isovalerate).

DISCUSSION

A reduction of rapeseed meal adverse effects was seen when half the maize starch was replaced by amylomaize starch. These favorable effects disappeared when a greater concentration of amylomaize starch was used in the diet. These observations support the idea that the influence of poorly digestible carbohydrates on bacterial enzymic activities depends not only on the origin of the polysaccharide (Mallett et al. 1988 ; Wyatt and Horn 1988) but also on its concentration.

The lowered branched-chain SCFA concentrations and the enhanced propionate concentration observed with the amylomaize starch-supplemented diet are in agreement with results of other workers (Mallett et al. 1988 ; Andrieux et al. 1989). However, contrary to our results, these authors observed an increased butyrate level using amylomaize starch. Since their protein source was different (casein), one may suggest that the use of a meal, i.e. of a complex material containing proteins and carbohydrates, may influence the microbial fermentation and thus the profile of SCFA in the cecal contents.

Our results support the hypothesis that changes in the carbohydrate fraction of the diet may modify the availability of glucosinolate derivatives *in vivo*. This effect may result either from a decrease in the population of bacteria with a "myrosinase-like" activity or from a direct pH-related inhibition of "myrosinase-like" activity.

Further investigations will be made to confirm these results and to determine the concentration of amylomaize starch in the diet to obtain the optimal improvements.

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Table 1. Composition of diets (g/kg dry matter)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Rapeseed meal (DARMOR 00)	390.00	390.00	390.00	390.00	390.00	390.00
Maize starch	531.40	265.70	132.85	265.70	265.70	478.26
Amylomaize starch	-	265.70	398.55	-	-	-
Potato starch						
- cooked	-	-	-	265.70	-	-
- raw	-	-	-	-	265.70	-
Inulin	-	-	-	-	-	53.14
Lysine-HCl	0.60	0.60	0.60	0.60	0.60	0.60
Corn oil	20.00	20.00	20.00	20.00	20.00	20.00
Vitamin mixture	18.00	18.00	18.00	18.00	18.00	18.00
Mineral mixture	40.00	40.00	40.00	40.00	40.00	40.00

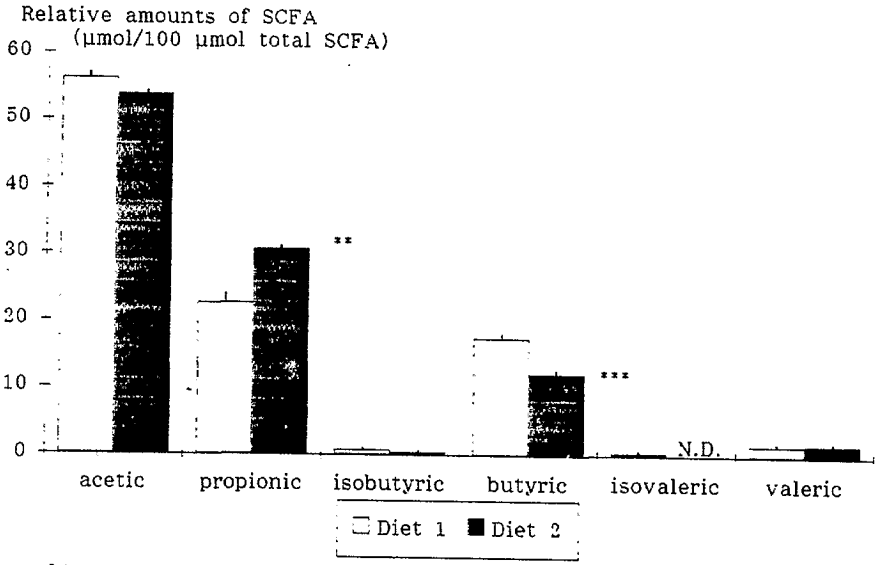
Table 2. Glucosinolate (GLS) content of Darmor 00 rapeseed meal (µmol/g dry matter)

progoitrin	25.0
gluconapoleiferin	1.5
gluconapin	7.1
glucobrassicanapin	2.1
sinalbin	-
gluconasturtin	-
4-OH-glucobrassicin	1.3
glucobrassicin	0.3
neoglucobrassicin	-
4-OCH3-glucobrassicin	-
Total alkenyls	35.7
Total indols	1.6
Total GLS	37.3

Table 3. Influence of the carbohydrate fraction of rapeseed meal-based diets on weight gain, glucosinolate target organs' weight and cecal pH in conventional rats

Groups	Weight gain (g)	Liver (g/100 g body wt)	Kidneys (g/100 g body wt)	Thyroid (mg/100 g body wt)	Cecal pH
Diet 1	109±5	5.00±0.02	0.88±0.02	16.2±1.3	6.45±0.13
Diet 2	127±1**	5.03±0.11	0.79±0.01*	12.4±0.1	5.86±0.08**
Diet 3	118±3	5.20±0.05	0.83±0.01	13.8±1.0	6.03±0.04
Diet 4	93±3*	4.73±0.08	0.94±0.01	15.6±1.9	6.30±0.10
Diet 5	101±4	4.70±0.11	0.87±0.02	16.6±1.5	6.19±0.09
Diet 6	86±2**	5.05±0.11	0.92±0.03	17.0±1.8	6.75±0.10

Results are expressed as mean ± SEM of the values (n=4). Mean values are significantly different from those for the control group (diet 1) : * P<0.05, ** P<0.01



Results are expressed as mean ± SEM of the values (n=4). Mean values are significantly different : ** P<0.01, *** P<0.001

Fig. 1. Influence of the carbohydrate fraction of rapeseed meal-based diets on relative amounts of SCFA in cecal content from conventional rats