association of lactose manufacturers

7, rue des Deux Eglises
B-1040 Bruxelles
Belgique
Tél. 230.10.10 - Fax 230.24.40

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THE TOLERANCE OF LACTOBIONIC ACID IN MAN

Summary of the Study

Lactobionic acid (4-0-β-D-galactopyranosyl-D-gluconic acid) is a white crystalline compound, which can be produced by oxidation of lactose. In food it combines a pH-decreasing effect with a sweet taste. These properties make lactobionic acid an interesting compound for application as a "novel food ingredient".

Before any application can be considered, knowledge about the tolerance in man is required.

This was evaluated in 18 healthy male subjects (average age 23 years). They were given 12g and 24g of lactobionic acid per day and a placebo in a double-bind randomized design, each dose during one week and in addition to their habitual diet. Each day the subjects scored their abdominal complaints, if any, and their defecation pattern. Fermentation of lactobionic acid in the colon of the subjects was assessed by measuring the amount of hydrogen in expired air. Interference of lactobionic acid with lactose digestion was studied in a separate experiment in 8 of the 18 subjects through a standard lactose tolerance test.

For most subjective indicators of abdominal complaints, the number of events reported was either low or did not show a clear relationship with lactobionic acid consumption. The significant increase in the number of events reported for flatulence on an increased intake of lactobionic acid ($P<0.01$) indicates that lactobionic acid is fermented in the colon. This is confirmed by the results of the measurements of hydrogen in expired air, for which the concentration also increased on a higher intake of lactobionic acid ($P<0.01$).

The lactose tolerance test results showed significant lower levels of glucose concentrations in blood when lactobionic acid is given with lactose as compared with lactose alone.

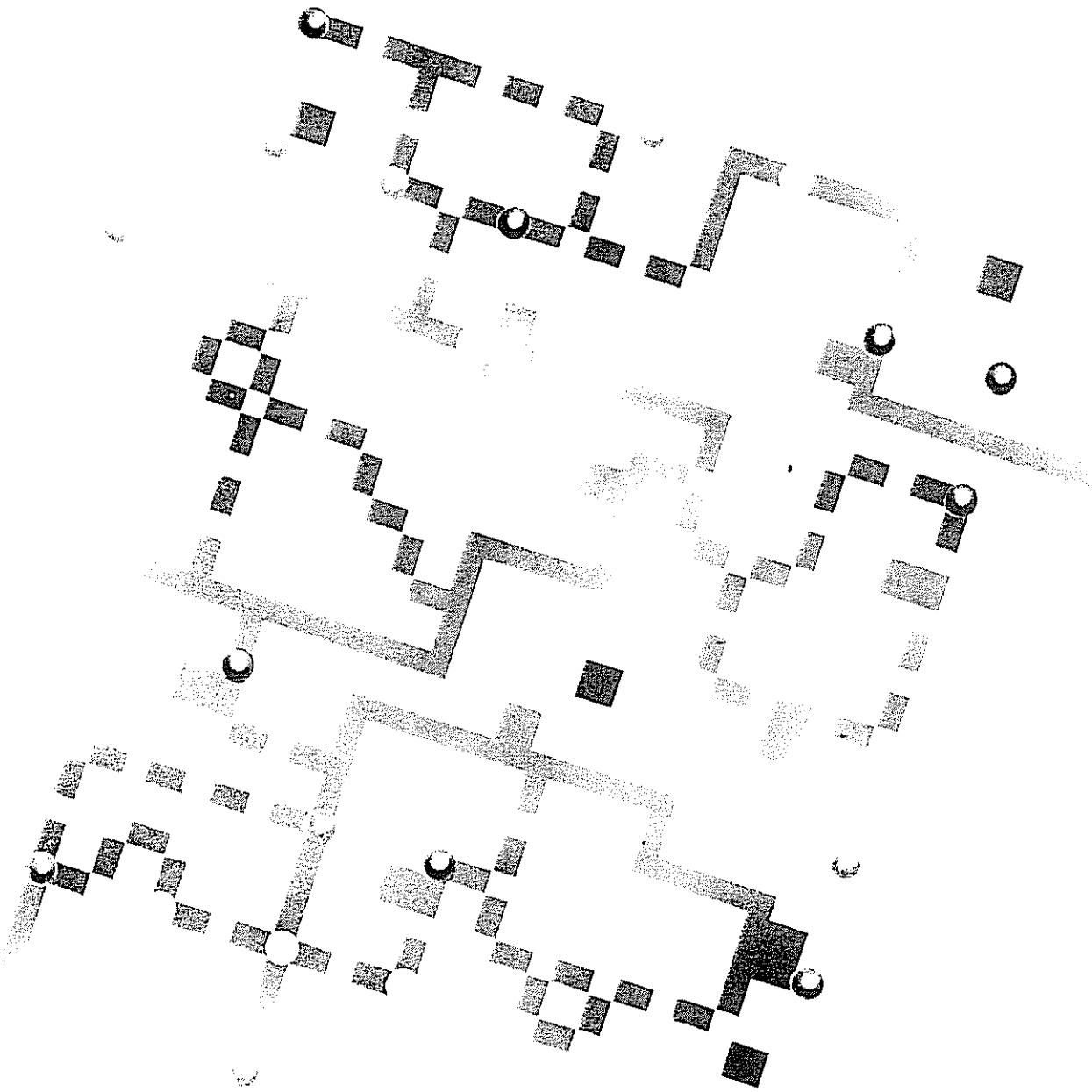
Findings

Tolerance for lactobionic acid in man was evaluated in 18 healthy male subjects, who were given 12 and 24 g of lactobionic acid and a placebo, each treatment during one week. Only the number of events reported for flatulence increased on a higher intake of lactobionic acid. Fermentation of the acid in the colon was confirmed by an increased production of hydrogen in expired air. Lactobionic acid showed a distinct influence on lactose digestion

Key Words

Lactobionic acid
Novel Foods
Hydrogenic production
Flatulence
Tolerance
Human studies
Fermentation
Lactose Digestion

TNO Nutrition and Food Research



TNO Nutrition and Food Research

P.O. Box 360
3700 AJ Zeist
Utrechtseweg 48
3704 HE Zeist, The Netherlands

Fax + 31 34 04 57 22 4
Phone + 31 34 04 44 14 4

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Tolerance of lactobionic acid in man

Authors:
Dr. W. van Dokkum
L.J.W. Wezendonk
P. van Aken-Schneijder
Ing. C. Kistemaker

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SUMMARY

Tolerance for lactobionic acid in man was evaluated in 18 healthy male subjects (mean age 23 years). They were given 12 g and 24 g of lactobionic acid per day and a placebo in a double-blind randomized design, each dosis during one week and in addition to their habitual diet. Each day the subjects scored their abdominal complaints, if any, and their defecation pattern. Fermentation of lactobionic acid in the cecum/colon of the subjects was assessed by measuring the amount of hydrogen in expired air. Interference of lactobionic acid with lactose digestion was studied in a separate experiment in 8 of the 18 subjects through a standard lactose tolerance test.

For most subjective indicators of abdominal complaints the number of events reported was either low or did not show a clear relationship with lactobionic acid consumption. The significant increase of the number of events reported for flatulence on an increased intake of lactobionic acid ($P < 0.01$) indicates that lactobionic acid is fermented in the cecum/colon.This is confirmed by the results of the measurements of hydrogen in expired air, for which the concentration also increases on an increasing lactobionic acid intake ($P < 0.01$). The lactose tolerance test results show significant lower levels of glucose concentrations in blood when lactobionic acid is given with lactose as compared with lactose alone ($P < 0.01$).

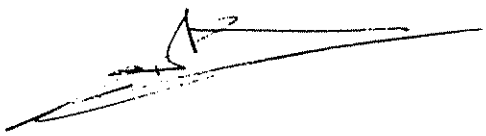
It is concluded that:

- Consumption of lactobionic acid shows clear signs of fermentation in the cecum/colon of the subjects.
- Lactobionic acid has a distinct influence on lactose digestion.
- Amounts up to 24 g of lactobionic acid are rather well tolerated by the group of 18 subjects.

STATEMENT OF AUTHENTICITY

We, the undersigned, hereby declare that the report following constitutes a true and faithful account of the procedures adopted and the results obtained in the tolerance study.

First author:



Dr. W. van Dokkum Date: 17 - 3 - 1994

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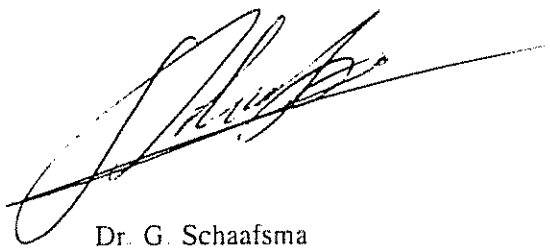


Dr. G. Schaafsma Date: 17 - 3 - 1994

1 INTRODUCTION

Lactobionic acid (4-0-β-D-galactopyranosyl-D-gluconic acid) is a white crystaline compound, which can be produced by oxidation of lactose (1). In food it combines a pH-decreasing effect with a sweet taste. These properties make lactobionic acid an interesting compound for application as a "novel food ingredient".

From a nutritional point of view, lactobionic acid can be considered as a resistant carbohydrate. This implies that lactobionic acid is not hydrolysed by the intestinal enzymes (2). Lactobionic acid may, however, be fermented by the intestinal bacterial flora in the cecum and colon. In this way, suitable quantities of lactobionic acid may have beneficial effects on bowel function. Excessive intake of lactobionic acid may, however, cause symptoms of intolerance, which are comparable to those of lactose intolerance. Lactobionic acid can, in addition, interfere with lactose digestion as lactobionic acid competes with lactose in binding to mucosal β-galactosidase (2,3). Apart from the potential application of lactobionic acid as a novel food ingredient for improving bowel function, lactobionic acid may form soluble complexes with minerals and trace elements. These complexes may potentially increase the availability of minerals and trace elements for absorption.

Before any application of lactobionic acid as a food ingredient can be considered, knowledge about the tolerance in man is required. The amount of orally administered lactobionic acid which can be tolerated by man without adverse events must be assessed. Results of such a study will be required before any other investigation on the envisaged beneficial effects of lactobionic acid can be carried out

The aim of the study therefore is to investigate the tolerance of lactobionic acid after oral administration.

2 MATERIALS AND METHODS

2.1 Subjects

Eighteen healthy male subjects (mean age 23 years) were selected for the study. Normal health was assessed at pre study screening. This included a medical history, physical examination, vital signs and routine clinical laboratory tests (haemoglobin, haematocrit, leucocytes, AST, ALT, alkaline phosphatase, γ-GT, creatinine, total bilirubin, urea, glucose, sodium and potassium in blood or serum). Food intake, including milk and dairy products, was assessed as part of a general questionnaire. Only subjects who were used to an average Dutch diet according to the National Food Consumption Survey of 1987-1988 were selected. Some relevant characteristics of the subjects at pre study screening are given in Table 1

The study protocol was approved by the TNO external Medical Ethics Committee and all subjects signed informed consent forms.

2.2 Location

The study was performed by the TNO Centre for Controlled Clinical Trials in Zeist and was monitored by the Department of Human Nutrition. All analyses were performed at TNO in Zeist.

2.3 Experimental design

Each subject received two doses of lactobionic acid and a placebo during one week each in a double-blind randomized design (see Appendix 1). The test substance and placebo were equally divided over the three meals. The subjects continued their normal daily routines and consumed their habitual diet during the study period. They were, however, instructed to avoid the consumption of those foods which are known to influence their habitual bowel function considerably such as sugar alcohols (xylitol, sorbitol, lactitol, present in e.g. chewing gum, licorice and products for diabetics), bran, lactulose and excessive lactose (through dairy products).

The amounts of lactobionic acid to be administered were 12 g and 24 g per day. These amounts are not considerably exceeding those that are to be expected in a possible application of lactobionic acid in foods.

During each period of one week the tolerance for lactobionic acid was evaluated by subjective assessment of abdominal complaints and defecation pattern. Fermentation of lactobionic acid in the

cecum and ascending colon was assessed by quantitative analysis of hydrogen in expired air.
Interference with lactose digestion was studied in a separate experiment with 8 of the 18 subjects through a standard lactose tolerance test, with or without added lactobionic acid, according to a double-blind cross-over design.

2.4 Methodology and procedures

Lactobionic acid was administered, dissolved in 150 ml orange juice (for breakfast and lunch) and 200 g low-fat yogurt (for dinner). The amounts per serving were 4 and 8 g, respectively, corresponding to two treatments of 12 and 24 g per day, respectively. Orange juice and yogurt, both without lactobionic acid served as placebo.
Tolerance for lactobionic acid was evaluated by subjective assessment of abdominal complaints. To this end, a relevant questionnaire was completed each day. This questionnaire referred to complaints such as vomiting, nausea, belching and flatulency, which were recorded during a period of two hours after each meal. At the end of the day the subjects recorded their defecation pattern on the same questionnaire (see Appendix 2, the actual text was in Dutch). These questionnaires were checked daily by the staff. The subjects were not housed in the metabolic ward of the institute, but they came there daily (between 17.00 and 19.00 hours) to complete the questionnaire, to evaluate their complaints and to obtain the lactobionic acid-enriched orange juice or yogurt or the placebo (non-enriched orange juice/yogurt). Moreover, they consumed a hot meal consisting of a soup, a main course and the experimental yogurt in the dining room of the metabolic ward. Each week the same sequence of the various soups and main courses was applicable.
Fermentation of lactobionic acid by the bacterial flora in the cecum of the subjects was assessed at the end of each experimental week by measurement of the amount of hydrogen in expired air with the Lactoscreen Hydrogen Breath Tester (Hoek Loos, Amsterdam). Breath samples of 20 ml were collected in duplicate in plastic syringes, one hour after the start of the consumption of the hot meal.

The lactose tolerance test was performed in a separate experiment, one week after the first experimental period of three weeks, in 8 of the 18 subjects. The test was executed after an overnight fast on two mornings separated by a wash-out period of two days. The subjects received 40 g of lactose with or without 10 g of lactobionic acid in a double-blind cross-over design. To this end, 0.5 g of citric acid was added to the pure lactose solution to mask the taste of lactobionic acid.
Blood samples were collected by finger puncture at 0, 15, 30, 60 and 90 minutes for glucose determination and expired air was analysed for hydrogen in duplicate at 0, 60, 120, 180 and 240 minutes. The zero-point was directly before the consumption of the test drink.

2.5 Statistical evaluation

Results regarding abdominal complaints and those of the hydrogen measurements and lactose tolerance test were analysed statistically by analysis of variance.
For abdominal complaints the various sources of variance (subjects, weeks and meals) were taken into account. For the lactose tolerance test the repeated measurements model was applied.

3 RESULTS

Seventeen of the 18 subjects completed the first part of the study. One subject was excluded from the study at day 14 because of (possible allergic) eczema in the face and wrist. From the results of the treatment with antibiotics (he recovered within a short period of time) and microbiological testing it was concluded that no apparent relationship existed with the consumption of lactobionic acid. The remaining 17 subjects could complete the experiment without serious complaints or adverse events.
Relevant results of the reported abdominal complaints and those of the defecation pattern are summarized in Table 2 and in Figures 1 and 2.
Nausea was scored 19 times in a total of 1134 periods after a meal (=2%), abdominal distention 86 times (8%) and abdominal cramps 32 times (3%). No adverse event of vomiting was reported. No relation could be detected between the above mentioned abdominal complaints and the consumption of lactobionic acid and probably this reflects normal dietary habits and normal gastrointestinal function. Also the defecation pattern was not statistically significant different between treatments. The scores for borborygmia and flatulence were reasonable normally distributed after transformation. No statistically significant difference between treatments could be detected for borborygmia and belching, although a tendency for more complaints on an increasing lactobionic acid intake is visible (see Table 2 and Figure 1). For flatulence a significant increase was observed on an increasing amount of lactobionic acid intake ($P< 0.01$), both on a daily basis of intake and for all meals separately. For both borborygmia and flatulence the reported events indicate that lactobionic acid is fermented in the cecum and colon of the subjects. This is confirmed by the results of the measurements of hydrogen in expired air which are summarized in Table 3 and Figure 3: a statistically significant increase of the production of hydrogen on an increasing lactobionic acid intake ($P< 0.01$).

Figure 4 shows the results of the lactose tolerance test with respect to the concentration of glucose in blood of the subjects. Figure 5 presents the results of the hydrogen concentration in expired air. Interference of lactobionic acid with lactose digestion is indicated by a significant lower level of blood glucose as compared with the consumption of lactose without lactobionic acid ($P< 0.01$). The higher

levels of hydrogen in expired air after consumption of lactose plus added lactobionic acid reflect fermentation of the acid and the non digested lactose in the cecum/colon of the subjects. This confirms the findings of the measurements of the hydrogen in expired air at the end of each experimental week, preceeding the lactose tolerance test.

4 DISCUSSION AND CONCLUSIONS

The aim of the study was to investigate whether intake of lactobionic acid in amounts to be envisaged when this acid will be applied in food products, would produce serious adverse events in man. The fact that all subjects could complete the study (the excluded subject after 2 weeks did not show signs of lactobionic acid intolerance) gives already an indication that up to 24 g of lactobionic acid, divided over the day and part of the habitual diet, is well tolerated. Although several elements of the subjective scoring of abdominal complaints were obvious, the differences between treatments were only significant for flatulence. This, together with the observed increased production of hydrogen on an increased lactobionic acid intake, makes it clear that lactobionic acid is being fermented in the cecum and colon. The results of the lactose tolerance test confirm this conclusion. Fermentation, however, is not being considered as an unwanted change. Moreover, bowel function and defecation pattern were not affected. Since lactobionic acid is fermented in the distal intestine, it could be considered as having dietary fibre properties. More detailed studies on the metabolic activities and composition of the intestinal flora seem indicated.

The significant influence of lactobionic acid on lactose digestion could also be demonstrated. No adverse events were observed during the lactose tolerance test, which means that the slightly impaired lactose digestion does not seem to be a serious problem.

From the results of this study it is concluded that:

- The consumption of lactobionic acid produces clear signs of fermentation in the cecum and colon of man.
- Lactobionic acid has a distinct influence on lactose digestion
- Amounts up to 24 g of lactobionic acid per day are rather well tolerated by the group of 18 subjects.

It is recommended that fermentation of lactobionic acid will be studied in more details in order to investigate possible positive effects of lactobionic acid on human gastrointestinal function.

TNO NUTRITION AND FOOD RESEARCH

Dr. W. van Dokkum

Dr. G. Schaafsma

DOW-POA

5 REFERENCES

1. Veringa, H.A.
Fermentive production and application of low-molecular compounds from lactose (whey).
Proceedings Symposium "Milk compounds in food and non food", February 27, 1991, Wageningen. NRLO report 91/4, The Hague, 1991.

2. Harju, M.
Lactobionic acid as a substrate of β-galactosidases.
Milchwissenschaft 45 (1990), 411-415.

3. Nishizuka, Y. and Hayaishi, O.
Enzymic formation of lactobionic acid from lactose.
J. Biol. Chem. 237 (1962), 2721-2729.

Table 1. Characteristics of the test subjects (means ± SD, N= 18)	
Age (years)	23.2 ± 2.3
Weight (kg)	77.4 ± 8.1
Height (cm)	184.5 ± 6.9
BMI (weight/height^2)	22.7 ± 1.9
Blood haemoglobin (mmol/l)	9.8 ± 0.5
Blood glucose (mmol/l)	4.8 ± 0.7

Table 2. Abdominal complaints and defecation pattern. Number of events reported per week (means ± SD)

		Placebo (N=18)			12 g lactobionic acid (N=18)			24 g lactobionic acid (N=17)		
		Br*	Lu*	Di*	Br	Lu	Di	Br	Lu	Di
Belching	mean	6.7	6.9	10.1	6.3	7.4	12.7	5.2	8.4	11.4
	SD	8.3	8.5	10.0	6.3	8.4	12.9	5.4	8.4	10.4
Borborygmia	mean	1.3	1.2	2.3	2.8	3.7	3.2	1.8	2.1	3.4
	SD	2.2	2.3	3.9	4.6	6.2	5.2	3.3	2.7	5.4
Flatulence**	mean	4.1	3.8	8.7	7.7	9.7	15.1	8.5	11.9	17.9
	SD	4.5	4.6	7.4	8.1	11.3	11.8	9.6	17.2	17.4
Number of defecations	mean	10.0			10.0			9.7		
	SD	4.4			4.8			3.9		
Consistency stools		normal/softer			normal/softer			normal/softer		
Amount stools		normal			normal			normal		

* Br, Lu, Di = after breakfast, lunch and dinner, respectively

** Significant increases of the number of events reported on an increasing lactobionic acid intake ($P < 0.01$)

Table 3. Hydrogen concentration in expired air at the end of each period indicated (in ppm, means ± SD)

	Placebo (N=18)	12 g of lactobionic acid (N=18)	24 g of lactobionic acid (N=17)
1st measurement	21 ± 12	29 ± 25	53 ± 34
2nd measurement	21 ± 10	27 ± 15	38 ± 30
Mean values *	21 ± 10	28 ± 19	46 ± 30

* Significant increases on an increased lactobionic acid intake ($P < 0.01$)

Figure 1. NUMBER OF EVENTS FOR BELCHING

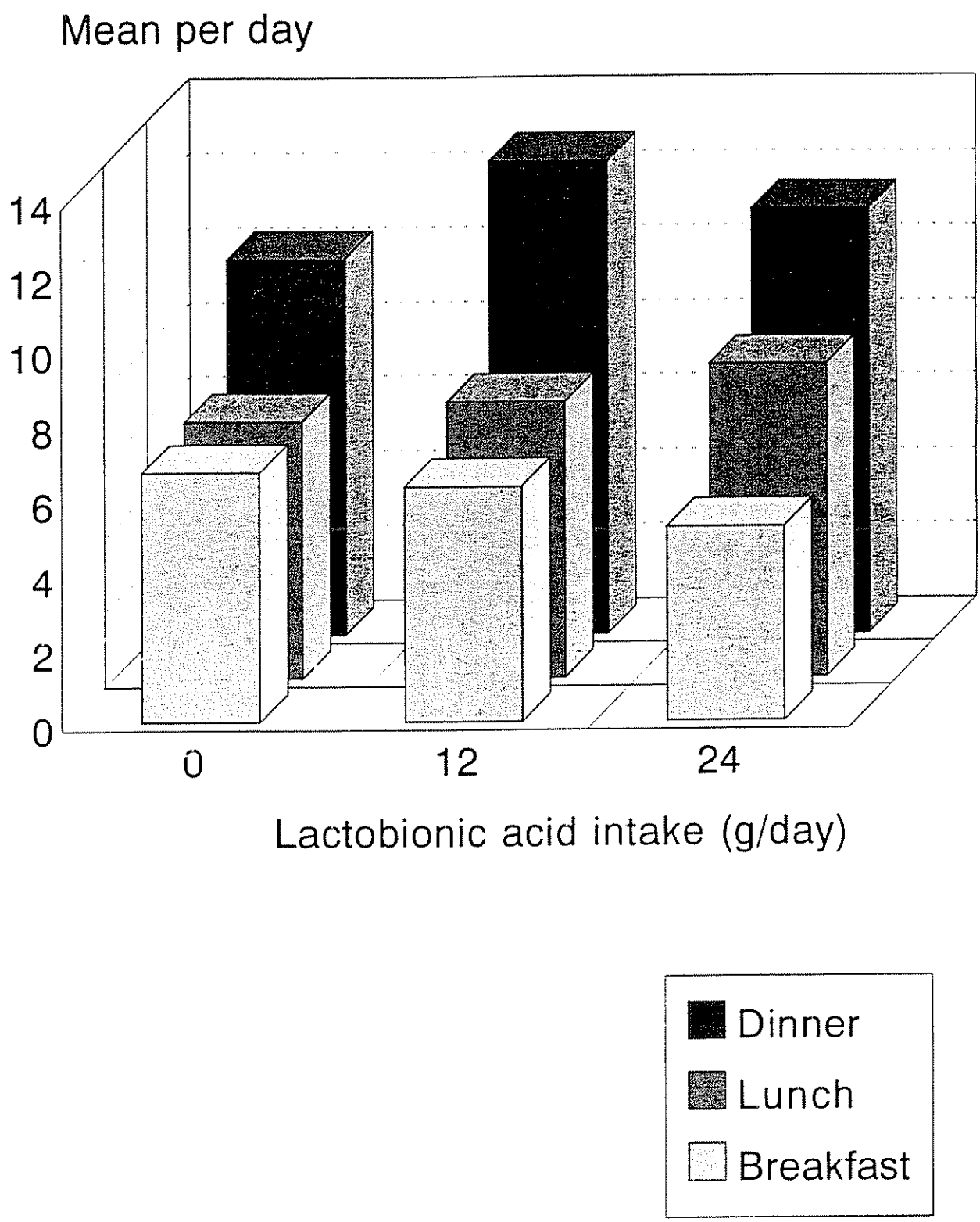


Figure 2 NUMBER OF EVENTS FOR FLATULENCE

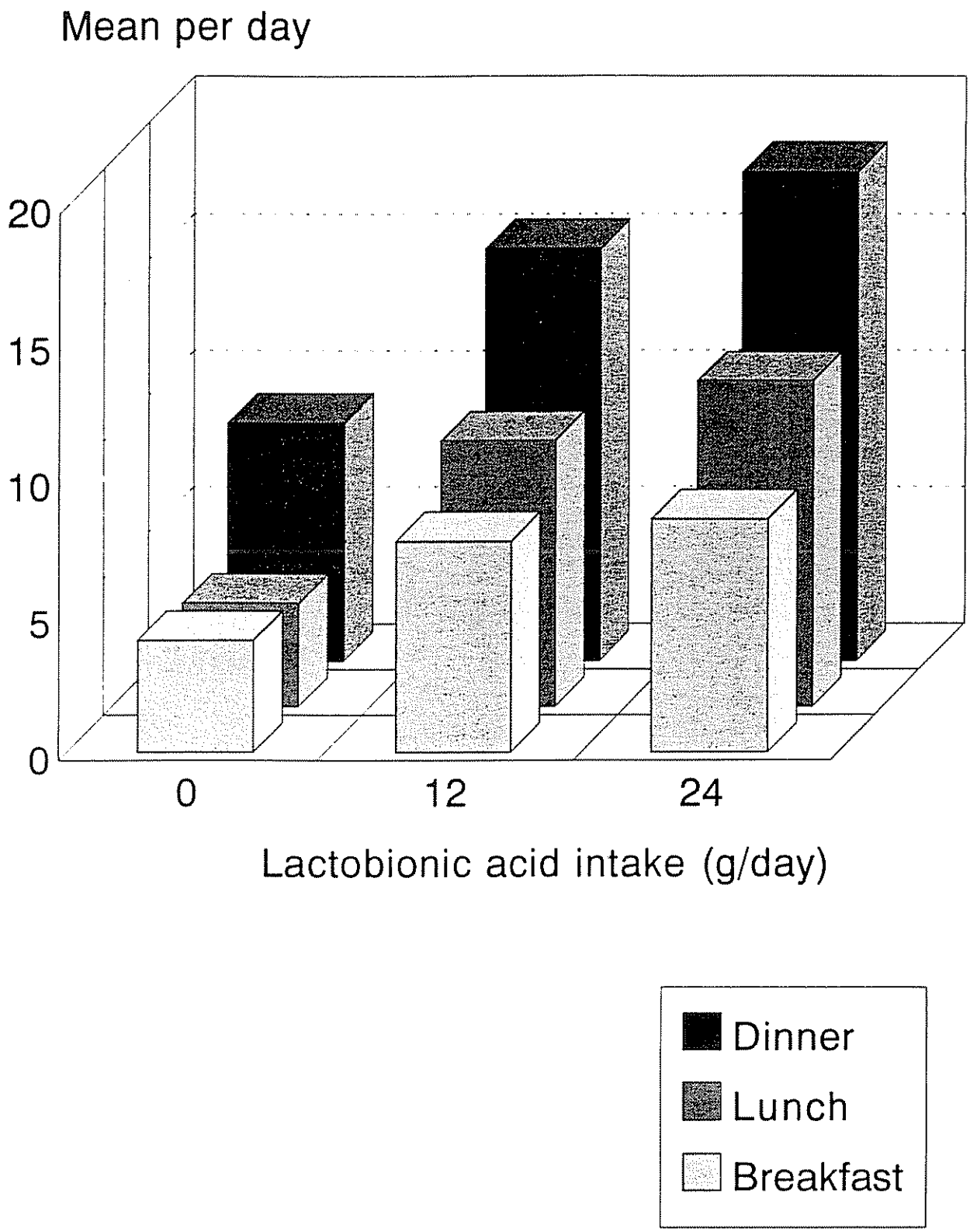


Figure 3 HYDROGEN CONCENTRATION IN EXPIRED AIR AT THE END OF EACH PERIOD OF SEVEN DAYS ON THREE DIFFERENT DOSES OF LACTOBIONIC ACID INTAKE

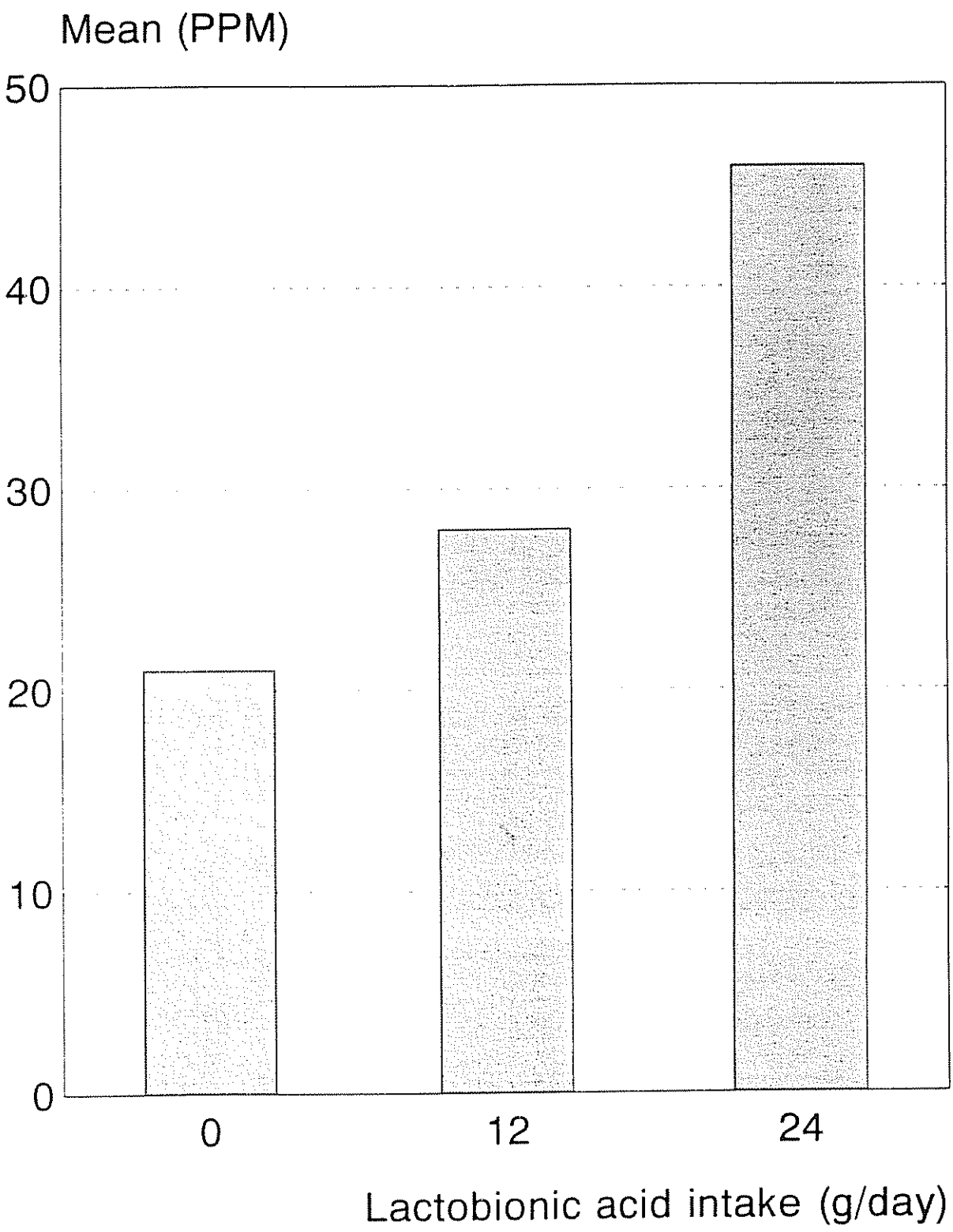


Figure 4 GLUCOSE CONCENTRATION IN BLOOD OF EIGHT SUBJECTS AFTER INTAKE OF 40 G LACTOSE AND 40 G LACTOSE PLUS 10 G LACTOBIONIC ACID

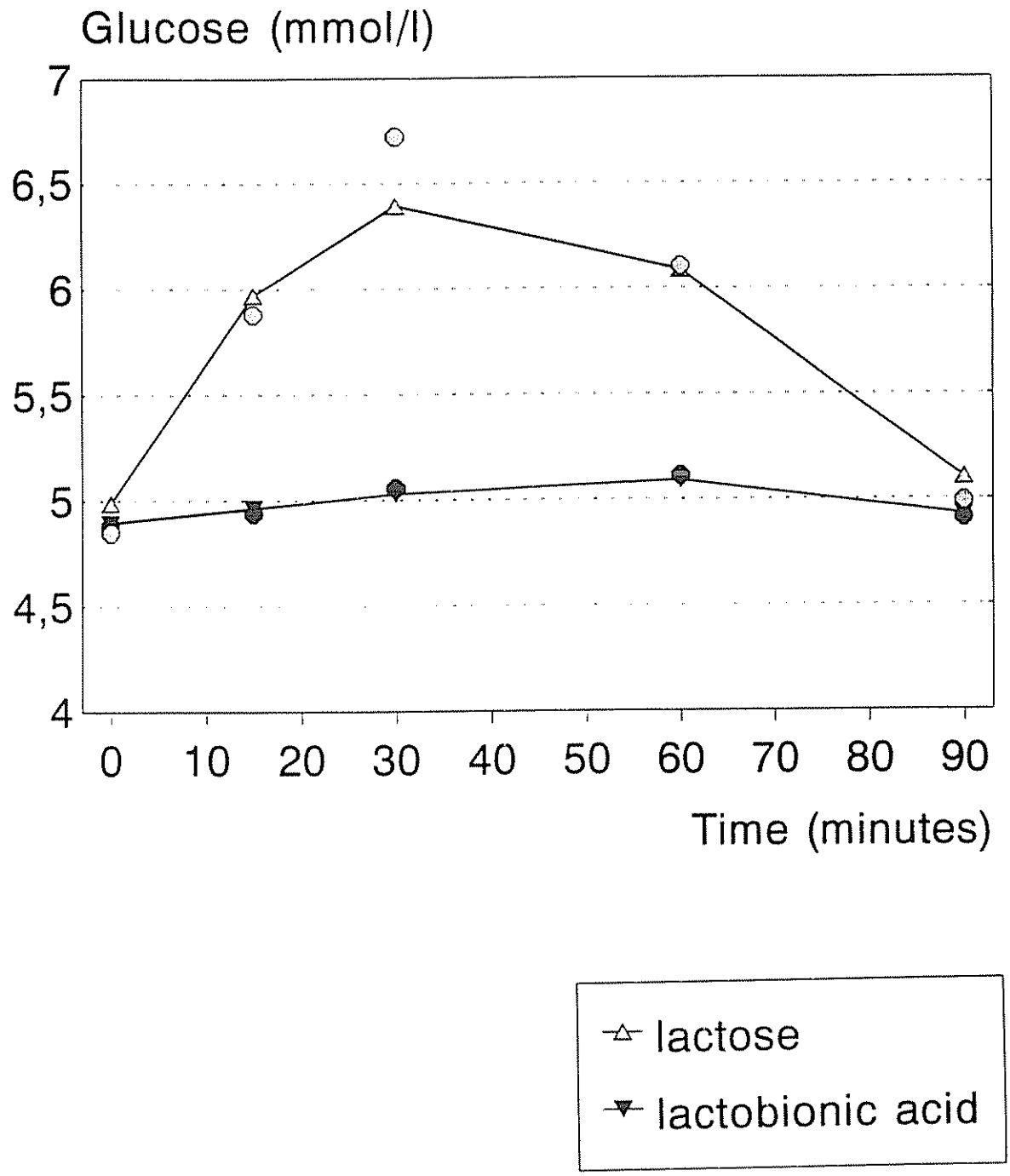
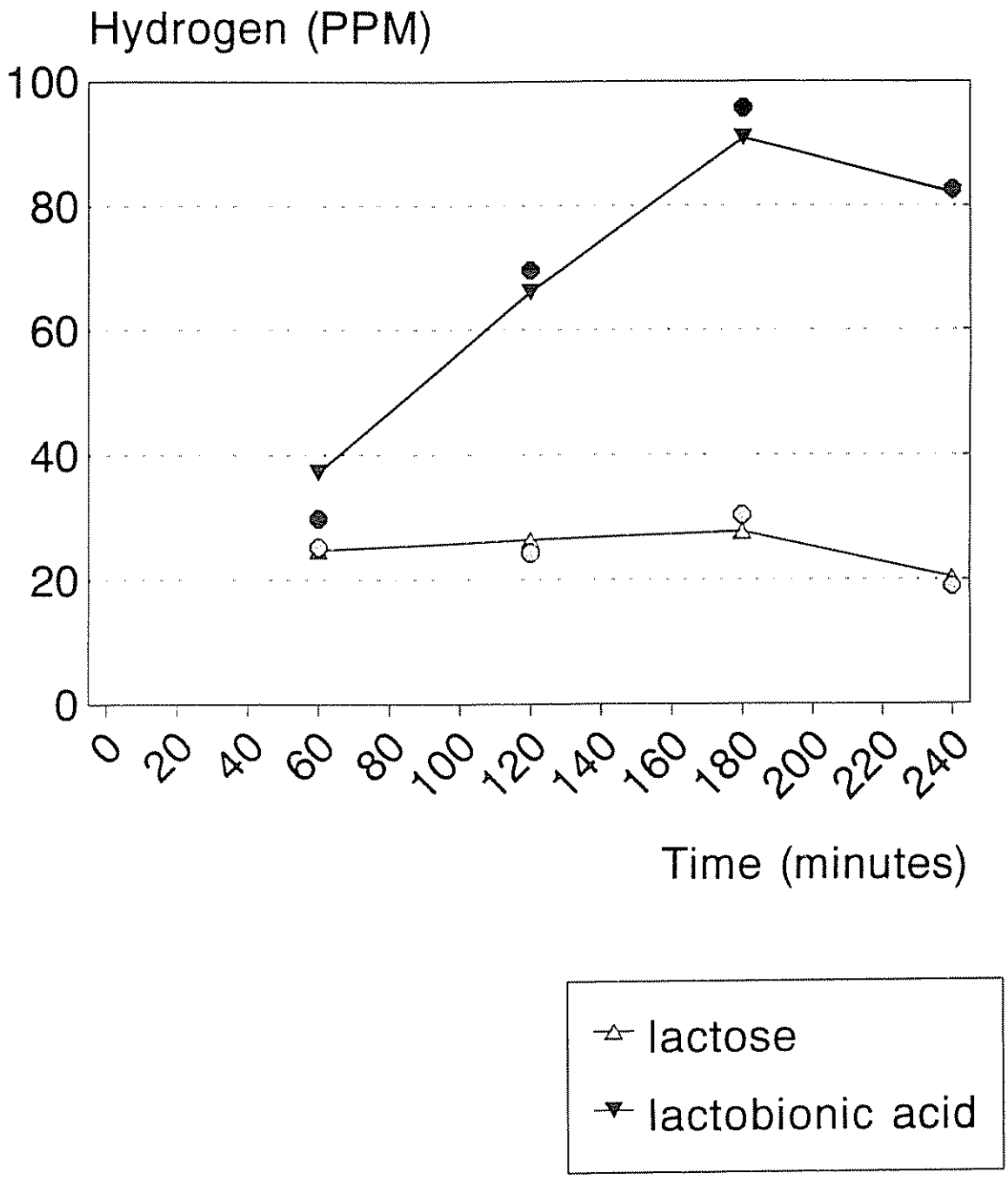


Figure 5 HYDROGEN CONCENTRATION IN EXPIRED AIR OF EIGHT SUBJECTS AFTER INTAKE OF 40 G LACTOSE AND 40 G LACTOSE PLUS 10 G LACTOBIONIC ACID



Appendix 1 EXPERIMENTAL DESIGN

The 18 subjects were randomly assigned to the three treatments according to the following scheme.

Subject nr.	Treatment order
1	ABC
2	ACB
3	BAC
4	BCA
5	CAB
6	CBA
7	ABC
8	ACB
9	BAC
10	BCA
11	CAB
12	CBA
13	ABC
14	ACB
15	BAC
16	BCA
17	CAB
18	CBA

The treatments were 12 g of lactobionic acid per day, 24 g of lactobionic acid per day or a placebo. The key regarding the corresponding letter was only known to the dietitian.

The lactose tolerance test was executed in 8 of the 18 subjects according to the following scheme. (L=lactose only, L+LB=lactose + lactobionic acid)

Subject nr.	First day	Second day
2	L	L+LB
3	L	L+LB
4	L	L+LB
5	L+LB	L
6	L+LB	L
7	L+LB	L
11	L	L+LB
14	L+LB	L

Appendix 2 QUESTIONNAIRE ABDOMINAL COMPLAINTS AND DEFECATION PATTERN

Subject nr.: __ Day of the study: __ Date: _____

1. Did you consume the test drink at breakfast? Yes No

   If not, why not? _____

2. Did you consume the test drink at lunch? Yes No

   If not, why not? _____

3. How many stools did you have the past 24 hours? ___

4. How was the consistency of the stools?

   __ harder than normal

   __ normal

   __ softer than normal

   __ much softer than normal

   __ not applicable (no stools)

5. What was the amount of the stools?

   __ much more than normal

   __ more than normal

   __ normal

   __ less than normal

   __ not applicable (no stools)

Abdominal complaints*	Number of events			Remarks
	Dinner**	Breakfast	Lunch	
Belching				
Nausea				
Vomiting				
Abdominal distention				
Borborygmia				
Abdominal cramp				
Flatulence				

* Until 2 hours after the meal ** Previous day