

## Feeding behavior in rats on a complete diet containing Concanavalin A

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**Summary** — *Canavalia ensiformis* is a tropical legume which could be used in animal feeding. However, it contains a lectin, Concanavalin A (Con A) which is harmful to animals. When rats are presented with a diet containing Con A, rejection of the food appears very soon after the beginning of ingestion. In order to examine this rejection phenomenon 3 studies were carried out. The rejection was found not to be due to a neophobic response, nor could it be attributed to a conditioned taste aversion. The gastric intubation study suggested the hypothesis that it could be the binding of the lectin to the glycosylated molecules from the gut membrane which impaired absorption and hence food intake.

**rat / food intake / neophobia / conditioned taste aversion / Con A**

**Résumé** — **Comportement alimentaire du rat sur un aliment contenant de la Concanavaline A.** *Canavalia ensiformis* est une légumineuse tropicale ayant un rendement élevé en graines qui pourrait être utilisée pour l'alimentation animale; mais ces graines contiennent une lectine toxique, la Concanavaline A (Con A). Cette toxicité se manifeste rapidement, dès le premier repas, par une diminution de la prise alimentaire. L'ingestion par le rat de son aliment habituel contenant la Con A permet d'étudier ce phénomène de refus. Ce rejet de l'aliment contenant Con A n'est pas dû à une réponse de néophobie, ni à une aversion gustative conditionnée. C'est peut-être la présence dans l'estomac et/ou le tube digestif de la lectine qui, en s'adsorbant aux groupements glycosylés de la parois, réduit la prise alimentaire en empêchant l'absorption intestinale.

**rat / prise alimentaire / néophobie / aversion gustative conditionnée / Con A**

## INTRODUCTION

*Canavalia ensiformis* (Jackbean) is a tropical legume showing a high potential yield of seeds. Digestibility of carbohydrates and amino acids is 94% when the ground seeds are extruded. It could be included in poultry feed (Léon, 1989). However, ripe unprocessed *Canavalia* beans are harmful to animals (Skerman, 1977; Wyss and Biczjel, 1988): a reduced food intake is observed when untreated *Canavalia* flour is added in substantial amounts to animal diets. This phenomenon appears soon after the beginning of the meal. Concanavalin A (Con A) purified from the seeds exhibits the same deleterious effect on food intake (Léon *et al*, 1991). Con A is a lectin. The toxicity of raw seeds is attributed to the fact that lectins when binding to glycoconjugates located on the luminal surface of the gut cause morphological changes (Donatucci *et al*, 1987; Pusztai *et al*, 1990): disruption of the intestinal brush borders of enterocytes and atrophy of the intestinal villi of the small intestine (Lorenzson and Olsen, 1982).

The measurement of food intake in animals under steady-state controlled conditions provides the required baseline for investigations of the underlying mechanisms. In addition, correct quantification of the amounts eaten and of the pattern of intake over time is a basic requirement in a physiological approach.

Laboratory techniques that permit the automated recording of meal patterns and the comparison of responses throughout the diurnal cycle are well known (Le Magnen and Devos, 1980). It is now well established that ingestive responses are adjusted both to the nutritive properties and metabolic needs through a learning process (Le Magnen, 1984). In this conditioning, the post-ingestive activity of the ingested foods serves as an unconditioned

stimulus and the orosensory activity becomes a conditioned stimulus to eat or not to eat, and in the first case to ingest a small or large amount.

In order to explain the feeding behavior of rats presented with a diet containing Con A, 3 studies were carried out.

In a first experiment the neophobic effect of the new food was examined (Le Magnen, 1963, 1987).

In a second experiment, we tested whether the rejection of the food containing Con A was due to a conditioned taste aversion, which, like neophobia, appears to be involved in feeding behavior as a defense mechanism against poisoning. It has been repeatedly shown that the association of illness and ingestion of a food with a new chemosensory cue induces a strong and persistent aversion to this flavored food (Revusky and Bedarf, 1967).

In a third experiment we tested the effect on consumption of the usual diet of a solution of Con A injected directly into the stomach *via* an oesophageal tube. Short-circuiting oro-sensory information could provide clues about the direct effect of the drug on the intestinal walls. The ability of lectins to bind to the cells, illustrated by the hemagglutinating properties of Con A, is due to their specific binding to sugar residues (Sharon and Lis, 1972). The affinity of Con A for D-mannose residues is high (Goldstein and Poretz, 1986). Thus, we tested the potential inhibition of ConA toxicity by intragastric administration of D-mannose.

## METHODS

Male adult Wistar rats were used. They were individually housed in Plexiglas cages placed in a quiet, temperature-controlled room ( $23 \pm 1$  °C) with a 12-h light-dark cycle (1 pm–1 am dark). They had free access to water and food (UAR

A04: proteins 17%, carbohydrates 70%, fats 3%, salt mixture 5%, vitamins 1%, cellulose 4%, metabolizable energy: 2.9 kcal/g) at all times. Concanavalin A was extracted and purified from Jackbean seeds using the methodology described by Léon *et al* (1990). The haemagglutinating activity and high pressure liquid chromatography spectrum of the preparation isolated was similar to that of Con A obtained from a commercial source (Sigma C2010). Con A was added to the experimental diet at the concentration level of 3% which was shown to inhibit food intake in chicks (Léon *et al*, 1991).

The free food intake was graphically recorded by continuously monitoring the weight of the food cup using an electric strain gauge microbalance. A metal cover with a hole in the middle was placed over the cup in order to prevent spillage.

The results were analyzed in terms of 24 h, night-time and daytime food intake, meal number and meal size. A meal was defined as a period of continuous eating preceded and followed by a least 40 min without eating (Le Magnen and Devos, 1980).

### Statistical analysis

One-way analysis of variance for repeated measurements followed by a Dunnett's *t*-test for *ad hoc* comparisons with the control was used in experiment 1. Student's *t*-tests were undertaken

in experiment 2. A two-way factorial ANOVA was used in experiment 3 (Statview).

### EXPERIMENT 1: NEOPHOBIA

The 24-h feeding behavior was studied when rats were presented with 2 new foods: a diet containing 3% Con A and a diet containing 3% casein. Eight rats (BW = 342 ± 4.6 g) were used. They were fed the regular diet *ad libitum* for 9 d. Then, on test d 1, rats of group 1 (1–4) received the casein diet, rats of group 2 (5–8) received the Con A diet. Food cups were introduced at the end of the diurnal period (1 pm) for 24 h.

After test d 1, all rats received the regular diet for another 5 d. On the following test day (test d 2), group 1 rats received the diet containing Con A and group 2 rats received the diet containing casein.

### Results and discussion

Food intakes (table I) were compared in the 3 situations: regular diet, casein diet

**Table I.** Night, day food intakes and feeding parameters. Data are means ± SEM.

	Regular	Casein	Con A	P
First meal				
Latency	41.3 ± 3.0	46.1 ± 13.1	26.3 ± 11.6	NS
Size (g)	2.7 ± 0.2	3.2 ± 0.7	0.6 ± 0.2*	< 0.05
Duration (min)	38.2 ± 5.4	20.8 ± 7.6	5.8 ± 2*	< 0.05
Night FI (g)	21.5 ± 0.6	21.8 ± 0.7	13.2 ± 1.0*	< 0.05
Meal number	6.8 ± 0.2	5.9 ± 0.3*	5.8 ± 0.6*	< 0.05
Meal size (g)	3.2 ± 0.2	3.9 ± 0.3	2.3 ± 0.5*	< 0.05
Day FI (g)	4.2 ± 0.5	4.1 ± 0.7	3.9 ± 0.5	NS
Meal number	3.6 ± 0.5	3.1 ± 0.6	2.4 ± 0.3	NS
Meal size (g)	1.2 ± 0.1	1.4 ± 0.1	1.7 ± 0.3	NS

\* *P* < 0.05 significantly different from regular values; NS: non significant; FI: food intake.

and Con A diet. Under the Con A diet, the size of the first meal of the night was significantly smaller and of shorter duration than with the other diets. However, the latency between food cup presentation and this first meal was the same in the 3 situations. Night intake was significantly reduced when animals had the Con A diet. The number of meals on the Con A diet was not different from that on the casein diet but meal size was significantly reduced. Day-time food intake was not significantly modified.

Thus, no neophobia appeared with the new diet containing casein. The reduction in the first meal size under Con A diet was not due to neophobia since, as already mentioned (Le Magnen, 1963), when rats manifested a neophobic response, it affected only the first meal and thereafter rats had a normal food intake. This Con A-induced reduction in food intake persisted throughout the night. In the present experiment the low intake was maintained under the Con A diet and this may be interpreted as a manifestation of the aversion to Con A.

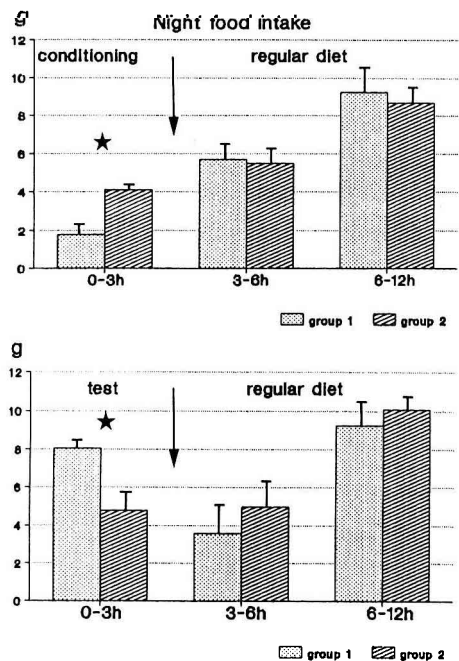
## EXPERIMENT 2: CONDITIONED TASTE AVERSION

In the present study, the ingestion of a new food (Con A diet) was paired with a new odor (amyl acetate) which is well appreciated by rats (Larue, 1973). Ten adult rats were used (BW = 344 ± 4.9 g). They had free access to food and water. All rats received their regular diet for 5 d. At the end of d 5, in group 1 (rats 1–5), a diet containing 3% Con A to which amyl acetate (1 ml/kg) was added was substituted for the regular diet for 3 h at the beginning of the nocturnal period. In group 2 (rats 6–10), rats received the regular diet odorized with amyl acetate. After this 3-h food intake (conditioning period) all

rats were returned to their regular normal diet. Five days later, on day 6, all rats received the regular diet odorized with amyl acetate for 3 h (test period). Then the regular non odorized diet was restored to all rats.

## Results and discussion

Food intake during the conditioning and test periods were compared (fig 1). On the conditioning day, group 1 rats presented with Con A diet consumed significantly less food than group 2 rats. The latency to the first meal was longer in group 1, but the difference did not reach statistical significance (72.6 ± 9.3 min vs 39.0 ± 17.2



**Fig 1.** Nocturnal food intake over conditioning and test days. During the conditioning period, group 1 rats received the regular diet containing Con A + amyl acetate; group 2 rats the diet odorized with amyl acetate. During the test period both groups received the regular odorized diet. \*  $P < 0.05$ .

min; NS). Over the following nocturnal period, when all rats were returned to the regular diet, group 1 rats partly compensated for their previous lower food intake. During the 3-h test-period, 6 d later, the latency to eat of rats in group 1 was significantly longer than that group 2 ( $79.2 \pm 20.4$  min vs  $19.0 \pm 11.6$  min;  $P < 0.05$ ). The first meal was larger in group 1 than in group 2 whereas group 1 rats had a larger food intake during the whole 3-h. Rats of group 2 had the same intake on conditioning and test days. If the aversion induced by pairing amyl acetate with intake of a new food had caused malaise in the rat on the test day, according to the well known conditioned taste aversion phenomenon, the presentation of a food odorized with the same flavor would have led to a total or partial rejection of that food. However, in the present experiment, rats which had ingested the Con A diet odorized with amyl acetate, did not reject the regular food odorized with amyl acetate; on the contrary, after a significantly greater latency to eat, they consumed nearly 4 times as much food as on the conditioning day. The explanation for this huge intake is not obvious; at least it can be concluded that no conditioned taste aversion induced the rejection of the diet containing Con A.

### EXPERIMENT 3: EFFECT OF GASTRIC INTUBATION

Eighteen rats (BW = 350 g on average) received 5 ml of one of 4 solutions: 0.8% NaCl, the same solution containing 300 mg Con A, the same solution containing 1 g mannose or the solution containing 300 mg Con A + 1 g mannose. The solutions were administered *via* an oesophageal tube in random order, 3 or 4 days apart, at the end of the diurnal period.

### Results and discussion

The first meal latency after gastric intubation was slightly but not significantly higher after mannose and Con A + mannose than after NaCl or Con A solutions (table II). The size of the first meal after intubation was smaller after Con A and Con A + mannose, but the duration of this first meal was not significantly different in the 4 situations. Both meal size and meal number were reduced after Con A ingestion over the 12-h night period, although the meal number was not significantly different in the first 6 h period. The following diurnal intake was significantly increased after mannose. The resulting 24 h food intake was significantly lower after Con A ingestion and intermediate with Con A + mannose.

Food intake is reduced after binding of Con A to the stomach mucosa. The addition of mannose partly inhibits the effect of Con A (Liener, 1986; Pusztai *et al*, 1990). The reduced nocturnal food intake observed after mannose and Con A + mannose is probably due to the caloric value of the mannose solution: one g of mannose represents 4 kcal, *ie* about 1.4 g of the regular diet. This is nearly the difference in food intake observed between NaCl and mannose on the one hand and Con A and mannose + Con A on the other hand.

The persistent and marked inhibitory effect of the initial load of Con A on the subsequent nocturnal food intake of a regular diet does not change the latency to feeding of rats. The reduction of the first meal of the night is smaller when compared to the following nocturnal ones, suggesting that the Con A effect is accentuated by the presence of food in the digestive tract. With regard to the dose chosen in this study: 300 mg of Con A corresponds to the ingestion of 30 g per day of a food containing 1% lectin (*ie* 30% *Canavalia ensiformis*). It has been observed in the chicken

(Léon *et al*, 1991) that an inverse linear relation exists between ingestion and concentration of lectin in the food (0 to 1% lectin). A higher dose gives a lower food ingestion. The ratio, 1 g of mannose to 300 g of Con A was obtained by hemagglutinating *in vitro* inhibition tests. On the other hand it has been shown, by adding mannose to the chicken feed, that there was a partial inhibition of the toxic effect of Con A (Léon *et al*, 1989).

## GENERAL DISCUSSION

Thus, neophobia was not shown by the present study. However the first meal was interrupted quickly (6 min) when intestinal

absorption has already begun in rats. This is different from the results in chickens (30 min; Léon *et al*, 1991). It could perhaps be due to the lack of chewing in chickens. These differences in the digestive treatment in the 2 species could be responsible for these discrepancies.

Regarding the condition taste aversion results, there is no real conditioned taste aversion; nevertheless rats presented with Con A, eat later but they increase food intake during the first meal. It seems that rats behave as if they feel relieved after the first min of consumption.

The gastric intubation study confirms that the effects of phytoagglutinins on the activity of the intestinal mucosa enzymes (Rouanet and Besançon, 1979) are re-

**Table II.** First nocturnal meal, night and day food intakes and feeding parameters after intubations at the beginning of the night (MN = meal number; MS = meal size). Data are means  $\pm$  SEM.

<i>n</i>	NaCl (16)	Con A (15)	Mannose (10)	Con A + Man (7)	F values (1.44)		
					Con A	Man	Interact Con A x man
1st meal							
Latency (min)	59.8 $\pm$ 11	66.2 $\pm$ 23	112.1 $\pm$ 16	90.0 $\pm$ 15	NS	NS	NS
Size (g)	2.1 $\pm$ 0.1	1.7 $\pm$ 0.3	2.0 $\pm$ 0.2	1.4 $\pm$ 0.3	4.3 *	NS	NS
Duration (min)	16.8 $\pm$ 3.6	18.6 $\pm$ 4.2	13.1 $\pm$ 2.2	9.4 $\pm$ 1.7	NS	NS	NS
Night FI (g)							
(0-6 h)	9.8 $\pm$ 0.4	4.8 $\pm$ 0.6	8.1 $\pm$ 0.7	6.3 $\pm$ 0.6	32 **	NS	7.5 *
MN	3.4 $\pm$ 0.2	2.8 $\pm$ 0.3	3.3 $\pm$ 0.3	3.3 $\pm$ 0.3	NS	NS	NS
MS (g)	2.9 $\pm$ 0.2	1.7 $\pm$ 0.2	2.5 $\pm$ 0.2	2.0 $\pm$ 0.2	17 **	NS	NS
Night FI (g)							
(0-12 h)	19.6 $\pm$ 0.5	9.6 $\pm$ 1.1	18.3 $\pm$ 0.8	14.4 $\pm$ 1.0	52 **	NS	10 *
MN	6.9 $\pm$ 0.3	5.3 $\pm$ 0.5	6.6 $\pm$ 0.4	6.4 $\pm$ 0.4	4.7 *	NS	NS
MS (g)	2.9 $\pm$ 0.1	1.2 $\pm$ 0.1	1.4 $\pm$ 0.1	1.8 $\pm$ 0.2	21 **	NS	NS
Day FI (g)							
(0-24 h)	4.8 $\pm$ 0.4	4.1 $\pm$ 0.5	5.7 $\pm$ 0.5	6.1 $\pm$ 0.8	NS	7.8 *	NS
MN	4.1 $\pm$ 0.3	3.4 $\pm$ 0.3	4.3 $\pm$ 0.3	3.4 $\pm$ 0.3	4.8 *	NS	NS
MS (g)	1.2 $\pm$ 0.1	1.2 $\pm$ 0.1	1.4 $\pm$ 0.1	1.8 $\pm$ 0.1	NS	11 *	NS
24 h FI (g)	24.4 $\pm$ 0.6	13.7 $\pm$ 1.3	24.1 $\pm$ 0.9	20.4 $\pm$ 1.1	42 **	NS	9.9 *

\*  $P < 0.05$ ; \*\*  $P < 0.01$ .

sponsible for the reduced food intake. Comparing various lectins has shown (Pusztai *et al*, 1990) that most of them link at the first exposure to the brush border, inducing a hyperplastic growth of the rat small intestine and delaying animal growth. Moreover, it has been demonstrated (Nakata and Kimura, 1986) that ingested Con A remained unaltered during digestion and was rapidly excreted in the faeces. The excreted Con A was bound in the faeces to a glycoprotein which is found in the stomach, the small intestine and the caecum of rats (Nakata and Kimura, 1990). The inhibition of rats food intake by Con A is accentuated after the first meal and partly compensated by mannose distribution (in experiment 3), suggesting a digestive origin. The effect of Con A on food intake could be hypothetically explained by a binding of the lectin to glycoprotein residues of digestive tract cell membranes, thus, impairing the digestive process. Recently, Marcongenty *et al* (1991) demonstrated, *in vitro*, the possibility of a transmucosal transport of Con A across the rabbit ileum. Thus, the hypothesis of a metabolic action of Con A cannot be completely rejected.

## CONCLUSION

The systematic study of the feeding behavior of the rat presented with a diet containing Con A has shown that animals reject this diet very rapidly. This reaction cannot be explained by a neophobic response of the rat; neither can it be attributed to a conditioned taste aversion.

Gastric intubation studies show that it is the presence of Con A in the stomach or further down the digestive tract which impairs the subsequent food intake.

This result suggests that the binding of the lectin to the glycosylated molecules from the gut membrane by virtue of its in-

terference with the intestinal absorption could be responsible for the reduction of food intake.

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