

Observation

## COMMERCIAL RODENT FEED AS AN OCCASIONAL CAUSE OF MORBIDITY AND MORTALITY IN A RAT BREEDING COLONY

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In the last fifteen years there were several feed-related outbreaks of morbidity and mortality in the Institute's breeding colony of Wistar rats. The last event took place in April 1999, one month after the use of a new supply of the usual standard rodent feed. Animals did not thrive and manifested generalised oedema, hypoalbuminaemia, elevated liver enzymes, and high mortality. The effect of feed was assessed first by feeding a group of sick females during 14 days with either suspected feed (A-March) or with the earlier supply of feed (A-January) of the same producer. Then a group of healthy male rats Y59 from another breeding colony was fed either suspected feed (A-March) or feed from another producer (feed B). Although neither chemical nor microbiological deviation in feed analysis had been detected, decreased consumption and slower body weight gain in all animals fed with feed A-March suggested an association between this batch of feed and the increased morbidity in those animals. Eventually, the entire rat colony was put down and replaced with a new breed which was given a new brand of feed.

**KEY WORDS:** *failure to thrive, food analysis, hypoalbuminemia, liver cirrhosis, malnutrition*

Nutrition is one of the most important prerequisites for maintaining laboratory animals in good health condition. It affects not only their health status, but also the experimental results (1, 2). Variations in laboratory animal feed can have a great impact on animal growth, development and health. Two main premises have to be fulfilled; the feed must contain all essential nutrients in required concentrations and the level of contaminants must be minimal and harmless (1, 3). Unfortunately, actual composition of animal feed does not always meet the required values and/or comply with the producer's declaration on the label (2, 4-6).

*Beynen and co-workers* (5) described two types of diet variations; the first can occur among feeds produced by different manufacturers, and the second between batches of a particular brand

of feed from the same producer. In addition, commercial laboratory animal feed, especially the one composed of natural ingredients, can be contaminated with microorganisms or various toxic substances, such as pesticides, antibiotics, heavy metals, histamine and other (7-12). Contamination can occur at any stage of feed processing, in transport, distribution or storage.

According to the record kept by the Laboratory Animals Unit of the Institute for Medical Research and Occupational Health in Zagreb, Croatia, adverse health effects of commercial laboratory animal feed were reported on several occasions over the last 15 years. For example, the autumn of 1987 saw an extremely high rat mortality rate (over 50%) and almost no newborn rats (according to the minutes of the Institute's Laboratory Animals Commission meetings in

1987 and personal records from the laboratory notebook of M. Piasek, at that time a member of the Commission). Certain ingredients in animal feed used at that time (called feed S) were found to be directly related to rat morbidity and mortality. Feed ingredients were analysed repeatedly. Vitamin analysis at the Department of Poultry Diseases of the Faculty of Veterinary Medicine, University of Zagreb showed that feed S contained only 3,560-4,300 IU/kg of vitamin A and 13.2 mg/kg of vitamin E. The vitamin concentrations declared by the producer were 12,000 IU for vitamin A and 35 mg for vitamin E per kilogram of feed. Element analysis of feed S performed by the Institute's laboratory for the physiology of mineral metabolism showed about 3 to 5 times higher calcium content than the declared 1-1.2%. It was concluded that feed deficient in vitamins essential for fertility combined with extremely high calcium content was causally related to the breeding failure and high mortality rate in the rat colony at the end of 1987. The Institute filed a complaint and the producer was found guilty as charged by the court of justice. The producer had major financial difficulties at the time and stopped producing feed only to bankrupt shortly after these events.

Another outbreak of morbidity and mortality in the Institute's Wistar rat breeding colony took place in spring 1994 (13). Again, the suspicion fell on the feed as responsible for the illness of rat, but the feed analysis was not thorough this time. No definite proof of harmful effects of any particular feed ingredient was established. After the event, all sick animals were put down and a new breed introduced.

The last outbreak of serious morbidity and mortality in the Institute's Laboratory Animals Unit took place at the end of April 1999. Most rats in the breeding colony were dehydrated, hypodynamic, with bristled fur and pale ears, tail and paws. Their abdomen diameter was extremely enlarged. Feed and water consumption dropped. Young animals were more affected than older ones. One female and six male rats died at the age of 2 months. Of 24 female rats with litters at the time, 18 mother rats rejected their pups. Cannibalism became common. Body weights of survived pups were lower than average. These events occurred approximately one month after the Institute received the last shipment of

laboratory animal feed from the supplier who had been supplying the same feed to the Institute's breeding farm for two years. Other conditions in the Unit such as water supply, housing, care, and indoor microclimate were unchanged and under control. This practically total drop in food consumption, and the memory of earlier unfortunate events in 1987 and 1994, led us to assume that the last supply of laboratory feed with the production date of 1 March 1999 and the morbidity outbreak could be related.

We conducted two experiments to assess the influence of suspected laboratory feed on growth and general health in experimental animals. The first experiment was performed on young female Wistar rats bred in the Institute's Unit, who exhibited the described signs of illness. The aim of this experiment was to see whether the change in nutrition would improve their growth and general health status. The second experiment included healthy young Y59 male rats brought in from a colony bred elsewhere to see the effect of suspected feed on the growth and health of rats of different strain.

The feed suspected to cause animal morbidity underwent microbiological, chemical, toxicological and radiological analyses. Blood samples from sick animals were taken for biochemical analyses. Several animal bodies were sent to pathological and histopathological examination at the Croatian Veterinary Institute in Zagreb at the beginning of the experiments.

## MATERIALS AND METHODS

### *First experiment: Assessment of health effects of two supplies of the same brand of feed*

Food and water consumption and body weight gain were measured in thirty 2-month-old female Wistar rats from the Laboratory Animals Unit of the Institute for Medical Research and Occupational Health, Zagreb, who were fed on two feed supplies from the same producer. The baseline average rat body weight was 115 g (range: 86-139 g). The animals were randomly assigned into two groups of 15. The first group was fed with the batch produced on 1 March 1999 (feed A-March) which was suspected to cause animal illness. The second group was fed with

the batch produced by the same supplier on 13 January 1999 (feed A-January). Before the experiments started the animals were receiving feed A-March for one month and all of them exhibited signs of morbidity described above.

*Second experiment: Effect assessment of suspected feed in comparison to a different feed brand*

This experiment was conducted on twenty 2-month-old healthy male rats of strain Y59 taken from the colony bred at the Faculty of Natural Science of the Zagreb University where they were receiving imported feed which was not used in this experiment. Their baseline average body weight was 187 g (range: 133-236 g). They were randomly divided into two groups; the first group received feed A-March and the second imported commercial laboratory animal diet (feed B).

*Animal housing and measurements*

Animals from both experiments were kept in polycarbonate cages (40 cm x 25 cm x 15 cm) (Ehret, Germany), five per cage, with free access to feed and tap water. Each experiment lasted 14 days.

Body weight, feed and water consumption were measured every morning. Feed and water

consumption were measured per cage. Individual daily consumption was calculated as an average daily consumption of either feed or water for each group for 14 experimental days. Body weight gain is presented as cumulative average body weight gain for each group. Only total body weight gain was analysed by the Student's *t*-test using Statistica® for Windows (StatSoft 1995 package, release 5.0) at the level of significance of  $P < 0.01$ .

## RESULTS

### HEALTH EFFECTS OF TWO SUPPLIES OF THE SAME BRAND OF FEED

*Feed and water consumption*

Before the experiment started, the average feed consumption in both groups of female rats was very low: approximately 4-8 g per day. In comparison, the normal average feed consumption for the female rats of the same age is about 15 g, and the water consumption approximately 35 ml. The consumption in the first group, which continued to be fed with feed A-March, remained low throughout the experiment (Figure 1). The average consumption in the second group, which switched to feed A-January,

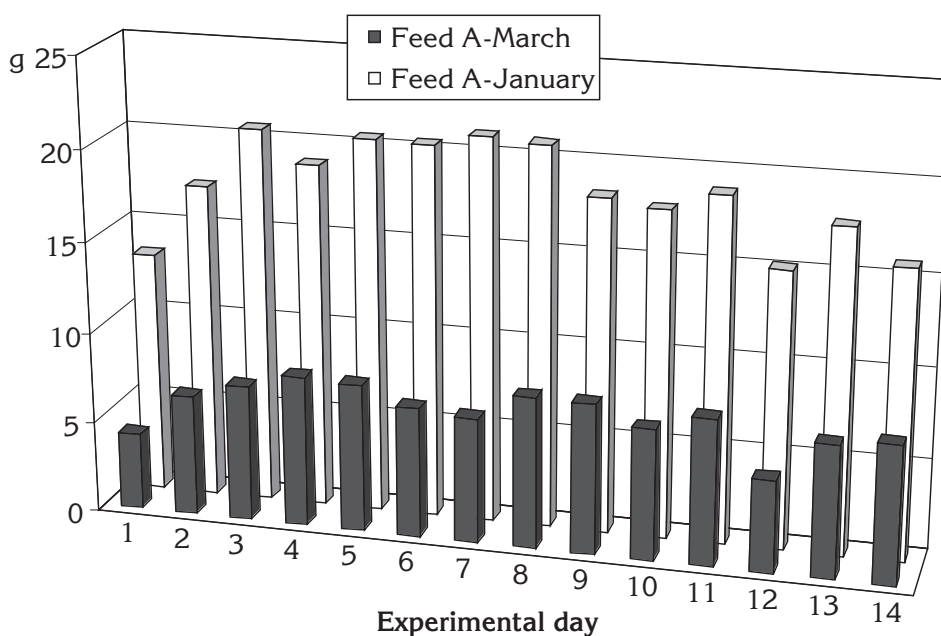


Figure 1 Average feed consumption by Wistar rats (g/day) (Experiment 1)

increased as soon as the first experimental day, and was approximately 2-3 times higher than in the first group throughout the experiment. Average water consumption was also 2-3 times higher in the group fed with feed A-January (Figure 2).

*Body weight gain and general health*

The average body weight gain also greatly differed between the two groups (Figure 3). In the group fed with feed A-March the highest daily body weight gain was only 3.13 g, and at the end of the experiment total body weight gain was

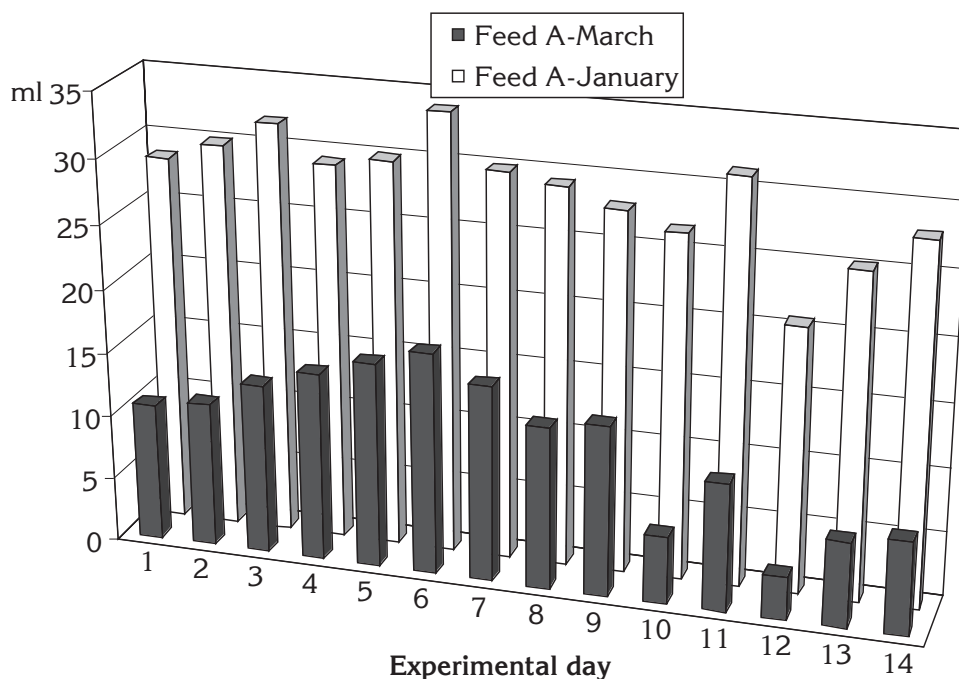


Figure 2 Average water consumption by Wistar rats (ml/day) (Experiment 1)

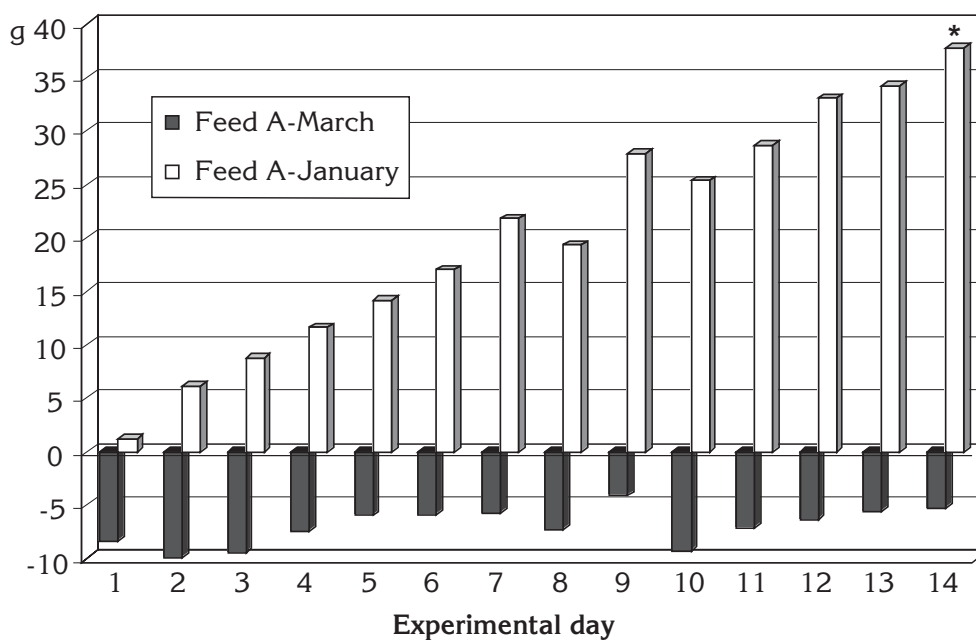


Figure 3 Cumulative average body weight gain by Wistar rats (g) (Experiment 1). \*Significant difference ( $P < 0.001$ ) between the groups in total body weight gain

negative (-5.3 g). In the second group fed with feed A-January, daily gain was significantly higher, up to 8.5 g. Total body weight gain was 37.7 g. Statistical significance of this difference was confirmed by Student's *t*-test (at the level of significance of  $P < 0.01$ ). In both groups of animals

symptoms of illness persisted throughout the experiment. Figure 4 shows the comparison between body weights of female rats in Experiment 1 fed with feed A-March and body weights of female rats of the same strain and age grown in the Unit in 1998 (14).

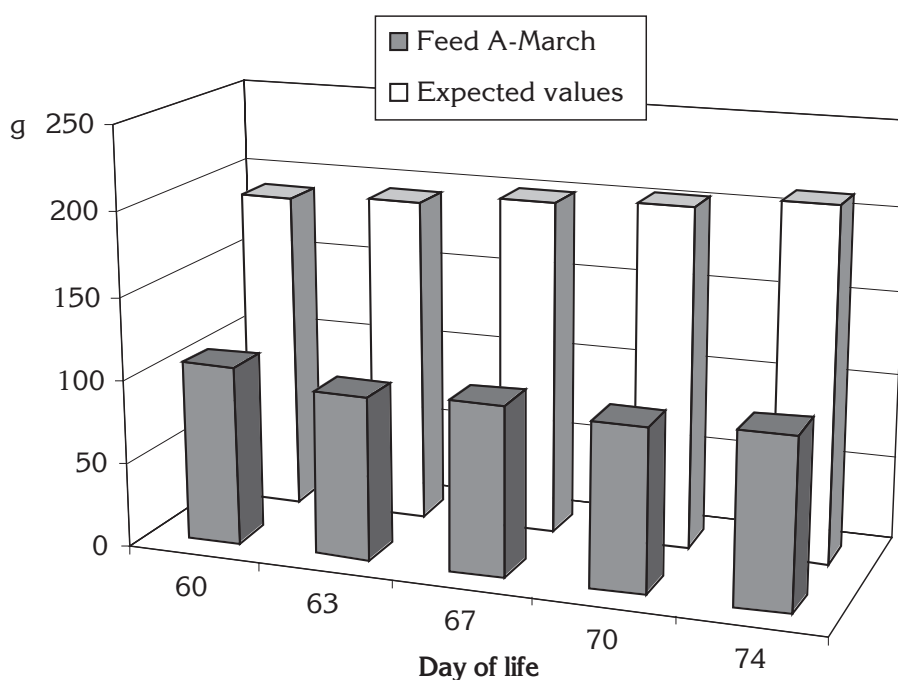


Figure 4 Body weights (g) of Wistar rats (Experiment 1) compared to the expected values (taken from body weight data recorded for the same strain, age and sex (breed 1998) (14)

## HEALTH EFFECTS OF TWO DIFFERENT BRANDS OF FEED

### Feed and water consumption

Average feed consumption markedly differed between the two groups of male rats from the first experimental day, and remained almost two times higher in the group fed with feed B (Figure 5) throughout the experiment. Water consumption was similar in both groups (data not shown).

### Body weight gain and general health

The average daily body weight gain in the group fed with feed A-March declined to negative value on the first experimental day (Figure 6). It remained negative or very low during the experiment. In the second group, fed with feed B, average daily gain was always positive, from 1.3 to 9 g. Total average body weight gain in this

group during 14 experimental days was 66.6 g, while in the group fed with feed A-March it was negative (-6.1 g). This difference was statistically highly significant ( $P < 0.001$ ).

Except for slower growth, animals in the group fed with feed A-March did not show signs of illness described in rats from the Institute's breeding colony.

## DISCUSSION

The results from both experiments in 1999 show that the intake of feed A produced in March 1999 was markedly lower than that of the supply produced in January, or of the imported brand (feed B). As expected, feed consumption had a significant impact on body weight gain in both experiments.

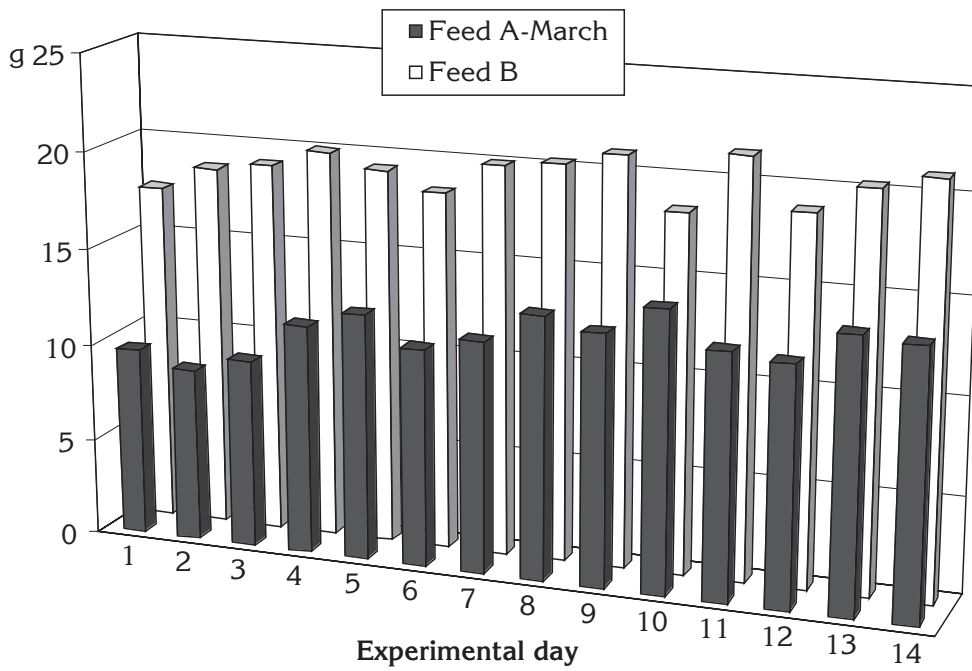


Figure 5 Average rat food consumption by Y59 rats (g/day) (Experiment 2)

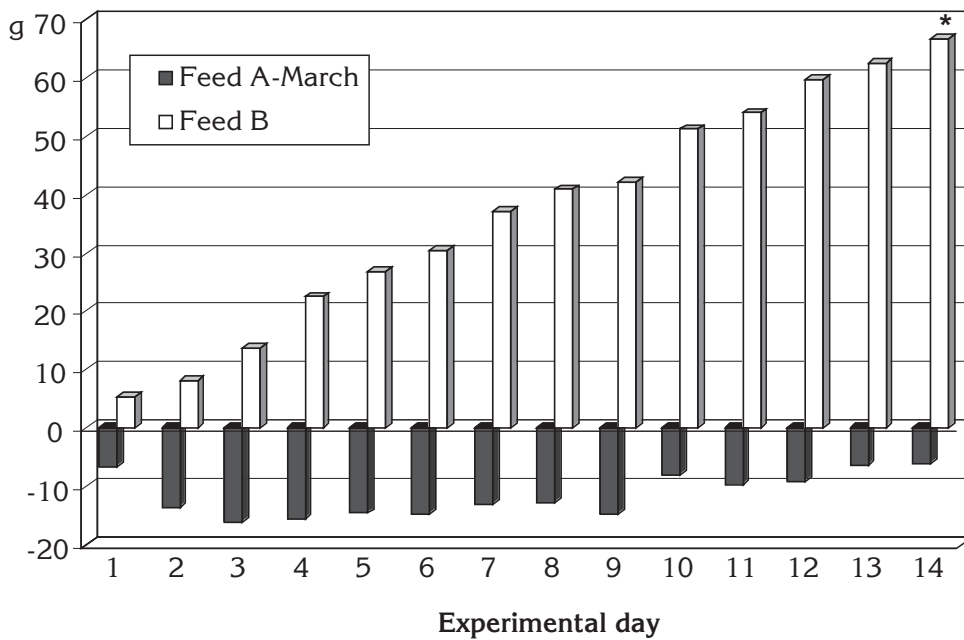


Figure 6 Cumulative average body weight gain (g) by Y59 rats (Experiment 2). \*Significant difference ( $P < 0.001$ ) between the groups in total body weight gain

Sick female rats that were receiving feed A-January did not recover entirely and exhibited most of the described symptoms by the end of the experiment. However, a sign of general health improvement was the significant increase in their

body weight gain. On the other hand, young males of Y59 strain from the second experiment showed no symptoms of illness. It could be argued that the exposure time was too short for the occurrence of these symptoms in male rats. However, even



that short a period was enough for the animals on feed A-March to grow slower.

Another factor could be the difference in age at which the two groups of rats were exposed to feed A-March; Wistar females from the Institute started to feed on feed A-March in the first and Y59 male rats in the second month of their life. It is possible that Wistar rats started to feed on A-March at the age critical for their further growth and development (post-weaning period). The most severe symptoms in the breeding colony were observed in the youngest animals. Rats older than six months did not show overt clinical signs of illness. It is known that older animals have lower nutrient requirement than young animals in the period of intensive growth and development. Therefore adult animals could be less sensitive to possible deficiency of essential nutrients. Furthermore, it is possible that a potential toxic substance in feed is less harmful to adult than young animals.

The fact that lower consumption of feed A-March and the consequent drop in body weight gain were found in healthy young males of different strain (Y59), breed and housing, confirms our hypothesis that the laboratory animal diet was responsible for the morbidity and mortality of animals in the Institute's Unit in spring 1999.

Although the feeds A-January and A-March are two batches of the same brand, it is very likely that their composition is different, and significant variations in diets based on natural ingredients have been known to occur. These fluctuations in feed composition are much lower in purified diets, based on refined, standardised ingredients (5).

Our considerations included possible contamination of feed A-March and we decided to analyse both supplies of feed A (A-January and A-March) microbiologically, chemically, toxically and radiologically, but no indication of toxic or other harmful component was found in either of them. The nutritional analysis also failed to reveal either excess or deficiency of major essential nutrients.

Histopathological examination of sick animals from the Institute's breeding farm of both sexes performed at the Veterinary Institute in Zagreb showed abundant oedemata in subcutaneous tissue, markedly reduced skeletal musculature, cirrhotic liver changes, splenomegaly, and myocardial degeneration with necrosis.

Hemosiderosis was found in the liver, spleen, and heart muscle (15).

Blood tests of sick animals also indicated liver damage. In comparison with normal values for laboratory rats according to *Ringler and Dabich* (16), we found slightly lowered total protein concentration, markedly lowered albumin concentration, and moderately elevated alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase. These biochemical changes, backed with histopathological findings remind us of protein-energy malnutrition syndrome. Some of the symptoms of starvation (inanition) in humans, such as apathy, failure to thrive, reduced mass of skeletal muscles, marked oedema, hypoalbuminemia, distended abdomen, decreased mass of myocardium with myofibrillar atrophy, oedema, and necrosis are very similar to symptoms observed in the sick rats (17). Starvation can induce liver cirrhosis in humans, but this is rare (18). Humans usually develop fatty liver (steatosis), with different degrees of fibrosis, from absent to severe (19). Cirrhosis is only occasional in adults (20-22), and has never been found in children, but significant liver injury with bridging fibrosis may be present (23, 24).

It is possible that suspected feed (A-March) was not toxic or harmful. There is a possibility that the described symptoms in Wistar rats were merely the consequence of extremely reduced dietary intake due to one or more unpalatable ingredients. This is supported by the fact that healthy Y59 rats refused to eat the food from the very beginning of the second experiment, before any toxic effect could be experienced. However, one can not completely rule out the existence of undetected toxic substance in the feed.

In conclusion, although we were not able to detect any harmful component in the feed in 1999, the fact that, for some reason, most probably feed-related, an extremely reduced feed intake occurred and led to morbidity and mortality in the Institute's rat breeding colony, can not and must not be ignored. It is important to stress that commercial feed for laboratory animals is not always safe, regardless of the manufacturer's declaration and/or inability to pinpoint the exact non-compliances from the declared specification. When it does not comply, the feed may affect the rate of morbidity and sometimes mortality in laboratory animals, with serious consequences

for scientific experimental results and finances in general.

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## Sažetak

### KOMERCIJALNO KRMIVO ZA GLODAVCE KAO POVREMENI UZROK OBOLJEVANJA I UGIBANJA U UZGOJNOJ KOLONIJI ŠTAKORA

Tijekom posljednjih petnaest godina u Jedinici za uzgoj laboratorijskih životinja soja Wistar Instituta za medicinska istraživanja i medicinu rada u Zagrebu, u nekoliko navrata došlo je do obolijevanja i ugibanja životinja u uzgajalištu kada je izravno ili neizravno kao uzrok tomu dokazano krmivo. Posljednji takav događaj zbio se u travnju 1999. godine. Približno mjesec dana nakon primjene nove pošiljke hrane za pokusne glodavce od dugogodišnjega domaćeg proizvođača (hrana A od ožujka), u životinja su se počeli pojavljivati znakovi bolesti i povećano ugibanje. U oboljelih štakora opaženo je usporeno napredovanje, edemi, hipoalbuminemija i povišene vrijednosti jetrenih enzima. Napravljena su dva kratka pokusa. Prvo je skupina ženki štakora Wistar iz istog uzgoja koje su pokazivale znakove bolesti hranjena 14 dana hranom za koju se posumnjalo da je uzrokovala obolijevanje (hrana A od ožujka) uz istodobno hranjenje druge (kontrolne) skupine prije proizvedenom hranom istog proizvođača (hrana A od siječnja). U štakorica hranjenih sumnjivom hranom (A od ožujka) opažena je znatno manja potrošnja hrane i vode, a ukupni prirast tjelesne mase bio je statistički značajno manji nego u štakorica hranjenih kontrolnom hranom (A od siječnja). U drugom pokusu uspoređivano je napredovanje dviju skupina zdravih mužjaka štakora soja Y59 iz drugog uzgajališta. Jedna skupina hranjena je sumnjivom hranom (A od ožujka), a druga hranom drugog proizvođača (hrana B). Skupina hranjena suspektnom hranom (A od ožujka) ponovno je pokazivala zaostajanje u rastu, čak i negativni prirast i imala manju prosječnu potrošnju hrane u odnosu na kontrolnu skupinu (na hrani B). Provedenim mikrobiološkim i kemijskim analizama hrane nisu se mogli otkriti štetni sastojci ili odstupanja u esencijalnim prehrambenim sastojcima. Međutim, izrazito zaostajanje u rastu životinja hranjenih hranom proizvedenom u ožujku 1999. u oba pokusa upućuje na to da je ta pošiljka hrane bila povezana s povećanim obolijevanjem i uginućem životinja u uzgojnoj koloniji tijekom proljeća 1999. godine. Nakon toga sve životinje u uzgajalištu morale su se eutanazirati, uveden je novi uzgojni soj i od tada se primjenjuje standardna hrana od drugog proizvođača.

**KLJUČNE RIJEČI:** *analiza hrane, ciroza jetre, hipoalbuminemija, malnutricija, zaostajanje u napredovanju*

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