To the editor:

As basic researchers in clinical nutrition area, it was with great interest that we read the recent Santos JF et al. article describing hepatic steatosis associated to changes in liver fatty acids profile in Wistar rats fed ad libitum a standard diet AIN-93, at time points of one and four months. We recognize the value of such investigation because a standard nutritionally balanced diet is important for the welfare of laboratory rodents and to ensure that experimental results are not biased by unintended nutritional factors. However, there are some major drawbacks in this study protocol that may limit the validity of its conclusions.

Current evidences support that ad libitum feeding is an uncontrolled variable in animal studies that may lead to metabolic disorders by accelerating body weight gain, decreasing glucose tolerance and peripheral insulin sensitivity, and increasing liver fat. When comparing to dietary restriction, ad libitum feeding is also associated to an increase in size of different organs due to degenerative and proliferative injury, including liver. Some of these alterations were observed in Santos JF et al. study using this kind of feeding and then may not be directly associated to the type of diet offered.

Moreover, Santos JF et al. compared nonindustrial manufactured AIN-93 diets to an industrialized commercial diet. We do not feel comfortable with such approach because the industrial process enrolls standardized safety procedures to produce diets for laboratory animals that not only ensure the proper animal development, but also avoid contaminations by pathogenic microorganisms. In this regard, diet pelletization requires appropriate well-controlled time, temperature and pressure of drying to avoid fungi fermentation and proliferation within the chow due to humidity, which can be better achieved industrially.

Unfortunately, Santos JF et al. did not detailed the mechanism underlying the pellet formation to support that potential fungi contamination did not occurs in provided AIN-93 diets and affected the hepatic response. Furthermore, the American Institute of Nutrition states that AIN-93 diets should be stored at 4°C and its deterioration monitored periodically, but these authors also did not report if this specific refrigeration temperature and deterioration monitoring was properly attended.

Based on these observations, it would seem crucial to us to maintain consistency in using comparatives commercial diets or laboratory diets offered at standardized amounts and at fixed times to avoid bias within studies assessing the physiological and biochemical impact of the AIN-93 diet intake in rats.

References


Priscila Garla, Raquel Susana Torrinhas, Alwedy Tessier, Felipe Aprobato, Ronaldo Oliveira Filho, Márcia Antunes and Dan Linetzky Waitzberg

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The critical reflection brought by the letter from Garla et al. about our findings on the effects of AIN-93 standard diet in Wistar rats presents interesting elements.

Concerning the arguments about the ad libitum regime in the diet offer, although well founded and indeed a possible triggering factor of the observed hepatic steatosis, we would like to remind that this procedure was similarly adopted for all groups and even then, the groups fed with the commercial diet did not exhibited (commercial diet group – 1 month) or exhibited a more modest change in the histological pattern (commercial diet group – 4 months), when compared to the groups fed with the AIN-93 diet; it means different metabolic effects of the diets and a worse impact caused by the AIN-93. Therefore, the argument that the AIN-93 formulation promoted hepatic steatosis, unlike the commercial diet and regardless of the offering period, becomes relevant.

It is pertinent to indicate, in addition to the group’s own experience in previous experimental protocols, that liver and even kidney changes were associated with the use of AIN-93 diet, according to reports from other authors, corroborating the findings of our investigation.

Regarding the handling of the formulation, an equally important concern, we recognize that the use of industrialized formulas offers greater standardization assurance of planned dietary treatments, but we take the opportunity to point out that besides the cautious control of AIN-93 diet preparation and storage (4°C for 15 days at maximum) in the study under discussion, similar liver abnormalities results were found in a recent study conducted by the group in which this diet was purchased ready to use from a specialized commercial laboratory, leading us to confirm consistent the data.

References


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