DIETS – CRITIC POINTS FOR *IN VIVO* TESTS, A COMPARATIVE ANALYSIS BETWEEN NATURAL AND PURIFIED DIETS

C. COMAN, E. VLASE

NIRDMI Cantacuzino, Splaiul Independenței No. 103, District 5, Bucharest, Romania
E-mail: enevlase@yahoo.com

Summary

Diets are an important variable for *in vivo* experiments, one which can now be controlled.

The natural diets which contain in composition vegetable and animal ingredients are most used. Because the ingredients sources are very different and the technologies for the production can differ from a supplier to another, the uniformity in the nutritive value is missing from a food batch to another. This lack of uniformity conducts to different results in the same studies.

Comparatively, the purified diets give to the researchers the benefit of having complete control over diet composition. The nutritionists can change the amount and type of any micro or macronutrient to researcher's specification. In addition, the animal in studies can be dosed daily via diet with pharmaceuticals or other compounds.

This review presents the major difference between this two types of laboratory animal diets and theirs possible interference with some type of studies.

Key words: diet, natural, purified, interference, experiments

The principle according with that the phenotype is the result of the interaction between genotype and medium is well known. If the genotype is invariable, the environmental factors (temperature, humidity, and food condition) are variables, variability that could be subtle sometimes, making that the previous results to not repeat. Consequently, the control of the environmental conditions is important for decreasing the variability of the results in animal experimentations. The research of the dieticians from 20 century has showed that the diet is a powerful environmental instrument, capable to induce variability to the research results making them hard repeatable and reproductively.

The natural diets, variability source for the *in vivo* testing results

The natural diets (fig. 1), which contain in composition vegetable (wheat, barley, oats, soya-bean, etc.) and animal (meat and fish flour) ingredients are most used. The natural diets are very appreciated by the animals, because they are very palatability, have a big availability on the market and are very cheap. Because of the big number of the ingredients used and the lack of the uniformity in the nutritive

value of each ingredient, the natural diets don't permit a very precisely and standard knowledge of the concentration of each nutrients principle. This thing produces a significant variation between consecutive food batches (9). This variation can be put in evidence by the absence of the repeatable results of tests done in similar conditions.

Fig.1. Natural diet

The fitoestrogen from soya-bean (constant ingredient in natural diets like protein source) is the most studied element from natural diets with big potential to induce variability in the results of *in vivo* test. Once ingested, the fitoestrogens are tied with the receptors for self estrogens and they produce a varied game of effects in skeletal development, in the development and function of male and female sexual glands, on central nervous system and on cardiovascular system that could interfere with a lot of studies and making that the result interpretations to be with no sense. The most important interference with experiments is presented below.

Interference with study on sexual development. The fitoestrogens because are involve in the development and function of male and female sexual glands, in acceleration of female puberty, in less development of male sexual glands, the decrease of testosterone (14,15) are very used in direct or indirect studies that involve sexual development.

Interference with study on tumor development. The isoflavones are fitoestrogens subclasses, which are present in soya-bean. The epidemiological studies effectuated on human populations that eat soya-bean show a less incidence in a lot of types of cancer. The experiments on laboratory rodents fed with diets that contain isoflavones have confirmed the epidemiological studies. The animals have less tumors and/or a delay in the development of mammary, hepatic, colon, prostate tumors (3,4,5,10). The results are sometimes contradictory, probably depending from a lot of factors like: quantity of isoflavones from diet, the duration of feeding, and the hormonal status of animals. With these elements well

known, the diet for animals in studies involving the tumor development is very important.

Interference with studies on induction of metabolic disorders. The hypertensive effect of NaCl excess from diet was diminish by the presence of ginesteine (fitoestrogens subclasses from soya-bean) to the rats involved in studies for the induction of arterial hypertension (8). The soya-bean isoflavones can reduce the cholesterol and serique triglyceride (2,6) and can prevent the development of hepatosteatosis. In studies for the obesity induction was observed that the rats fed with reach diets in fat but which use soya-bean like protein sources, the obtained body weight and the fat tissue were less comparatively with the rats fed with high rich fat diets but that use casein like protein source (13). The studies for the induction of diabetes can be compromised by the presence of soya-bean in diet by the reduction of insulin-resistance (7).

Interference with behavior studies. As estrogens receptors are presents even in brain is not a surprise that isoflvones from diets affect the behavior parameters. The effects on learning and memorizing processes, social interaction, anxiety, locomotors activities, pain and sensibility were reported in case of fitoestrogens excess (11).

With certainly can be sustained that there isn't a perfect diet in animal laboratory research, but at least, for making experiments that have a sense, we must try hard to eliminate the variability sources induced by the diets, especially the known sources, for obtaining biological responses repeatable and reproductively. To this aim the purified diets respond to. (fig. 2).

Fig.2. Purified diet

The purified diets

At the beginning of '70, the American Institute of Nutrition (AIN) recognized that is needed a standardization of laboratory rodents diets from the nutritional point of view but also a elimination of all nutritional factors that could interfere with

the studies, modifying the results. For this purpose was formed a committee, that conceived a diet made up from purified ingredients, named AIN-76A. The composition of AIN-76A diet is in table 1.

Table 1

AIN-76A diet

Ingredients	**Quantity** (gr.)	**Analytics parameters:**	
		Nutrient	Quantity %
Casein	200	Proteins	20.3%
DL – methionine	3	Carbohydrate	66%
Corn starch	150	Fat	5%
Corn oil	50	Other	8.7%
Sucrose	500		
Cellulose	50		
Mineral premix	35		
Vitamin premix	10		
Bitartrat choline	2		

In this diet the necessary of protein is assure by casein together with methionine added to satisfy her require in sulfur aminoacids. The carbohydrates are providing from the corn starch and sucrose, corn oil satisfies the fat requirement and pure cellulose satisfies the fiber requirement. Vitamins and minerals are added under the form of a specific mixture for rodents.

So, each nutrient is provided by a purified ingredient separately. It is true that, for example, casein contains vitamin traces and minerals in small quantities. Generally, this fact became important only then when the aim of the experiments is the induction of a deficiency in one of a vitamin or mineral present in casein. In this case, it can be used alcoholic extract from casein, to remove the fat and liposoluble vitamin traces or aminoacides mixtures to eliminate the traces of nutritive substance contained.

In 1993, because of the numerous nutritional and technique problems in connection with the diet on previous period (1970-1993), AIN-76A was revised (12). The two new derivates forms are the next:

AIN-93G that assures the necessary for growth, gestation and suckling to mice and rats (table 2).

AIN-93M that assures the necessary for maintaining to adult mice and rats (table 3).

Table 2

AIN-93G diet

Ingredients	**Quantity** (gr.)	**Analytics parameters:**	
		Nutrient	Quantity %
Casein	200	Proteins	20%
L – cysteine	3	Carbohydrate	64%
Corn starch	397.486	Fat	7%
Sucrose	100	Other	9%
Maltodextrina	132		
Cellulose	50		
Soya- bean oil	70		
t- Butilhidroqinona	0.014		
Mineral premix	35		
Vitamin premix	10		
Choline bitartrate	2.5		

Table 3

AIN-93M diet

Ingredients	**Quantity** (gr.)	**Analytics parameters:**	
		Nutrient	Quantity %
Casein	140	Proteins	14.2%
L – cysteine	1,8	Carbohydrate	73.1%
Corn starch	495.692	Fat	7%
Sucrose	100	Other	5.7%
Maltodextrina	125		
Cellulose	50		
Soya- bean oil	40		
t- Butylhydroquinone	0.008		
Mineral premix	35		
Vitamin premix	10		
Choline bitartrate	2.5		

Some major differences appeared in new diet AIN-93G comparatively with AIN-76A are:

- 7 gr. soya-bean oil/100 gr. food replace 5 gr. corn oil/100 gr. food to increase the quantity of linoleic acid;
- the corn starch replaced partially the sucrose
- the phosphorus quantity was reduced to eliminate the calcification problems on female rats kidneys.
- L-cysteine replaced DL – methionine like supplement in sulfur aminoacids
- the manganese concentration was reduced with 20% comparatively with old diets
- the vitamin E, K and B12 increased
- to the mineral mixture were added molybdenum, selenium, fluorine, nickel, bohrium, lithium and vanadium.

For maintaining the diet, AIM-93M, the fat quantity was reduced at 40 gr/ kg food from 70 gr/ kg food and casein quantity at 140 gr/ kg food from 200 gr/ kg food in AIM-93G. Because of a better equilibrium in essential nutritive elements, AIN-93 could prove to be a better choice than AIN-76A on short and long terms studies on laboratory rodents.

Conclusions

The natural diets represent a source of variability for the *in vivo* studies.

Te natural diets don't permit a very precisely and standard knowledge for the concentration of each nutrients principle.

The purified diets where the ingredients are refined permit to the researcher in nutrition to define more easily the nutritional requirement of animals.

From the selective removal or quantitative modification at one time for a nutritional factor from the diet is possible the precisely evaluation the effect done.

The possibilities of modification to a purified diet are practically unlimited, making from this a powerful research instruments.

References

1. **Ascencio, C., Torres, N., Isoard-Acosta, F., Gomez-Perez, F.J., Hernandez-Pando, R., Tovar, A.R.**, Soy affects serum insulin and hepatic SREBP-1 mRNA and reduces fatty liver in rats. Journal of Nutrition, 2004 134, 522-529.
2. **Bakhit, R.M., Klein, B.P., Essex-Sorlie, D., Ham, J.O., Erdman, J.W., Potter, S.M.**, Intake of 25 g of soybean protein with or without soybean fiber alters plasma lipids in men with elevated cholesterol concentrations, Journal of Nutrition, 1994, 124, 213-222.
3. **Barnes, S.**, Effects of genistein on in vitro and in vivo models of cancer. Journal of Nutrition, 1995, 125, 777S-783S.

4. **Barnes, S., Grubbs. C, Setchell, K.D.R., Carlson, J.,** Soybeans inhibit mammary tumors in models of breast cancer. Prog. Clinical Biology Research, 1990, 347, 239-253.
5. **Barnes, S., Peterson, T.G., Grubbs, C., Setchell, K.D.R.,** Potential role of dietary isoflavones in the prevention of cancer. Advances in Experimental Medicine and Biology, 1994, 354, 135-147.
6. **Carroll, K.K., Kurowska E.M.**, Soy consumption and cholesterol reduction: review of animal and human studies. Journal of Nutrition, 1995, 125, 594S-597S.
7. **Cederroth, C.R., Vinciguerra, M., Gjinovci, A., Kuhne, F., Klein, M., Cederroth, M., Caille, D., Suter, M., Newmann, D., James, R.W., Doerge, D.R., Wallimann, T., Meda, P., Foti, M., Rohner-Jeanrenaud, F., Vassalli, J., Nef, S.**, Dietary phytoestrogens activate AMP-activated protein kinase with improvement in lipid and glucose metabolism.Diabetes, 2008, 57:1176-1185.
8. **Cho, T.M., Peng, N., Clark, J.T., Novak, L., Roysommuti, S., Prasain, J., Wyss, J.M.**, Genistein attenuates the hypertensive effects of dietary NaCL in hypertensive male, rats.Endrocrinology, 2007, 148(11), 5396-5402.
9. **Coman, C., Savi, G., Cotor, G**., Şoarecele, animal de laborator, Alt Press Tour, Bucharest, 2008.
10. **Fitzsimmons, J.T.R., Orson, N.V., El-Aaaser**, Effects of soybeans and ascorbic acid on experimental carcinogenesis. Comparative Biochemistry and Physiology, 1989, 93A, 285-290.
11. **Lephart, E.D., Setchell, K.D., Handa, R.J., Lund, T.D.**, Behavioral effects of endocrine-disrupting substances: phytoestrogens, ILAR Journal, 2004, 45, 443-454.
12. **Reeves, P.G., Nielsen, F.H., Fahey, G.C.Jr**., AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet, J. Nutr. 1993, 123(11):1939-51.
13. **Torre-Villalvazo, I., Tovar, A.R., Ramos-Barragan, V.E., Cerbon-Cervantes, M.A, Torres, N**., Soy protein ameliorates metabolic abnormalities in liver and adipose tissue of rats fed a high fat diet. Journal of Nutrition, 2008, 138, 462-468.
14. **Weber, K.S., Setchell, K.D.R., Stocco, D.M., Lephart, E.D.**, Dietary soy-phytoestrogens decrease testosterone levels and prostate weight without altering LH, prostate 5 alpha-reductase or testicular steroidogenic acute regulatory peptide levels in adult male Sprague-Dawley rats. Journal of Endocrinology, 2001, 170, 591-599.
15. **Wisniewski, A.B., Klein, S.L., Lakshmanan, Y., Gearhart, J.P**., Exposure to genistein during gestation and lactation demasculinizes the reproductive system in rats, Journal-of-Urology, 2003, 169, 1582-1586.