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FORMULATION, PREPARATION AND CHEMICAL ANALYSIS OF PURIFIED DIETS FOR LABORATORY MICE AND RATS

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Abstract

The aim of this study was the preparation and determination of the main chemical parameters for 5 purified diets for laboratory mice and rats: 2 diet for maintaining and growth/breeding animal colonies and 3 diets for inducing metabolic disorders (atherosclerosis, diabetes type II and obesity). Diet recipes for maintenance and growth are the classic recipes and diets that induce metabolic syndromes attempts to replicate human food behaviour with unidirectional nutrition (excess cholesterol, excess fructose and excess fat). For all chemical parameters were established limits values necessary to achieve the aims pursued by manufacturing these diets. Diets were prepared in our laboratory. To all diets were made the following measurements for determining the gross chemical composition: protein, fat, fibres, ash, dry matter and cholesterol for atherosclerosis induced diet. It was also calculated gross energy. For comparison purposes, similar diets were purchased from a specialized company, diets that were analysed for the same chemical parameters in the same specialized laboratory. The results showed that the values of the analysed parameters were within the limits set by recipes and compared to acquired diets the values of most parameters are close to having a coefficient of variation lower than 10. The results allow the transition to the next phase of that study, respectively the administration of purified diets in mice and rats and in achieving the induction of metabolic syndromes.

Key words*: purified diets, metabolic syndromes, gross chemical composition.*

INTRODUCTION

Research of mechanisms for different morbid entities based on experimental models take increasingly higher in this research field. There are searched and established experimental models for understanding and treat some metabolic diseases, oncology diseases, toxicology, etc. The study of mechanisms of generation and development of pathologic entities such as diabetes or atherosclerosis concern broad categories of researchers from nutritionists to physiologists, pathologists, biochemists, etc.

At the same time, the expenses that mankind are assumed to understanding, preventing and combating these entities are very high. Along with human medicine, the veterinary one is increasingly involved in the study of these pathological entities providing appropriate experimental biological material more increasingly demanding by specialists, along with important contributions to the knowledge of their development mechanisms.

In procedures applied to animals, natural diets are the most used. They contain plant and animal various origin ingredients, have high palatability, are appreciated by animals, have high availability in the commercial market and are very cheap. Nevertheless due to the number of ingredients, different producers for each of these ingredients and higher variability in terms of nutritional value of each ingredient, successive batches of natural diets are very different regarding main chemical parameters (Savenije et al., 2010).

Studies on the induction of metabolic syndromes may not have the expected results because of the type of administered diet. Thus, the hypertensive effect of excessive NaCl dietary was diminished by the presence of genistein (soy phytoestrogens subclass) in rat diet trying to induce hypertension (Cho et al., 2007). Soy isoflavones have been shown also that can reduce serum cholesterol and triglycerides (Bakhit et al., 1994; Carroll et al. 1996) and can prevent the development of hepatosteatosis (Ascencio et al., 2004).

In studies on inducing obesity was observed that in rats fed with fat rich diets, but using soy as a protein source, the weight gained is less and body fat less than in rats fed with diets high

in fat but using casein as a source of protein (Torre-Villalvazo et al., 2008). Studies on the induction of diabetes may be compromised by the presence of soybeans in the diet by reducing the insulin resistance (Cederroth et al., 2008).

Purified diets have occurred in the early 1970s when the American Institute of Nutrition (AIN) created a committee that devised a diet consisting of purified ingredients (AIN-76A). The Committee acknowledged the need for a standardization of diets for laboratory mice and rats from a nutritional standpoint, but also eliminate all nutritional factors that can interfere with the study changing results.

Since then, AIN has changed diets receipts, currently standard purified diets for colonies of mice and rats being AIN-93 M and AIN-93 G (Reeves et al., 1993). Based on these diets were developed a variety of purified diets used for multiple experimental purposes. Because these ingredients are refined, (unlike cereals included in the composition of diets natural) diets purified allow researchers nutritionists to be able to define better the nutritional requirements of animals, but also through selective elimination or quantitative change at some point of a nutritional factor in diet can accurately assess the effect.

This means that there are virtually unlimited possibilities for modifications that can be made from a purified diet, making it a powerful research tool.

Purified diets to induce metabolic syndromes were manufactured in different forms with different amounts of added cholesterol for atherosclerosis induction (Xiangdong Li et al., 2011), fructose for diabetes induction (King et al. 2012) and fat for obesity induction (Buettner et al., 2007; Slavin et al., 2010).

Cantacuzino Institute (IC) is a manufacturer of laboratory animals and natural diets for laboratory animals, but Romanian research system requests also purified diets.

The long acquisition time, short validity time, long experiments and inconsistency of time between acquisition diets and animals purchase at optimal age and weight led to the idea of this project that aimed to standardizing purified diets manufactured at Cantacuzino Institute, induction of metabolic syndrome in rats and mice and standardizing animal models.

In this paper we describe the formulation, preparation of purified diets, their analysis and comparison with standard purified diets.

MATERIALS AND METHODS

Receipts

The purified diets are diets wherein each nutrient is provided by one or more of purified ingredients. In these diets, protein requirements are provided by casein with added methionine (to meet the sulphur amino acids). Carbohydrates are provided by fructose corn starch and soybean oil satisfies the necessary fat and pure cellulose supply fibres needs. Vitamins and minerals ingredients are added in the form of specific mixtures for mouse and rats.

Recipes were calculated using EXCEL (Professional Edition, Microsoft) based on analytical components of each ingredient, component declared by each manufacturer.

The recipes for the maintenance and growth of colonies of animals are similar to AIN - 93 M (AIN - 93M. IC) and AIN - 93 G (AIN - 93 G. IC).

Other recipes diets for induction of atherosclerosis (IC – Ath), diabetes type II (IC – Db) and obesity (IC - Ob), have been established based on information from the specialized literature (Nishina et al., 1990; Getz et al. 2005; Buettner et al., 2006; Rossmeisl et al., 2003).

Where the certificate was not specified ingredients required parameter, it was placed ideal value.

The ingredients for recipes were: casein (Ca caseinate), L-Cystina, corn starch, maltodextrin, soybean oil, sucrose (sugar), fructose, cholesterol, cholic acid, cellulose (Arbocel), lard, antioxidant, anhydrous milkfat, vitamin and mineral premix, methionine, calcium carbonate, calcium phosphate.

All the ingredients for purified diets were purchased by company Nutristar Romania from the following manufacturers: CCPA France, JRS Germany, Dyets USA and Corman Belgium.

Estimated values of the main chemical parameters for each of the recipes are highlighted in Table 1.

Table1. Estimated values of the main chemical parameters (in percent)

Parameter	AIN-93 G. IC	AIN-93 M. IC	IC - Ath	IC - Db	IC - Ob
Protein	17 - 20	11 - 14	16 - 19	15 - 20	20 - 25
Fat	5-8	3-5	18-22	3-6	33-35
Fibres	3-6	3-6	3-6	3-6	7-10
Dry substance	87 – 90	87 – 90	93 – 96	90 – 95	92 – 95

Preparation

Preparation process was at Cantacuzino Institute, Baneasa Station. Cantacuzino Institute is a producer of natural diets for laboratory animals, manufacturing process being carried out in a factory with a capacity of 1000 kg / hour.

Because of the small amount of purified diets needed for this study, they were manufactured in the laboratory.

For all recipes, the ingredients of purified diets were weighed separately for each recipe and placed in a dish. The solid ingredients (fat, cholesterol and butter) were melted in a bain-marie and added in the same dish.

After all ingredients were added, they were homogenized for 45 minutes and made into dough by the addition of bi-distilled water in a ratio of 10%.

Then the slurry was granulated in a granulator GRAMILL GRM30 through a sieve of 8 mm. All of the recipes were dehydrated and dried by keeping for 3 days at room thermostat in a temperature of 37^0 C. The diets were then stored in a refrigerator at 2-8^0 C to minimize oxidation.

Compared to manufacturing of natural diets, purified diets paste was made by adding water but do not by steaming as natural diets.

While the dehydration of natural diets is mechanically natural, purified diets was carried out by maintaining at thermostat. The storage is also different, natural diet was stored at room temperature, and purified diets at the refrigerator.

Chemical analyses

Chemical analyses were conducted at the Institute for Biology and Animal Nutrition Baloteşti, in the laboratory of chemistry and physiology of nutrition.

As a positive control, purified diets were purchased from the company Envigo Teklad Diets Italy: TD.94045 AIN 93-G; TD.94048 AIN-93 M; Adjusted TD.06414 Calories Diet (60 / Fat); TD.02028 Atherogenic Rodent Diet and TD.89247 60% fructose Diet.

For all the 10 diets were taken sample and counter sample which were sent to the laboratory.

In the chemistry laboratory were conducted chemical analyses following ISO standards and according to procedures recommended by FAO (FAO, 2011) (Table 2).

Table 2. Analysis method for chemical determination

Determination	Analysis Method
Dry matter 103^o C	Gravimetric method Regulation (EC) no. 152/2009 SR ISO 6496: 2001
Crude protein	Mineralization in the block and steam distillation method Regulation (EC) no. 152/2009 SR EN ISO 5983-2: 2009 / AOAC 2001.11
Crude fat	Extraction method with organic solvent Regulation (EC) no. 152/2009 SR ISO 6492: 2001
Crude fibres	Method with intermediate filtration Regulation (EC) no. 152/2009 SR EN ISO 6865: 2002
Crude ash	Gravimetric method Regulation (EC) no. 152/2009 SR EN ISO 2171:2010
Cholesterol (only for atherogenic diet)	Gas Chromatographic method ISO 12228:1999 / AOAC 994.10
Gross energy	Calculation

Statistics

To assess the comparative results of the chemical analyses at purified diets produced by Cantacuzino Institute and at purchased diets were calculated average, standard deviation and coefficient of variation using EXCEL software (Professional Edition, Microsoft) and GraphPad software (GraphPad 2016).

RESULTS AND DISCUSSIONS

Table 3 and 4 shows the results of gross chemical analysis compared with estimated values by calculation (in percent).

Table 3. The results of gross chemical analysis for AIN recipes (in percent)

Parameter	AIN-93 G. IC		AIN-93 M. IC	
	estimated	obtained	estimated	obtained
Protein	17 - 20	17.47	11 - 14	12.84
Fat	5-8	5.41	3-5	3.53
Fibres	3-6	3.24	3-6	3.64
Dry matter	87– 90	87.37	87– 90	89.58

Table 4. The results of gross chemical analysis for purified diets for metabolic syndrome induction

Parameter	IC - Ath		IC -Db		IC - Ob	
	estimated	obtained	estimated	obtained	estimated	obtained
Protein	16- 19	17.99	15- 20	18.45	20- 25	24.94
Fat	18-22	21.49	3-6	4.55	33-35	33.58
Fibres	3-6	4.14	3-6	5.69	7-10	9.69
Dry matter	93–96	95.9	90–95	93.77	92–95	96.52

A comparison between diets manufactured at Cantacuzino Institute and the standardized diets purchased from Envigo Teklad Diets shows close values, with one exception (Table5).

Table 5. The results of gross chemical analysis for produced and acquired purified diets

Purified diets	Parameter	Envigo Teklad	IC
growth/breeding	Crude protein	17.98	17.47
	Crude fat	6.07	5.41
	Crude fibres	1.92	3.24
	Crude ash	2.55	4.81
	Dry matter	90.12	87.37
	Gross energy	4217	3976
maintaining	Crude protein	13.12	12.84
	Crude fat	3.55	3.53
	Crude fibres	3.14	3.64
	Crude ash	2.71	4.3
	Dry matter	89.31	89.58
	Gross energy	3871	3825
Diets for atherosclerosis induction	Crude protein	17.46	17.99
	Crude fat	20.73	21.49
	Crude fibres	3.78	4.14
	Crude ash	2.86	4.97
	Dry matter	93.32	95.9
	Gross energy	5130	5070
	Cholesterol	0.6567	0.5731
Diets for diabetes type II induction	Crude protein	17.51	18.45
	Crude fat	0.43	4.55
	Crude fibres	5.49	5.69
	Crude ash	3.69	4.52
	Dry matter	87.86	93.77
	Gross energy	3787	4234
Diets for obesity induction	Crude protein	22.12	24.94
	Crude fat	29.07	33.58
	Crude fibres	8.42	9.69
	Crude ash	3.42	5.42
	Dry matter	87.86	96.52
	Gross energy	5425	6000

Statistical analysis in comparison of the two types of diets is presented in Table 6.

Table 6. Statistical results of comparison for produced and acquired purified diets

Purified diets	Parameter	Average	SD	CV
growth/ breeding	Crude protein	17.72	0.36	2.03
	Crude fat	5.74	0.46	8.13
	Crude fibres	2.58	0.93	36.17
	Crude ash	3.68	1.59	43.42
	Dry matter	88.74	1.94	2.19
	Gross energy	4096	170.41	4.15
Maintaining	Crude protein	12.98	0.19	1.52
	Crude fat	3.54	0.01	0.39
	Crude fibres	3.39	0.35	10.42
	Crude ash	3.50	1.12	32.07
	Dry matter	89.44	0.19	0.21
	Gross energy	3848	32.52	0.84
Diets for atherosclerosis induction	Crude protein	17.72	0.37	2.11
	Crude fat	21.11	0.53	2.54
	Crude fibres	3.96	0.25	6.42
	Crude ash	3.91	1.49	38.10
	Dry matter	94.61	1.82	1.92
	Gross energy	5100	42.42	0.83
	Cholesterol	0.61	0.05	9.61
Diets for diabetes type II induction	Crude protein	17.98	0.66	3.69
	Crude fat	2.49	2.91	116.99
	Crude fibres	5.59	0.14	2.52
	Crude ash	4.105	0.58	14.29
	Dry matter	90.81	4.17	4.60
	Gross energy	4010	316.07	7.88
Diets for obesity induction	Crude protein	23.53	1.99	8.47
	Crude fat	31.32	3.18	10.18
	Crude fibres	9.05	0.89	9.91
	Crude ash	4.42	1.41	31.99
	Dry matter	92.19	6.12	6.64
	Gross energy	5712	406.58	7.117

Purified diets are an important research tool for inducing several diseases, including metabolic syndromes that are most important. But making these diets and storage them poses some logistical problems what makes between finance, planning and execution of the procedure to pass a longer period of time. Therefore we felt that having experience of over 20 years in manufacturing natural diets and laboratory animals nutrition we can produce also purified diets.

Production of purified diets in laboratory conditions for small quantities not encountered problems; the technology is similar to that for manufacturing natural diets.

For purified diet formulas have been established and were calculated some values for crude chemical composition (protein, fat, cellulose and dry matter). These values were determined based on the values chemical

parameters diets to induce the desired metabolic syndrome (atherosclerosis, diabetes type II and obesity) and reproduce standard diets for maintaining and growth/ breeding colony of mice and rats. In calculating parameters was of special importance for the parameter values of each ingredient used in recipes diets, according to the analysis report.

The results obtained from chemical analyses have shown that all values obtained were within the limits anticipated, which confirms that when we know the nutritional values of each ingredient we can correctly predict the chemical composition of a diet.

Although purchased purified diets were accompanied by quality certificates and test reports, we found it necessary to do analyses of chemical composition because there are differences between laboratories, differences in method, apparatus, calculation, differences which can lead to variability in results (Bielohuby et al., 2010; Hristov et al., 2010). Analysed the diets, except for a few parameters, the variation in chemical composition values at the 5 diets of the two producers was low.

Standard deviation highlights the uniformity of data around the mean value and except for one parameter (gross energy), expected result because of the various ingredients used to produce diets. The coefficient of variation allows the comparison of statistical series in terms of standard deviation. It found a high coefficient of variation (CV = 14.29 - 43.42%) of all diets values of crude ash.

How values were higher in own diets than those purchased and reflects the total mineral raw ashes of a diet, we believe that the differences are due to vitamin and mineral premix used and its composition. A coefficient of variation was large and consisted of crude fibre on growth/breeding diet (CV = 36.17%), the percentage being higher on crude fibre to produced diet.

A paradoxical result was recorded of crude fat parameter for diet can induce type II diabetes. The coefficient of variation was 116.99, due to low percentage value of fat in the diet purchased. Although the analysis was repeated, because it was far from optimal and from received analysis reports, the result was maintained.

The gross chemical composition is different for the same feed, from one country to another, from one area to another, depending on the starting materials, technology of obtaining, the way of conservation. This makes the average data available in the literature to be insufficient to assess the nutritional value of a diet, which is why it were conducted extensive chemical gross tests for diets manufactured to be used in other experiments.

The results taken as a whole show similarity between diets produced and diets acquired. The values of chemical composition within the range expected, indicates that diets have been manufactured correctly and could go to the next stage of study, respectively the induction of metabolic syndromes by administering these diets. In the same time will continue to analyses for produced diets other nutritional parameters to determine sugars, amino acids, micro and macronutrients, vitamins.

CONCLUSIONS

Purified diets are a powerful research tool for studying induced metabolism syndromes.

The recipes of purified diets that were produced at Cantacuzino Institute were calculated based on the values of nutrition that should induce metabolic syndrome (atherosclerosis, diabetes type II and obesity), setting margins of values.

Results of gross chemical composition analysis showed that the values obtained were within the margins established.

Comparative analysis of own diets and similar standardized purchased diets showed that unless crude ash value all other values are superimposable.

The results allow us to move on to the next phase of that study by administering diets to induce metabolic syndromes.

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