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Types of sterilization in feed containing different lipidic sources for golden hamster (*Mesocricetus auratus*)

Tipos de esterilização em rações com diferentes fontes lipídicas para hamster golden (*Mesocricetus auratus*)

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Abstract
The Golden hamster has been gaining significance as a new experimental biomodel, finding use as a reliable diagnostic tool in biomedical research and for zoonosis. Authentic data in terms of digestibility, interactions among raw materials and essential nutrients, besides the influence exerted by various sterilization processes on animal behavior remain unclear. We aimed to assess the influence of sterilization, via autoclaving and irradiation, of pellet feeds prepared using salmon or linseed oil on the digestibility and plasma biochemical parameters in Golden hamsters. Randomized evaluations were conducted on 36 adult male Golden hamsters (*Mesocricetus auratus*), distributed in six treatments and six replications, namely: common salmon oil; radiated salmon; autoclaved salmon; common linseed oil; radiated linseed and autoclaved linseed. A remarkable effect of the sterilization was evident on the digestibility and protein solubility of the feed, which was lower for autoclaved diets. There was also a significant effect on blood parameters. Animals fed diets containing linseed oil showed lower blood glucose compared to the others. Thus, the inference reached was that while salmon and linseed oil can be used in laboratory hamster feeds, autoclaving disturbs the nutritional quality of the rations.
Key words: animal facilities; essential fatty acid, irradiation; laboratory animal; nutrition.

Resumo
O hamster Golden é um importante biomodelo utilizado na pesquisa biomédica e como meio de diagnóstico para zoonoses. Informações de qualidade, digestibilidade, interações das matérias primas e exigências nutricionais, assim como os efeitos decorrentes dos diferentes processos de esterilização sobre o desempenho animal ainda são desconhecidos. Objetivou-se com esse estudo avaliar os efeitos da esterilização por autoclavagem e irradiação de rações peletizadas, formuladas com óleo de salmão ou linhaça, sobre a digestibilidade e parâmetros bioquímicos

plasmáticos de hamsters Golden. Foram avaliados 36 hamsters Golden (*Mesocricetus auratus*), machos adultos distribuídos em delineamento experimental inteiramente casualizado com seis tratamentos e seis repetições, a saber: óleo de salmão comum; salmão irradiada; salmão autoclavada; óleo de linhaça comum; linhaça irradiada e linhaça autoclavada. Houve efeito significativo da esterilização sobre a digestibilidade e solubilidade proteica da ração, que foi pior para as rações autoclavadas. Também houve efeito significativo sobre os parâmetros sanguíneos. Animais alimentados com rações contendo óleo de linhaça apresentaram glicemia mais baixa em comparação aos demais. Conclui-se que o óleo de salmão e linhaça pode ser utilizado em rações para hamsters de laboratório, porém a autoclavação interfere na qualidade nutricional das dietas.
Palavras-chave: ácido graxo essencial; biomodelo; biotério; irradiação; nutrição.

Introduction

The Golden hamster (*Mesocricetus auratus,* Rodentia, Cricetidea) is a noteworthy biomodel and an essential tool in biomedical and veterinary research as well as a diagnostic medium, for instance, for Leishmaniasis. Although several scientific applications are present, only a few studies are available that focus specifically on discovering the zootechnical and ethological traits of these hamsters.

Normally, hamsters are subjected to similar management methods and techniques as those implemented for other animal biomodels. The method is the same in terms of the nutrition supplied, as no data is available regarding the specific nutritional needs of hamsters or feeding guides, or studies focusing on preference for shapes and palatability of the feed offered. The only published reference that is available for laboratory animals is obsolete and the information is limited to the needs of only mice and rats[1].

Although there are commercial extruded products, laboratory rodents are most commonly given food in pellet form. The rations for laboratory rodents are prepared specially to satisfy the nutritional and sanitary needs suggested by veterinarians, which in some instances require sterilization protocols.

Autoclaving, gamma or electron beam irradiation are the main sterilization processes adopted[2]. Both processes involve a decrease in the nutrient bioavailability, including the most essential ones, such as lysine (an amino acid) and few primarily liposoluble vitamins. Sterilization also lowers digestibility levels[3]. However, based on many factors like stability, sensitivity and length of exposure to physical agents, the percentage of losses of the numerous nutrients showed variations[4].

Digestibility tests are performed to evaluate a variety of foods or raw materials in terms of the nutrient availability. A significant indicator of quality is digestibility, which is expressed as an improvement in animal activities and cost-effectiveness of the production system.

The sterilization processes cause nutritional losses, which can exert a negative effect on the behavior of laboratory animals, particularly in terms of their reproductive efficiency. To overcome this issue, animals are often fed supplements with nutritional complexes in water or offered oil seeds such as peanuts, flaxseed or sunflower. To maintain balanced animal nutrition, the required fatty acids like linolenic acid (omega-6), linoleic acid (omega-3) and arachidonic acid are provided. Linolenic acid is naturally and abundantly available in corn and soybean oils, while linoleic acid occurs in green leafy vegetables, linseed oil and marine fish oils from tuna and salmon[5].

When the rations are formulated by enriching them with essential fatty acids they can reduce the anti-nutritional influence of the processes of physical sterilization on laboratory rodents growth and reproduction. However, before incorporating the different oil sources into commercial and experimental diets, they should first be accurately determined to ensure that animal performance remains at its maximum level. This study aimed at assessing the plasma biochemical parameters to ascertain the coefficients of apparent digestibility of the commercial diets prepared using a variety of oils and sterilization via the autoclaving and gamma irradiation processes.

Materials and methods

The experiment which involved 36 Golden hamsters (*Mesocricetus auratus*) was performed at the NBA 2 Central Animal Facilities of the Universidade Norte Fluminense, situated in the municipality of Campos dos Goytacazes-RJ, Brazil. The protocols were approved by the Ethics Committee for Animal Use (ECAU / UENF) - License No. 326/2016.

To evaluate the inclusion of essential fatty acids, two sources of oil, salmon and linseed were added to the pelleted feed in the versions: common, autoclavable and irradiated. Preparation and processing were done using the same raw materials to maintain the isonomy of the formulations. The steps of proximate bromatological analyses, as well as fatty acid and vitamin profiling (A, D, E, B2, B3 and B6) were conducted on the experimental rations, pelleted and post sterilization by irradiation and autoclaving. An analysis of the raw materials and rations produced in Quimtia Brazil's bromatology laboratory was then done. An analysis of the fatty acid profile of the salmon and linseed oils was performed in the Laboratory of Oils and Fats of the Faculty of Food Engineering of Universidade Estadual de Campinas- UNICAMP and the vitamin levels were analyzed by CBO Laboratories of Analyzes Ltda.,Valinhos-SP, Brazil, as shown in Table 1.

A completely randomized experimental design was adopted in a scheme involving subdivided plots (two oil sources x three sterilization modes) and included six treatments and six replicates using one animal each. The feed tested were formulated according to nutritional requirements of laboratory animals(1) and allocated among the experimental groups as mentioned: Common salmon oil (CSO); Irradiated salmon (RS); Autoclaved salmon (AS); Common Linseed oil (CLO); Irradiated linseed (RL); and Autoclaved linseed (AL).

Table 1. Bromatological composition analyzed of rations for laboratory rodents with different oil sources and sterilization method

Nutrients	Lipidic Sources					
	Salmon			Linseed		
	Common	Irradiated	Autoclaved	Common	Irradiated	Autoclaved
Dry Matter %	88.0	88.0	88.0	88.0	88.0	88.0
Crude Protein %	22.5	22.5	22.5	22.5	22.5	22.5
Ethereal Ether Extract %	7.0	7.0	7.0	7.0	7.0	7.0
Crude Fiber %	7.0	7.0	7.0	7.0	7.0	7.0
Mineral Matter %	9.0	9.0	9.0	9.0	9.0	9.0
Crude Energy Kcal/Kg	4,016	4,016	4,016	4,016	4,016	4,016
Lysine %	1.4	1.4	1.4	1.4	1.4	1.4
Methionine %	0.5	0.5	0.5	0.5	0.5	0.5
Calcium %	1.4	1.4	1.4	1.4	1.4	1.4
Phosphorus %	0.8	0.8	0.8	0.8	0.8	0.8
Linoleic Acid (ω-6) %	1.36	1.36	1.36	1.81	1.81	1.81
Linolenic Acid (ω-3) %	1.5	1.5	1.5	1.5	1.5	1.5
Vit. A UI/Kg	11520	11520	25500	11520	11520	25500
Vit. D3 UI/Kg	2304	2304	2100	2304	2304	2100
Vit. E UI/Kg	28.8	28.8	60.0	28.8	28.8	60.0
Riboflavin (B2) mg/Kg	5.76	5.76	10.93	5.76	5.76	10.93
Niacin (B3) mg/Kg	57.67	57.67	60.11	57.67	57.67	60.11
Pyridoxine (B6) mg/Kg	6.65	6.65	12.25	6.65	6.65	12.25

Values analyzed by Quimtia Brazil's bromatology laboratory. Composition of the mineral and vitamin supplement * (mg / kg): minerals - Na 2700; Co 1.50; Cu 10.0; Fe 50.0; Mn 60.0; If 0.05; Zn 60.0. Vitamins (Vit) - K3; thiamine (B1); riboflavin (B12); folic acid; pantothenic acid; biotin and choline. * The composition of the vitamin supplement varied according to each vitamin.

Utilizing a double-port autoclave with a Red 500 L capacity, sterilization of the autoclavable rations was done for 60 minutes cicle and 121 ºC each cicle. Gamma irradiation at a dose of 10 kGy was conducted by CBE Sterigenics®, Cotia, SP. Once the weighing and standardization of the lots were accomplished, all rodents were distributed randomly in their experimental units. There they were left under conditions of fasting for six hours, to ensure complete gastrointestinal emptying. Subsequently, they were supplied with the test rations together with green ferric oxide, which acted as the initial intestinal transit marker. The rate of the passage of the feed in hours was computed from the time of the feed consumption to the time of fecal excretion.

All animals were maintained under a photoperiod of 12/12 hours of light and dark. They had access tresto water at will and feed at the rate of 100 g / day. They were left for four

days to adapt and then for a further seven days. The feed consumed and total daily fecal matter excreted were then collected and measured, to facilitate measurement of the coefficient of apparent digestibility. Every day, fecal excreta and remaining feed were weighed, stored in labeled pots and finally frozen. At the completion of the collection period, the total feed intake and total fecal excretion were determined. The apparent digestibility coefficient (ADC%) was assessed employing the following formula[6]:

$$ADC\ (\%) = \{[ingested\ mass\ (g) - Excreted\ mass\ (g)] / Ingested\ mass\ (g)\} \times 100$$

When the digestibility assay was done, the rodents were anesthetized using 10% isofluorane and whole blood was drawn for the biochemical analyses via cardiac puncture. After conditioning the samples in the BD Vacutainer 2.0 mL EDTA tubes, they were transferred to the Laboratory of Clinical Analysis at the UENF Veterinary Hospital. Analyses were done using the Labtest kits employing the Labtest Labmax Plenno equipment for the following parameters: glucose (GLU), triacylglycerol (TRI), total cholesterol (CHOL), protein (PTN), albumin (ALB), alanine aminotransferase (ALA), aspartate aminotransferase (ASP), creatine (CRE), uric acid (UC), alkaline phosphatase (AP), gamma-glutamyl transpeptidase (GGT) and urea (URE).

Post exsanguination, all rodents were euthanized, adopting the method of inhalation of continuous CO_2 flow in a hermetic chamber, based on the recommendations in the CONCEA Guidelines on Euthanasia Practice (2015).

The “mean feed intake” and “final live weight” variables were estimated and statistical analysis were performed employing mixed models following the PROC MIXED procedure of the Statistical Analysis System (SAS System, Inc., Cary, NC, USA) software. Tukey’s test was applied when significant differences were noted. We used the Shapiro wilk test of normality for the variables “passage rate”, “apparent digestibility coefficient” and “feed intake in body weight relation (%)”, it were rejected to normal distribution, after that we evaluated three other distribution gamma, lognormal and exponential distributions. The best distribution was the lognormal and was selected using of the corrected akaike information criterion[7]. This is the output data from the referred procedure adopting the GLIMMIX procedure of the Statistical Analysis System (SAS System, Inc., Cary, NC, USA) software. In the event of any significant difference, the Tukey test was applied.

Results and discussion

Statistically significant differences were noted among the apparent digestibility coefficients of the rations ($P < 0.05$). However, no significant effect was observed for the other performance variables, as evident from Table 2.

Autoclaving caused a marked drop in the apparent digestibility coefficient of the feed and decreased solubility of the KOH protein in the rations (Figure 1). Prior irradiation induced no changes in the dietary protein quality, suggesting that this method is effective for the preservation of the nutritional profile of the feed for laboratory animals. These findings may be justified according to the results described by Ford[8] who determined the influence exerted by the different times and temperatures of autoclaving and

irradiation processes on protein digestibility in the feed rations for rats. Sterilization at 121 °C for 60 minutes or 134 °C for three minutes was observed to induce a decrease in the digestibility and biological value of the protein and many of the essential amino acids. They also reported that irradiation exerts no effect on the nutritional value of the rations, and is the reason for the sterilization method to be preferred for laboratory rodent feed.

Table 2. Mean data and standard deviations of the digestibility assay and feed protein quality according to the type of sterilization and oil sources in the Golden hamsters rations

Ration/ Oil	Live Weigth (g)		Feed consumption (g/a/day)	FC/LW (%)	Rate Passage (hours)	Fecal output (g/a/dia)	ADC (%)	CP (%)	
	Initial	Final						Before	After
CSO	142.9	142.3 ±7.8	11.08 ±1.2	7.8 ±7.7	03:31:30 ± 29:33	6.0 ±0.8	57.4a ±4.5	23.10	--
RS	149.9	148.8 ±7.3	11.64 ±2.0	7.8 ±8.0	03:35:00 ± 29:07	5.2 ±0.6	55.6a ±5.3	22.20	24,10
AS	148.4	149.1 ±8.9	11.81 ±0.8	7.9 ±7.6	03:29:00 ± 40:46	4.9 ±0.4	45.0b ±4.2	22.90	22,20
CLO	157.1	153.9 ±7.6	11.62 ±1.6	7.6 ±7.9	03:30:40 ± 27:48	6.1 ±0.4	51.9ab ±3.9	23.60	--
RL	148.0	154.1 ±9.6	11.85 ±0.8	7.7 ±7.6	03:34:10 ± 32:23	6.0 ±0.7	50.7ab ±3.7	22.00	23,60
AL	145.6	147.3 ±9.9	11.38 ±1.6	7.7 ±7.7	03:21:30 ± 35:36	6.1 ±0.4	45.1b ±3.9	23.90	23,30
P value	-	0.4188	0.8934	0.9889	0.7072	0.8751	0.0001*		

FC/LW : Feed consumption in relation to live weight; Apparent digestibility coefficient (ADC); CP: Crude protein in ration; Sol. KOH: solubility of protein in potassium hydroxide, before and after autoclaving process; (CSO) Common salmon oil; (RS) Radiated salmon; (AS) Autoclaved salmon; (CLO) Common Linseed oil ; (RL) Radiated linseed and (AL) Autoclaved linseed.* Significant effect, by the Tukey test ($P < 0,05$).

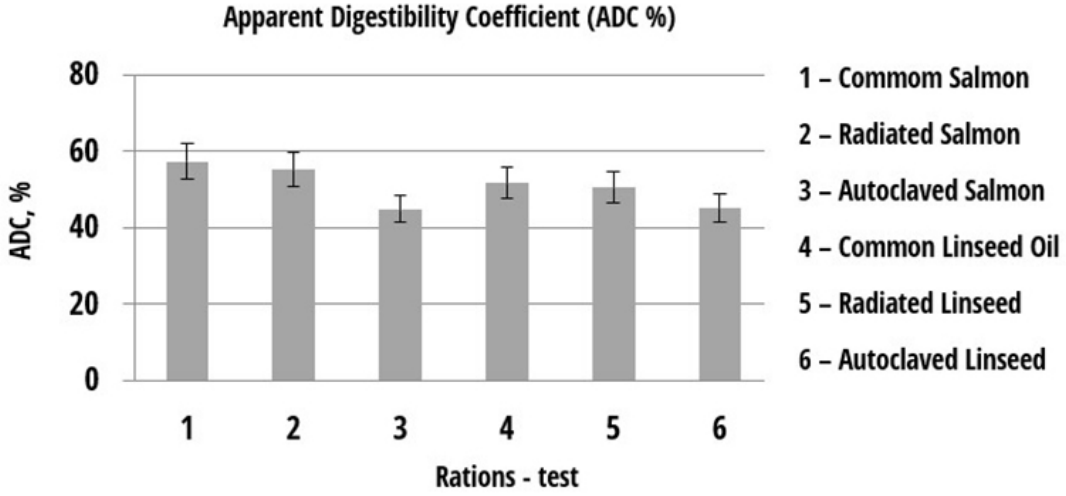

Figure 1. Apparent digestibility coefficient of diets with different sources of oil and sterilization methods for Golden hamsters.

In terms of the oil source utilized, the reference feed revealed a significantly reduced apparent digestibility coefficient (ADC) than did salmon oil or the linseed ration. The nonautoclaved diets presented ADC values equivalent to that of the autoclaved rations, even without being subjected to this sterilization process. Drackley[9] reported that the oil content and fatty acid profile exerted a direct effect on diet digestibility, with a proportional decrease in the ADC in response to the rise in long chain lipids.

These results corroborated with the findings of Faria and Stabille[3] who reported a linear decline in the solubility of protein in KOH, as well as in the digestibility coefficient of commercial rations that had been subjected to varying autoclaving times. According to the authors, the autoclaved rations in 60-minute cycles reveal nutritional losses that are the cause for poor performance efficiency in Wistar rats.

Feed consumption was not influenced by the oil source, nor the sterilization procedures. The ethology of the Golden hamster, similar to all rodents, uses the pelleted feed as a substrate for wearing of the incisor teeth. This behavior has a direct impact on the assessment of feed consumption in experimental trials because the entire feed is effectively not eaten by the animal. Therefore, it is essential that in protocols that calculate the feed consumption, techniques that permit the recovery of food waste rejected by the laboratory animal be preferred[3].

For rodents, feed hardness is beneficial, as explained earlier, but palatability is normally one of the consumption regulators, particularly in diets high in oil content. Pellet hardness increases by approximately 60% after autoclaving, which is a positive factor for rodents but a negative one for other mammalian orders[10].

Despite the idea that autoclaving reduces palatability and feed intake, this effect was not observed in the present study. The darkening and release of odors in autoclaved diets occur due to the Maillard reaction that may have desirable and undesirable effects[11]. However, with the results obtained, feed intake by Golden hamsters was not influenced by this phenomenon, including food selection, which was not evidenced. One plausible reason could be that hamsters generally chew the pelleted ration into extremely small fragments thus increasing the exposure of the surface area of the dietary particles to enzyme action. This observation may explain why all feed showed similar passage rates, implying that they remained in the gastrointestinal tract for a similar length of time .

With respect to the biochemical parameters, the oil source was noted to significantly influence the plasma glucose concentration ($P < 0.05$). Animals fed diets containing linseed oil had a reduction in glycemic levels when compared to those fed diets with salmon oil.Other biochemical parameters revealed no significant differences, as shown in Table 3.

Hematological and biochemical values for hamsters had a close bearing to those reported in the literature. This is commonly found because of the evaluation protocols employed, including gender, age, environment, diet, lineage, management and sensitivity of the analyses. However, the findings in the present study showed compatibility with the results reported in other research papers[12,13].

In Golden hamsters, the fatty acid profile and the proportional relationship between

monounsaturated, polyunsaturated and saturated fatty acids was observed to exert a direct effect on biochemical parameters linked to presence of saturated fat in the blood and white subcutaneous and visceral adipose tissue depositions[14].

Table 3. Mean data of serum biochemical parameters in fasted adult Golden hamsters according to the type of sterilization and oil sources in the rations

Treatment	GLU	TRI	CHOL	PTN	ALB	ALA	ASP	CRE	AC	AP	GGT	URE
Common Salmon Oil	241.0	140.7	34.3	5.2	2.5	29.0	61.7	0.3	2.3	5.0	15.0	57.0
Radiated Salmon	225.0	125.0	33.3	5.5	2.6	35.7	77.7	0.4	2.4	5.7	16.7	54.7
Autoclaved Salmon	240.3	152.0	40.7	5.4	2.4	29.0	41.7	0.4	1.8	6.7	20.0	55.3
Average	235.3a	139.2	36.1	5.4	2.5	31.2	60.4	0.4	2.2	5.8	17.2	55.7
Common Linseed	143.7	119.4	63.3	5.4	2.5	30.7	65.3	0.3	1.9	5.0	19.0	56.0
Linseed Oil	159.0	167.0	43.3	5.0	2.3	29.7	47.3	0.4	2.1	5.3	18.7	58.0
Autoclaved Linseed	136.4	114.2	26.4	5.2	1.6	22.3	38.6	0.3	1.8	5.2	17.0	55.0
Average	146.4b	120.2	44.3	5.2	2.1	27.6	50.4	0.3	1.9	5.2	18.2	56.3
P-valor	0,007*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Glucose (GLU), triacylglycerol (TRI), total cholesterol (CHOL), protein (PTN), albumin (ALB), alanine aminotransferase (ALA), aspartate aminotransferase (ASP), creatine (CRE), uric acid (UC), alkaline phosphatase (AP), gamma-glutamyl transpeptidase (GGT) and urea (URE). * Significant effect, different letters in the column differ from each other by the Tukey test ($P < 0,05$).

Although strong deviations were noted, the findings present a clear picture of the nutritional metabolism and dietary influences on the plasma biochemical profile in the Golden hamster; however, it is essential that further studies are conducted to interpret the responses observed in the experimental procedures.

Conclusions

Salmon and linseed oils are suitable to use in the dietary formulations for adult Golden hamsters. Linseed oil diets reduce blood glucose in Golden hamsters. The oil source does not influenced the digestibility of the feed, however sterilization by autoclaving reduces it when using salmon oil in the rodents ration.

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