Effect of autoclaving diets use for growing rats: digestibility and performance

Haroldo Garcia de Faria[1]*, Sandra Regina Stabille[2], Patrícia Liliane Lee Ng[1], Renata de Britto Mari[2] and Valdeci Aparecido Mota[2]

*[1]Biotério Central, Universidade Estadual de Maringá. [2]Departamento de Ciências Morfofisiológicas, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brasil. *Author for correspondence. e-mail: hgfaria@uem.br*

ABSTRACT. Experiments were carried out to evaluate the use of autoclaving diets for growing rats. To evaluate the performance, rats born from females receiving or not autoclaving diets during the gestation were distributed in a randomized experimental design, with two treatments and a one-animal experimental unit. The diet used for female rats in gestation did not influence the weight of the rats either at birth or during the weaning period. As for digestibility and performance assays, rats from both kinds of females (with and without autoclaving diets during gestation) were distributed in a 2x2 factorial outline (normal or autoclaving diets until weaning versus normal or autoclaving diets after weaning). There was a reduction in the digestive use of the autoclaving diet gross energy after weaning and a worsening in the alimentary conversion in the period up to 42 days. The protein solubility in KOH test is a good indicative of the diet's nutritional quality loss.

Key words: autoclaving, performance, digestibility, Wistar.

RESUMO. **Efeitos da utilização de ração autoclavada para ratos em crescimento: digestibilidade e desempenho.** Foram conduzidos experimentos para avaliar o fornecimento de ração autoclavada ou não para ratos em crescimento. Para analisar o desempenho, ratos nascidos de fêmeas recebendo ração autoclavada ou não durante a gestação, foram distribuídos em um delineamento experimental casualizado com dois tratamentos e unidade experimental de um animal. A ração fornecida para ratas em gestação não influenciou o peso dos ratos ao nascer nem ao desmame. Nos ensaios de digestibilidade e desempenho utilizaram-se ratos oriundos de fêmeas que receberam ração autoclavada ou não na gestação distribuídos em um esquema fatorial 2x2 (ração normal ou autoclavada até a desmama versus ração normal ou autoclavada após a desmama). Evidenciou-se redução no aproveitamento digestivo da energia bruta da ração autoclavada fornecida após a desmama e piora na conversão alimentar no período até 42 dias. O teste de solubilidade da proteína em KOH é um bom indicador da redução da qualidade nutricional da ração.

Palavras-chave: autoclavagem, desempenho, digestibilidade, Wistar.

Introduction

Producing animals in laboratory makes necessary to ensure appropriate diets, so they can reach their maximum genetic potential. The nutrition of such animals is made by the supply of rations or diets that should contain ideal concentrations of several components essential for the animal's good development. Besides the ration chemical composition, the procedures related to its handling and storing, such as cleanness, disinfection and sterilization should also be monitored and cared for, in order to avoid the presence of pathogenic microorganisms that could minimize the animal's performance.

Among many sterilization techniques for eliminating the microorganisms present in rations, there is autoclaving with temperatures over 100ºC. Some lab animals have used autoclaving with temperatures above 121ºC for 20 minutes. However, exposure to heat may result in loss of vitamins and denaturation of proteins, interfering in the diet's nutritional

value (Zimmerman and Wostmann, 1963; Coates, 1984; Neves, 1996).

The autoclaving may present positive or negative effects. Among the positive effects, there is the capacity of conserving food while promoting the denaturation of enzymes that cause its deterioration. Denaturation also eliminates the action of natural hazardous proteins that might be present in the food (Faria and Stabille, 2001). However, the denaturation provoked by autoclaving causes conformation changes in the protein itself, such as the opening of its molecules which, in many cases, may increase its chemical reactivity, creating degeneration or complexation reactions with other substances of the system, resulting in changes in its functionality and nutritious value. The presence of pigments, carbohydrates, phenol and other reactive substances in the system, while still warm, increases the probability of protein deterioration (Sgarbieri, 1996).

The protein solubility in KOH test is thought to be a good indicator of protein quality loss for some kinds of food, such as soybean meal (Parsons *et al.*, 1991) and canola meal (Anderson-Hafermann *et al.*, 1993). This test is based on the extraction and determination of nitrogen fraction of a soluble sample in a KOH solution, allowing the researcher to detect any food overheating (Anfar, 1992). However, the capacity of being used as an indicator in protein quality of rations that underwent heat sterilization has been scarcely studied.

The aims of the present work are: a) to assess the performance, before and after weaning, of rats (*Rattus norvegicus*) whose mothers had received autoclaving diets; b) verify the effects of autoclaving on nutrients digestive use; and c) verify the sensibility of protein solubility in KOH test as an indicator of the reduction in diet protein quality due to autoclaving.

Material and methods

Three experiments were carried out to determine the digestibility and the effects of the use of autoclaved diet (15 minutes at 120ºC) on the perfomance of Wistar rats.

Experiment 1: Performance of rats up to weaning, born from females rats receiving autoclaving diets

To assess the performance of rats born from females receiving autoclaving diet, we employed a second generation of 50 broods of Wistar female rats, equalized into eight rats, from birth to weaning at 21 days of life. During the gestation period, 25 females received normal diet and 25 were fed with autoclaving diet. We used a commercial rat ration of reference whose analyzed composition is shown in Table 1.

Table 1. Chemical composition of the rats commercial ration used as reference[1].

Nutrients	%
Dry matter	89.88
Crude protein	22.23
Crude fiber	5.73
Calcium	0.92
Phosphorus	0.87
Gross energy (kcal/kg)	3976

[1]The analysis were carried out at the laboratory of Animal Nutrition – DZO – UEM.

The animals were distributed randomly, with two treatments and 25 repetitions each. Each experimental unit was composed of one animal, housed in 40x33x17cm polypropylene box (length, width and height). The animals received water and food *ad libitum* and were kept in a room with established photo period and room temperature (average of 21ºC).

The broods were weighed at birth, at seven days, at 14 days and at weaning, at 21 days of age.

The diet supplied to the females during the gestation and the 21-day period was also weekly weighed. The autoclaving diet and the normal diet were submitted to protein solubility in KOH analysis test (Anfar, 1992).

The data related to the performance up to weaning were submitted to variance analysis, using the Saeg software (1997). The model used for data analysis is presented below.

The statistical model employed to analyze the animals' performance characteristics (initial body weight, final body weight and daily weight gain) was:

$Y_{jk} = \mu + A_i + e\ E_{jk}$, where:

Y_{ik} = recorded value of the studied variables related to the individual k, of gender j, born from females receiving autoclaving diet at the time i,

μ = the general constant,

A_j = effect of the time i on autoclaving (i = 0 and 15 minutes)

E_{jk} = random error associated to each observation.

We employed F test at 5% to compare the means.

Experiment 2: Digestibility test

48 Wistar rats, at 21-days of age, were employed in a digestibility test to assess the experimental diet dry matter (DM), crude protein (CP) and gross energy (GE) digestive use capacity.

The rats, born from mothers fed with normal or autoclaved ration, were distributed in a 2x2 factorial scheme (autoclaving or normal diet until weaning versus autoclaving or normal diet after weaning), with the diet supplied being the same up to weaning (Table 1). The weaned animals were individually distributed in metabolic cages with food & water dispensers and a device for excrement collection, with four treatments and 12 repetitions.

The experiment lasted for 14 days: seven days for their adjusting to the diet and cages and seven days for collecting excrement. We employed the reference method for *in vivo* digestibility determination (Perez *et al*., 1995).

Excrement of each animal was totally collected in the morning, conditioned in plastic bags and stored in a freezer at a temperature of -18ºC. Later on, the excrement of each animal was weighed, homogenized and placed into a forced ventilation stove at 60ºC, for 72 hours. Afterwards, they were ground and disposed in properly identified glasses for dry matter, gross protein and gross energy laboratorial analyses, in accordance with Silva (1990), for the calculation of the respective digestibility coefficients.

Experiment 3: Performance test

200 Wistar rats (100 males and 100 females), aged 21-70 days, coming from broods whose females had received autoclaving or normal diets were employed on the performance test. They were distributed in a 2x2 factorial scheme (autoclaving or normal diets up to weaning versus autoclaving or normal diets after weaning), receiving the same diet supplied up to weaning (Table1). The animals were housed in 40x33x17cm polypropyilene boxes (length, width and height) and randomly distributed, with four treatments and ten repetitions, with the experimental unit being five animals. The animals were weighed at the beginning of the experiment at 21, 28, 35, 42 and 50 days and at the end of the experiment, at 70 days of age. The diet supplied, as well as the leftovers, were also weighed at the animals' weighings. We used the weight gain and the diet consumption to accomplish the performance analysis.

Statistical analysis

The rats' digestibility and performance coefficients data, from weaning to sacrifice, were submitted to variance analysis, using Saeg software (1997). The model employed for analysing the data is presented below:

$Y_{ijk} = \mu + N_i + A_j + NA_{ij} + e_{ijk}$; where

Y_{ijk} = relative observation from the individual k, coming from the brood receiving diet j up to weaning, receiving diet i after weaning;

μ = effect of diet i before weaning (i_1 = normal diet,

i_2 = 15-minutes autoclaving diet;

N_i = autoclaving effect on i up to weaning (i_1=reference diet, i_2= 15-minute autoclaving diet;

A_j = autoclaving effect on j after weaning, (j_1=reference diet, j_2=15-minute autoclaving diet;

NA_{ij} = interaction between the autoclaving diet supplied up to weaning and the autoclaving diet j supplied after weaning;

e_{ijk} = random error associated to each observation.

To compare the means we empoyed F test at 5%.

Results

Experiment 1: Performance of rats up to weaning, born from females rats receiving or not autoclaving diets

The results obtained in the analyses revealed that there was a decrease in protein solubility in KOH for autoclaving diet, as shown in Figure 1.

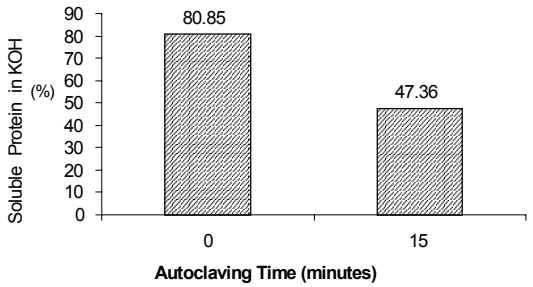

Figure 1. Levels (percentage) of soluble protein in KOH found on the diets due to autoclaving time (minutes).

The animal performance indicator parameters are shown at Table 2.

Table 2. Live weigh at birth and at weaning at 21 days e daily weight gain from birth up to weaning of Wistar *Rattus norvergicu* fed with normal diet (non-autoclaving) and autoclaving diet (at 120ºC for 15 minutes)

	Normal diet	Autoclaving diet		
		Live weight at birth		
			General mean	CV%
Males	6.22[a]	6.12[a]		
Females	6.17[a]	6.12[a]		
Means	6.19	6.12	6.17	5.37
		Weight at weaning (21 days)		
Males	46.7[a]	44.5[a]		
Females	45.7[a]	44.6[a]		
Means	46.2	44.6	44.42	15.3
		Daily weight gain from birth to weaning		
Males	1.93[a]	1.82[a]		
Females	1.88[a]	1.83[a]		
Means	1.90	1.82	1.86	16.85

Means followed by the same letters in the same line do not differ by F teste (p>0.05).

Experiment 2: Digestibility test

We did not observe any interaction (p>0.05) between normal and autoclaving diets supplied up to weaning and the diets supplied after weaning, regarding the apparent digestibility coefficients for dry matter (DCDM), crude protein (DCCP) and gross energy (DCGE), as shown in Table 3. However, we noticed a negative effect (p<0.05) for the autoclaving diet supplied after weaning for DCGE.

On the other hand, the use of normal and autoclaving diets, supplied before or after weaning, did not influence (p>0.05) the dry matter and gross protein digestive capability.

Table 3. Means of dry matter digestibility coefficient (DMDC), crude protein (CPDC) and gross energy (GEDC) of autoclaving diets supplied or not to weaned rats, coming from broods whose females did or did not receive autoclaving diets during the gestation period and until the weaning.

	Before weaning		After weaning			
	Normal	Autoclaving	Normal	Autoclaving	Means	*CV%
DMDC (%)	59.12[a]	58.9[a]	58.7[a]	59.3[a]	59.01	5.10
CPDC (%)	81.6[a]	81.4[a]	81.2[a]	81.7[a]	81.5	2.38
GEDC (%)[1]	56.0[a]	54.5[a]	57.3[a]	53.2[b]	55.28	8.36

[1]Means followed by the same letter in the same line do not differ (P>0.05) by F test
***CV:** variation coefficient

Experiment 3: Performance test

We did not observe any interaction (p<0.05) between normal and autoclaving diets given up to the weaning and the diets supplied after weaning for the performance parameters up to 70 days of age (Table 4).

The food conversion was better (p<0.05) for the animals who received normal diets after weaning for the 42-days period analyzed.

We did not observe any changes regarding the diets' visual aspect.

Table 4. Live weight (LW), daily diet consumption (DDC) and food conversion (FC) of rats from 21 to 70 days of age, according to the diets supplied (autoclaving or normal) to weaned rats coming from broods whose females received autoclaving or normal diets during the gestation period and until weaning.

	Performance analysis					
	Before weaning		After weaning			
	Normal	Autoclaving	Normal	Autoclaving	Means	CV%
			Live weight (LW)			
LW21 (g)	42.0[a]	45.2[a]	42.8[a]	44.4[a]	43.6	12.39
LW35 (g)	102.5[a]	110.4[a]	108.0[a]	104.9[a]	106.4	10.73
LW42 (g)	143.4[a]	148.6[a]	148.5[a]	143.6[a]	146.0	11.42
LW49 (g)	174.02[a]	177.2[a]	177.4[a]	173.7[a]	175.6	14.36
LW70 (g)	239.6[a]	233.3[a]	242.8[a]	230.1[a]	236.5	14.74
			Daily diet consumption (DDC)			
DDC21-35 (g)	11.16[a]	11.9[a]	11.5[a]	11.6[a]	11.5	10.37
DDC21-42 (g)	13.7[a]	14.3[a]	13.9[a]	14.09[a]	14.0	9.84
DDC21-49 (g)	17.9[a]	18.9[a]	18.3[a]	18.6[a]	18.4	11.19
DDC21-70 (g)	22.1[a]	22.2[a]	22.3[a]	22.0[a]	22.2	9.42
			Daily weight gain (DWG)			
DWG21-35 (g)	4.32[a]	4.65[a]	4.65[a]	4.32[a]	4.49	11.34
DWG21-42 (g)	6.83[a]	7.07[a]	7.07[a]	6.83[a]	6.95	11.42
DWG21-49 (g)	6.21[a]	6.32[a]	6.33[a]	6.20[a]	6.27	14.35
DWG21-70 (g)	4,89[a]	4.76[a]	4.95[a]	4.69[a]	4.82	14.74
			Feed: Gain Ratio			
FC21-35	2.58[a]	2.57[a]	2.47[a]	2.67[b]	2.58	5.04
FC21-42	2.00[a]	2.03[a]	1.96[a]	2.06[b]	2.01	5.33
FC21-49	2.90[a]	3.03[a]	2.92[a]	3.01[a]	2.97	11.19
FC21-70	4.89[a]	4.76[a]	4.95[a]	4.69[a]	4.82	8.72

[1]Mean with different letter on the same line differ (p<0.05) by F test. *CV: variation coeficient.

Discussion

Experiment 1: Performance of rats up to weaning, born from mothers receiving or not autoclaving diets

The assessment of gross protein soluble in KOH for normal and autoclaving diets revealed a decrease of protein solubility in KOH for the autoclaving diet. These results indicate an effect of autoclaving, reducing the diet's protein quality. Similar results were obtained by Faria and Stabille (2001) with autoclaving diets for growing rats. Araba and Dale (1990) and Parsons *et al*. (1991), when studying the effects of soybean meal processing, also verified a decrease in protein quality. The same authors stated that the protein solubility in KOH test is a good indicator for the protein quality reduction, which we can confirm at the present work.

The performance indicative parameters revealed that the animals born from mothers receiving normal or 15-minutes-autoclaving diets presented, at birth, a mean live weight of 6.19g and 6.12g, respectively (Table 2). At weaning, with 21 days of age, the mean live weight of animals born from mothers fed with normal diet was 46.2g while the animals born from mothers fed with autoclaving diet was 44.6g. The mean daily weight gain, from birth up to weaning, of animals whose mothers received normal and autoclaving diets were 1.90g and 1.82g, respectively (Table 2).

The statistical analysis showed that the differences on weight and on daily weight gain between animals from the two groups were not significant ($p>0.05$). The results obtained from birth up to weaning showed that, although there was a worsening in the diet nutritional quality due to the thermic treatment, this did not influence the rats' performance during the experimental period. This fact is most likely due to the high quantity of protein found in the reference diet employed, around 22%, well above the 15% recommended by the NRC (1995). Therefore, although there was loss in the diet nutritional quality, it was not enough to harm the animals during the gestation period and the period from birth to weaning.

Experiment 2: Digestibility test

We did not notice any interaction ($p>0.05$) between the normal and autoclaving diets supplied up to weaning and the diets supplied after weaning for dry matter, gross protein and gross energy digestibility coefficients (Table 3). However, a negative effect was observed ($p<0.05$) related to the autoclaving diet supplied after weaning for the gross energy apparent digestibility coefficient. On the other hand, the use of normal or autoclaving rations supplied before or after weaning did not influence ($p>0.05$) the dry matter and crude protein digestive use capacity.

The smaller energy utilization, observed for the animals receiving autoclaving diets after weaning, may be related to Maillard's reaction, which influences the nutritional value of food, as suggested by Hurrel (1990) who, when studying Maillard's reaction, concluded that a sharp drop on digestibility may be related to it.

Experiment 3: Performance test

No interaction was observed ($p>0.05$) between normal and autoclaving diets supplied up to weaning and the rations supplied after weaning on the performance parameters up to 70 days of age (Table 4).

The food conversion was better ($p<0.05$) for animals that received normal diets ater weaning at the 42-days period analyzed. The bad results for food conversion, observed on animals that received autoclaving diets after weaning, may be attributed to a smaller gross energy digestibility noticed at the digestibility test, since the daily weight gain and the daily ration consumption were not affected.

The protein solubility in KOH is an indicative of the effect of autoclaving in the reduction of protein quality. Araba and Dale (1990) and Parsons *et al*. (1991), when studying the effects of soybean meal processing, stated that protein solubility in KOH is a good indicator in the reduction of the protein quality. Although the laboratorial analyses show a drop in the amount of soluble protein, this does not seem to be the reason for a worsening in food conversion during the periods from 21 to 35 and from 21 to 42 days of age, since the digestibility test did not show problems related to protein digestibility but to energy.

Faria and Stabille (2001), when studying the effects of different autoclaving times on the nutritional quality of diets for growing rats, up to 35 days, also noticed a worsening in food conversion with 30, 45 and 60 minutes autoclaving rations at 120ºC. The authors attributed the worsening, observed in the performance characteristics, to the products, caused by Maillard's reaction during the autoclaving process, harming the nutrients availability, especially the proteins, since the ration consumption was not affected.

No alteration was observed regarding the visual aspect of the ration. However, Faria and Stabille (2001) noticed that autoclaving above 15 minutes produce changes in the ration such as its darkening,

a characteristic indicating Maillard's reaction. This reaction happens between proteins and reducing sugars, and results in the development of brown pigments as a reaction product between the glucose and the glycine. The reaction of the amino group (-NH2) with the carbonilo (-CHO) or cetonic (-C) -) reducing carbohydrate groups takes place even in relatively low temperatures, due to the high activation energy of this kind of reaction (Sgarbieri, 1996).

The results obtained in the present work agree with those obtained for other animal species. Parsons *et al*. (1992), when studying the effects of overheating on soybean meal, observed a worsening in the performance of chickens. Also, Anderson-Hafermann *et al*. (1993) noticed a worsening in the nutritional quality of canola meal, due to thermic treatment.

Other studies, aiming to assess the effects of different autoclaving time (0, 30, 60 and 90 minutes) on sunflower meal nutritional quality, also revealed a worsening in the performance of chickens (Zhang and Parsons, 1994). Zhang *et al*. (1996), when evaluating the effects of overprocessing on peanut meal autoclaving (0, 20, 30, 40, 50, 60 and 90 minutes) at 120ºC, observed a reduction from 78% to 56% in the soluble protein in KOH. Later on, these meals were tested in diets for chickens and a worsening was observed in the productive performance when the meal was autoclaved for more than 40 minutes.

With the colza meal and colza pie heated up at 130ºC for 0, 10, 20, 40 and 80 minutes, Pastuszewska *et al*. (1998) noticed a decrease in the levels of soluble protein in KOH: from 89.2% to 44.6% for the pie and from 58.6 to 43.4% for the meal. The tests *in vivo* accomplished with rats showed the negative effects of food heating in the animals' performance.

Previously mentioned Maillard's reaction inactivates mainly lysine, since the bonding of this amino acid with the carbonilo compound makes the peptide bonding adjacent to the adding compound resistant to hydrolysis by digestive enzymes. The final result is a darkening of the product, as observed in the autoclaving rations. Because of the production of dark pigments, the reaction is also known as a non-enzymatic darkening reaction (Sgarbieri, 1996).

Aburto *et al.* (1998), when studying ways of using overprocessed soybean meal, concluded that the use can be achieved if there is a supplementation with lysine, calculating the supplementation levels through the levels of soluble protein in KOH.

Conclusion

Considering the conditions in which these experiments were carried out, we can conclude that the 15-minute-autoclaving at 120ºC of diets supplied to the female rats in gestation does not influence the animals' weight either at birth or at weaning. There was a decrease in the digestive use of gross energy, which may explain the worsening of food conversion observed in the growth test at the 42-day- period. We may also conclude that the test of protein solubility in KOH used in autoclaving diets is a good indicator of reduction for protein nutritional quality.

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