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Ponderal evolution, food efficiency ratio and protein net efficiency ratio, determined in wistar rats fed diets with different protein sources [1]

Evolução ponderal, quociente de eficiência alimentar e quociente de eficiência liquida da proteína, determinados em ratos da linhagem wistar mantidos em dietas com diferentes fontes proteicas [1]

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Abstract: This study examined the viability of acid silage of Nile tilapia (Oreochromis niloticus (Linnaeus) on nutritional indices of Wistar rats: weight gain (GP), dietary intake (ID), food efficiency ratio (QEA) and relative protein net efficiency ratio (RNPR). Diets using silage obtained after 30 days and 90 days of storage were compared with diets containing casein, NUVILAB and aproteic diets. During 15 days (5 for adaptation and adjustments and 10 for samples), 40 male rats, weighing on average 61.42 + 3.09g, were housed in metabolic cages, followed by a completely randomized experimental design with five isoproteic and isocaloric diets, of which protein content (10.3 + 0.07% of protein) was supplied by casein, new silage, old silage, autoclavable Nuvilab CR-1 (unpurified diet closed formula of Nuvital Nutrients Ltda) containing (22.5% of protein) and aproteic diet as protein sources and 8 repetitions. There was a significant difference ($p < 0.05$) for weight gain (GP), dietary intake (ID), food efficiency ratio (FER), protein net efficiency ratio (PNER) and relative protein net efficiency ratio (RPNER), which could establish a linear comparison between weight gain of rats and their time in the respective diets, in days. Then, a diet consisting of tilapia acid silage harvest during30 days had better quality than those stored for 90 days, being diets containing proteins as casein and Nuvilab superior and aproteic diets did not present satisfactory results.

Keywords: acid silage, fish harvest, nuvilab, diets, rats.

Resumo: No presente estudo investigou-se a viabilidade da silagem ácida da despesca de tilápia do Nilo (Oreochromis niloticus (Linnaeus) sobre os índices nutricionais de ratos Wistar: ganho de peso (GP), Ingestão de dieta (ID), quociente de eficiência alimentar (QEA) e o quociente de eficiência líquida proteica (NPR). Foram comparadas dietas com silagens obtidas após 30 dias e 90 dias de armazenamento e dietas com caseína, NUVILAB e dietas aproteicas. Durante 15 dias (5 para adaptação e ajustes e 10 para coletas), 40 ratos machos, com peso médio de 61,42 + 3,09 g, foram alojados em gaiolas de metabolismo, seguindo-se o delineamento experimental inteiramente casualizado, com 5 dietas isoprotéicas e isocalóricas, cujo conteúdo protéico (10,3 + 0,07% de proteína), foi fornecido por caseína, silagem nova, silagem antiga, Nuvilab CR-lautoclavável (dieta não purificada de fórmula fechada, da Nuvital Nutrientes Ltda), contendo (22,5% de proteína) e dieta aprotéica como fontes protéicas e 8 repetições. Verificou-se diferença significativa ($p < 0,05$) para as variáveis ganho de peso (GP), ingestão de dieta (ID), quociente de eficiência alimentar (QEA), quociente de eficiência da proteína (PER) e quociente de eficiência líquida da proteína (NPER), sendo possível estabelecer relação linear entre o ganho de peso dos ratos e o tempo nas respectivas dietas em dias. Portanto, dietas compostas de silagem ácida da despesca com 30 dias foram superiores `as armazenadas durante 90 dias, sendo que dietas com caseína ou Nuvilab foram superiores e dietas aproteicas não apresentaram resultado satisfatório.

Palavras-chave: silagem ácida, despesca de pescado, nuvilab, dietas, ratos.

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Introduction

The artificial diets have been used in a decisive wayin fish cultivation, both as an activity factor of ecological sustainability or of technical-economic viability. The food item represents between 40 and 70% of the operating cost of pisciculture and the most expensive component is represented by the protein (FERNANDES et al., 2001). The high cost of protein sources, associated with

environmental pollution, require reevaluation of both the sources and the levels of protein used in commercial feeds (DA SILVA & ANDERSON, 1998). Diets for fish should contain a mixture of ingredients with adequate amounts of protein, energy, vitamins and minerals. The amount of each ingredient used depends on several factors, including requirements for the species at issue, palatability, cost, availability and proper processing (VIDOTTI et al., 2002; CARVALHO et al., 2006).

Protein sources of animal origin, usually of superior quality to those of vegetable origin, have a good balance of essential amino acids and better flavor (VIDOTTI et al., 2003). Fishmeal has traditionally been used in commercial feeds for fish. However, the offer's reduction, the variability in their composition and the high costs have forced nutritionists to seek alternative sources that can support the emerging demand of feed for aquaculture (FERNANDES et al. 2001). The fish acid silage is a product obtained from mixed and crushed residues in an acidic environment (FERRAZ DE ARRUDA et al., 2007). The enzymes in raw material separate the protein and dissolve it, while the acid prevents the action of microorganisms (BORGHESI et al., 2008). The final product is a high quality protein source and minerals to feed animals hardl obtained by other technological methods (SEIBEL et al., 2003) or new food development (MACH DIEP & NORTVEDT, 2009).

During silage preparation, amino acids are relatively stable, but, in acid hydrolysis, we observe a decrease of tryptophan and an increased stability of histidine. Tyrosine gradually separates from the aqueous phase by crystallization and methionine is stable in an acidic environment (LAZZARI et al. 2006). According to (MACH DIEP & NORTVEDT, 2009), only 8% of amine nitrogen turns into ammonia, in silage of codfish viscera stored for 220 days at 27^{o}C, which is very important. Tryptophan tends to decompose in acid silages but methionine and histidine are more stable (VIDOTTI et al., 2003).

The autolyzed material is characterized by a degradation of the original protein material of fishery, the state of peptides, oligopeptide and amino acids, to a greater or lesser extent, depending on the technique used in their preparation (VIDOTTI et al., 2003), this degradation results in an increase of the level of non-protein nitrogen components (suchas free amino acids, ammonia, mono and dimethylamine), as indicated in the study of acid silage of codfish viscera (FERRAZ DE ARRUDA et al., 2007).

Among the advantages of producing acid silage in relation to fishmeal, the process is independent of scale, the preparation is rapid, with reasonable cost, allowing the immediate use of the product, not requiring its storage in a refrigerator in addition to meliorating the problem of residues disposal (SOARES et al., 2001). The objective of this study was to evaluate the ponderal evolution, the food efficiency ratio and the protein net efficiency ratio, determined in wistar rats fed diets with different protein sources.

Materials and Methods

The raw material for the production of fish chemical silage was composed by Nile tilapia (*Oreochromis niloticus (Linnaeus*) harvest deriving from commercial cultivation, in the region of Indaiatuba, São Paulo, fragmented into crusher, electrical equipment model ML-4.0/Weg-uline, summarizing 5 kg in each batch. Then it was homogenized, weighed and evenly distributed in hard plastic containers, adding formic acid in the ratio of 3% (p/p) of the acid solution volume for the residue mass (FERRAZ DE ARRUDA et al., 2007; FELTES et al., 2010).

The agitation of the material was often held to spread the enzymes, thus accelerating the rate of liquefaction (MACH DIEP & NORTVEDT, 2009) obtaining tilapia chemical silage, which was made in the daily control of pH to remain close t 4.0. Then the material was subjected to a drying process at room temperature, the silage remained in the environment ($27^{o}C \pm 3^{o}C$) for 90 days. The samples were processed following the

procedures described in the Figure 1 flowchart.

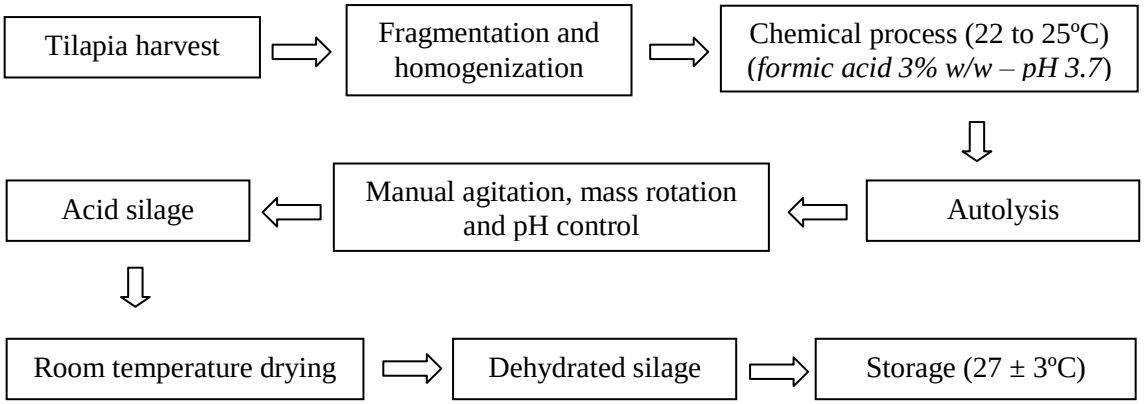

Figure 1. Flowchart of the processing for the production of acid silage of Nile tilapia harvest.

Fourty male Wistar albino rats, between 21-23 days, newly weaned, provided by the Biotery Center from the State University of Campinas (UNICAMP) were used. The animals were weighed on arrival (67.24 ± 3.09g) and submitted to a period of acclimation to the individual growth cage environment, for four days, with a non-purified diet (Nuvital®). After this period, the animals weight (61.42 ± 3.09g) was verified and they were divided into five groups with eight animals each, with the same average body weight, and placed in metabolic cages for five days to adapt to specific experimental diets for each group. Over the next ten days, passed the experimental period for calculation of weight gain (GP) and rats dietary intake and of nutritional indices, food efficiency ratio (QEA), protein net efficiency ratio (NPR) and relative protein net efficiency ratio (RNPR). The consumption of diets, which were offered, as well as of water, at will, was also determined.

The environmental conditions of the Biological Assays Laboratory were 22 ± 2^{o}C of temperature, 50-60% of relative air humidity and a 12 hour light-dark cycle with automatic rotation. The study protocol was previously approved by the Unicamp Ethics Committee on Animal Experiments. Upon completion of the biological assays, diets were prepared of which composition is specified in

(AOAC, 2000).The composition of the mineral mixture used was specified in the United States Pharmacopoeia- USP (AOAC, 2000) and the vitamin mixture to the fortification of animal diet in (NUTRITIONAL BIOCHEMICALS CORPORATION, 1977/1978).

The experimental diets were composed by: casein, new silage, old silage and autoclavable Nuvilab CR-1 (unpurified diet closed formula of Nuvital Nutrients Ltda) containing (17.4% of protein) and aproteic diet as protein sources. The diets were isoproteic (12.3 ± 0.2%) and isoenergetic (391.5 ± 2.3 kcal/100g). The consumption of diets was monitored during the fifteen-day trial period, as well as the rats ponderal evolution. The animals were weighed during the experimental period, with intervals of five days, in semi-analytical balance.

In assessing the NPR index, the method described was employed by (BENDER & MILLER, 1953) with animals being kept in individual cages with "ad libitum" water and food, for a period of 10 days and 5 of adaptation. In addition to the groups that received diets with proteins under study, control groups were always fed aproteic and casein diet. During the tests, the animals' weight gain and the amounts of diet consumed were controlled. After the period specified, the NPR index was calculated using the equation: RNPR=[(GP test + P.P. aprot)/P. ing].

GP test = weight gain in the group fed diet containing the protein under study.

PP aprot.= weight lossof the group fed the aproteic diet.

P ing =protein under study, ingested.

The statistical experimental design used in biological assays was the completely randomized experimental design (PIMENTEL GOMES, 1985). For comparison between means, the variance analysis was made, which if different from the F test, was analyzed according to Tukey.

Results and Discussion

The evolution of the average weights of animals on various diets, over the last 15 days of experiment, is shown in Figure 2. It was

observed that, for all diets, it was possible to establish a linear comparison between the weight gain of rats and their time in the respective diet, in days. These comparisons were, in all cases, positive and high, with Pearson correlation coefficients, r, close to unity, represented by the correlations: casein (y = 64.6388 + 4.7543 x, r = 0.9421), Nuvilab (y= 61.5490 + 5.5024 x, r = 0.9131040), new silage (y = 62.3966 +2.9671 x, r = 0.8951), old silage (y = 61.6925 + 0.78625 x, r =0.8701) and aproteic diet (y = 61.9822 + (-0.70467 x), r = 0.9126.

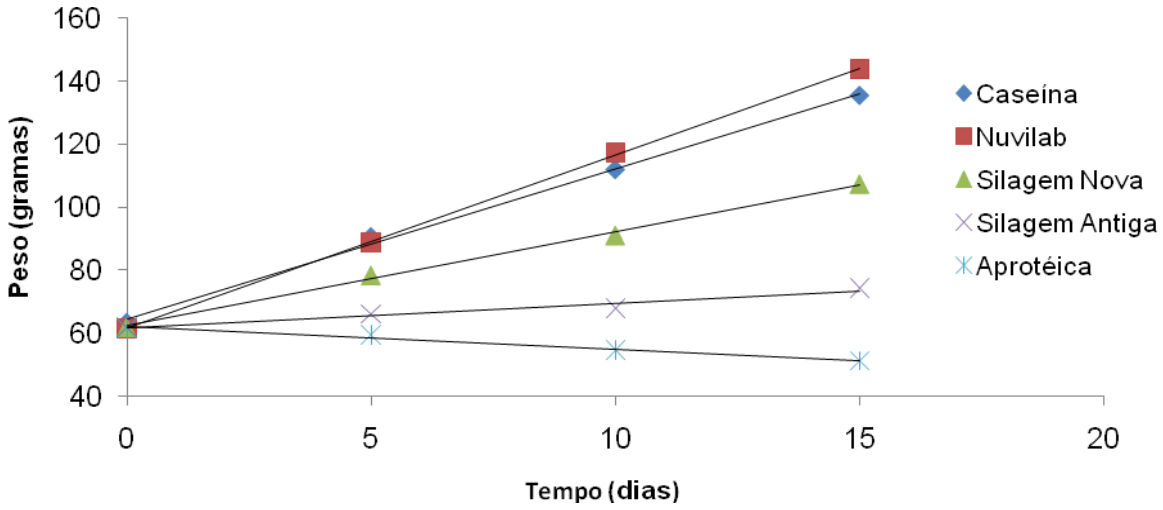

Figure 2. Linear regressions for comparisons between the average weight of Wistar rats, in grams, and the experiment time, in days, for several balanced diets, when fed diets of which protein content (10%) was supplied by casein, new silage, old silage and aproteic diet. Groups of eight animals were used, with average initial weight of 61.42 ± 3.09 g.

Table 1 illustrates the results of nutritional assessment assays with tilapia silage which may explain, satisfactorily, the parameters profile in determining the food and the protein efficiency ratios with acid silage of Nile tilapia for 10 days of experiment. It was observed that the different diets, casein, Nuvilab, new and old

silage, influenced ($p < 0.05$) the variables, weight gain (GP), dietary intake (ID), food efficiency ratio (QEA) protein net efficiency ratio (NPR) and relative protein net efficiency ratio (RNPR).

Comparing the treatments, the rats on Nuvilab diet (54.77 ± 6.88) had the weight gain ($p < 0.05$) thus differing from the others, being superior to those reported by Espe et al. (1999), which working with Nuvilab diet (52.77 ± 4.84) revealed a good use of this studied diet, due to the higher protein content, 22.5% compared to the others. However, regarding the old silage, the rats' weight gain was statistically smaller ($p < 0.05$) when compared to the new silage, because a reduction in the nutritive value with increasing degree of autolysis of fish silage has been reported in experiments with rats (Espe et al., 1989), trouts (Beerli et al., 2004) and pigs (Silva & Landell Filho, 2003), probably due to the reaction of carbonyl compounds deriving from the breakdown of hydroperoxides, with autolyzed protein amino acids, decreasing the nutritional value of silage (CISSE et al., 1995).

Table 1. Nutritional assessment assays with acid silage of Nile tilapia waste in Wistar rats previously adapted to diets with 10.2 +0.07% of crude protein for 10 days

Diets[2]	Weight gain (g)	Dietary intake (g)	QEA[1]	NPR[2]	RNPR[3]
Casein	$44.92^{b} \pm 6.76$	$190.71^{a} \pm 22.43$	$0.24^{ab} \pm 0.02$	$2.58^{a} \pm 0.30$	$0.99^{a} \pm 0.05$
Nuvilab	$54.77^{a} \pm 6.88$	$209.87^{a} \pm 21.03$	$0.26^{a} \pm 0.02$	$1.18^{c} \pm 0.10$	$0.46^{b} \pm 0.03$
New silage	$28.86^{c} \pm 3.46$	$145.21^{b} \pm 27.01$	$0.20^{b} \pm 0.03$	$2.22^{b} \pm 0.32$	$0.86^{a} \pm 0.13$
Old silage	$8.14^{d} \pm 3.30$	$80.85^{c} \pm 14.49$	$0.10^{c} \pm 0.04$	$0.22^{d} \pm 0.06$	$0.08^{c} \pm 0.02$
Aproteic diet	$-8.13^{d} \pm 5.80$	$51.30^{b} \pm 15.70$	$0.09^{c} \pm 0.07$	$0.02^{c} \pm 0.08$	$0.01^{a} \pm 0.04$

Values followed by different letters in the column differ among themselves by Tukey test ($P<0.05$). [1]QEA - food efficiency ratio; [2]NPR - protein net efficiency ratio; [3]RNPR - relative protein net efficiency ratio.

Confirming these results (Mach diep & Nortvedt, 2009) verified that diets consisting of freshly prepared silage were significantly superior to silages stored for long periods of time. However, the determination of the amount of available amino acids actually usable by the animal, as opposed to the total present in food, is essential, since the actual nutritional value of a protein does not depend solely on its content of essential amino acids, but also on the bioavailability (Viana et al., 1999; Vidotti et al., 2003).

In order to determine the best time to add the most proper silage in diets, (Carvalho et al., 2006) found differences ($p < 0.05$) between new and old silage, in the average weight gain, however, for the feed conversion and NPR, there were no differences ($p > 0.05$) found among treatments.

In dietary intake (ID), it was verified that there was no difference ($p > 0.05$) between casein (190.71 ± 22.43) and Nuvilab (209.87 ± 21.03) which were significantly superior to the other treatments at 10 days. Compared to Nuvilab, the old silage was the one with lower dietary intake ($p < 0.05$).

In the food efficiency ratio (QEA), casein (0.24 ± 0.02) and Nuvilab (0.18 + 0.10) diets were similar ($p > 0.05$) and after 10 days, the rats on Nuvilab diet showed higher (QEA), being very closed to the casein and new silage, thus differing from the old silage, which showed lower (QEA).

In net protein net efficiency ratio (NPR), the standard diet, casein (2.58 ± 0.03), was superior ($p < 0.05$) to other treatments.

The new silage treatment was significantly superior to Nuvilab, existing difference between new silage and casein at a 5% level, it requires some special care to any conclusions between these two treatments, as the new silage showed a NPR well above to the other diets at a 5% level. In the relative net protein net efficiency ratio

(RNPR), new silage (0.86 ± 0.13) was superior ($p < 0.05$) to the other treatments.

The present results correlate with another studies (Pimenta et al., 2008 and Lazzari et al., 2006) which reported an explanation for the reduction of the protein value of silage stored for long periods of time, that the free amino acids and peptides are formed in such silages very quickly, initiating a process of catabolic degradation even before the synthesis of proteins. Espe et al. (1999), in studies accomplished using diets supplemented with fish silage at a rate of 6 to 8%, did not found any impact on consumption. In pigs during the growing and finishing phases, Beerli et al. (2004) verified that the increase of fish silage level (0, 2, 5 and 8%) in food and the daily weight gain and the feed conversion were not similar in all treatments in relation to the diet consumed.

Studies of Feltes et al. (2010) using Tilapia finger lings fed with Tilapia silage at levels of 10, 20, 30%, instead of a commercial ration, showed that the weight offinger lings was lower than those using only commercial ration.

Bermudez et al. (1999), in studies using fish silage to feed pigs in replacement to soybean meal in the proportion of 0, 50 and 75%, assessed the daily weight gain and found no significant difference between treatments, emphasizing that the treatment with a 75% replacement showed the best performance.

Carvalho et al. (2006) and Fagbenro & Jauncey (1999) stated that, the use of a freshly prepared silage with 30 days, has a higher nutritional value compared to silage stored for more than 90 days, because there are evidences that the essential amino acids, specially, leucine, isoleucine, lysine and tryptophan, are gradually destroyed probably by interactions with oxidized lipids, allowing the development of toxic substances (BOSCOLO et al., 2004), which explains the low nutritional value found for that silage, as demonstrated by the rats low use of dietary protein. Thus, the storage time

choice is crucial for the expected response, as a quality criterion for fish silage (FELTES et al., 2010).

Conclusions

The use of acid silage of Nile tilapia stored for 30 days, show eda higher nutritional value compared with silage stored for more than 90 days and diets containing proteins as casein and Nuvilab are superior. Aproteic diets did not present satisfactory results.

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