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Effect of Dietary Supplementation of Fermented Fish Silage on Growth Performance in Male Wistar Rats

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Abstract

The effect of dietary supplementation of fermented fish silage on the growth performance of male Wistar rats was investigated. The rats were fed with feeds prepared with fermented fish silage of three different fish species *viz.,* jew fish, tilapia and silver carp. Body weight gain and nutritional performances of different groups of rats were assessed. There was no significant difference observed in the rate of body weight gain in groups supplemented with fermented fish silage and acid silage supplemented rats. However, the groups of rats fed with Jew fish silage showed better weight while Apparent Nitrogen Digestibility of all the experimental groups fed silage were found to be significantly less than that of control groups. Protein Efficiency Ratio was almost similar in all groups except for silver carp silage supplemented groups which showed significantly low value. The study indicated that the fermented silage prepared from jew fish was found to have better nutritional qualities.

Keywords*:* Fermented fish silage, growth rate, protein efficiency ratio, true digestibility, amino acid composition

Introduction

The shrimp trawling results in the landings of many low value fishes, most of which were thrown back in the sea. The industrial fish processing for human consumption yields only 40% edible flesh and the remaining 60% is thrown away as waste (Raa & Gildberg, 1982). Fish silage is a liquid product that can be prepared from whole fish or parts of fish (fish waste) that are liquefied by the action of endogenous gut enzymes of the fish in presence of added acid (acid silage) or the in-situ production of the acid by the microorganisms added along with some carbohydrate source (fermented silage). Fish silage has a number of added advantages over fish meal which is usually preferred as animal protein source in feeds. When compared to acid silage, fermented fish silage is found to be cheaper and has probiotic effect which is advantageous for animals. Lactic acid bacteria are known to possess antibacterial properties attributed to major end products of their metabolism such as lactic acid, acetic acid, hydrogen peroxide and peptide compounds termed bacteriocins. Fermented silage production is a better option for utilizing trash fish and fishery waste where cheap carbohydrate by products are also available. Effect of different levels of fermentable carbohydrate on the microbial level and the degree of hydrolysis has been reported by Zynudheen et al. (2008). Acidified or fermented fish silage should be considered as potential feed component for animals because of its high nutritional value and appropriate microbiological and chemical quality (Gulsun et al. 2015). However experimental studies on the nutritional influence of fermented fish silage are scanty. In the present study an attempt has been made to assess the effect of dietary intake of fermented silage on the growth performance of male albino rats.

Materials and Methods

The amino acid standards were procured from Sigma Chemicals USA, and Tryptophan from Sisco Research Laboratories (SRL). All other reagents were of analytical grade.

Wistar strain male albino rats, 120-125 g, were selected for the study. The animals were housed in polyurethane cages under hygienic conditions and maintained at normal room temperature. The

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animals were allowed food and water *ad libitum*. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC).

Tilapia (*Oreochromis mossambicus*) of size 10-18 cm and weight 40-75 g were collected from brackish water farms near Cochin, Silver carp (*Ctenopharyngodon idella* 15-20 cm and 200-250 g) were collected from a fresh water farm near Kottayam, and Jew fish (*Johnius dussumerie*) of size 15-20 cm and weight 100-200 g were collected from local markets in Cochin. *Lactobacillus plantarum* type culture (No.1425) procured from Microbial Type Culture Centre, Institute of Microbial Technology, Chandigargh, India was revived and repeatedly sub-cultured in MRS broth and used. Jaggery was procured from the local market. The fish was homogenized in a Bowl Chopper (Tecator 1094) for 5-10 minutes and cooked in a steel vessel for 30 minutes with jaggery. It was cooled to room temperature and *Lactobacillus plantarum* culture containing 10^9 CFU ml^{-1} was added @ 5% (W/V) of weight of homogenate. The whole mass was mixed thoroughly, fermented for 15 days under sterile condition and dried in a mechanical drier till the moisture levels was below 10%. Acid silage was prepared from rohu filleting waste with 3.5% formic acid. After hydrolyzing for 14 days it was dried and used. The fecal matter was collected for analyzing the nitrogen content.

The details of five feed formulations used are as follows.

Feed 1: Basal feed supplied by M/s Sai Feeds, Bangalore, India was used as control diet.

Feed 2: Dried fermented Jew silage at 10% level added to the basal diet.

Feed 3: Dried fermented tilapia silage at 10% level added to the basal diet.

Feed 4: Dried fermented silver carp silage at 10% level added to the basal diet.

Feed 5: Dried formic acid silage at 10% level added to the basal diet.

The proximate composition of feeds used was determined by standard methods (AOAC 2000). The amino acid composition was determined as per the procedure of Ishida et al. (1981) and tryptophan was estimated as per the method of Sastry & Tummuru (1985). Protein efficiency ratio (PER) was calculated as the weight gain per gram of protein consumed. Net protein utilisation (NPU) was calculated as the ratio of nitrogen retained in the body to the nitrogen intake. Feed Conversion Ratio (FCR) was calculated as the ratio of feed intake to gain in weight. Apparent nitrogen digestibility (AND) was calculated as the ratio of the difference of the ingested and faecal nitrogen to the ingested nitrogen and expressed as percentage. True digestibility (TD) was calculated as ratio of the difference of the ingested nitrogen and fecal nitrogen from which metabolic nitrogen was deducted to the ingested nitrogen and expressed as percentage.

The quality of protein was evaluated chemically using values of amino acids obtained and those of FAO reference patterns to determine the chemical score (FAO/WHO, 1973).

Seven days after acclimatization, the animals were divided into five groups of five animals each. Groups 1, 2, 3, 4 and 5 were fed with feeds 1, 2, 3, 4 and 5 respectively. Weekly average weight gain of the rats was noted. The experiment was carried out for 35 days.

Results expressed as mean±SD. Statistical analysis between the means using ANOVA and Duncan's multiple range comparison test were carried out to test the significance of variance. Statistical package used in the study was SPSS 10.

Results and Discussion

Proximate composition of feed used for the study is depicted in Table 1. The control feed which contained casein as protein source had 54% protein and nearly the same levels of protein were found in other samples also. But in case of acid silage the protein content was found to be 43.1%. This could be due to higher moisture levels and ash content in acid silage. The crude fat content in all the samples were higher than that of the control group. A similar proximate composition of fish waste silage based feed was reported by Kenan et al. (2015).

The amino acid profile and chemical score of feeds used are given in Table 2 and 3 respectively. In all the compounded samples including acid silage, amino acids like aspartic acid, threonine, serine, glycine and alanine were found to be in higher levels than the control feed; whereas valine,

histidine and agrinine were at lower levels. The differences in amino acid levels might have affected the growth rate of different groups. The chemical score was found to be less in case of all amino acids except tryptophan. The reduction in amino acid score in different types of silages has been reported by Vidotti et al. (2003).

Fig. 1 shows the percentage of weight gain in normal and experimental groups of rats. Rats fed with Jew fish silage showed better weight gain indicating better quality of the silage produced from the species. Control group and tilapia silage fed group showed similar weight gain and acid silage and silver carp silage showed marginally lesser weight gain. According to Yone et al. (1986) and Baraquet & Lindo (1985), the microbial fermentation alters the composition of crude protein and dry matter and increases volatile nitrogen, which could have resulted in the difference of weight gain in different experimental groups. Fagbenro & Jauncey (1994) observed that the weight gain of *Clarias gariepinus* fed with fermented whole tilapia silage in combination with other fillers like soyabean meal, feather meal, poultry waste meal etc. was not significantly different.

Table 1. Proximate composition of silage incorporated feed used for rat feeding study (values in %).

	Moisture	Crude Protein	Carbohydrate	Crude fat	Ash
Control	9.56 ± 0.43^{c}	54.07 ± 0.24^{d}	28.21 ± 0.38^{b}	3.18 ± 0.11^{a}	5.80 ± 0.31^{a}
Jew fish	8.51 ± 0.12^{b}	51.16 ± 0.80^{b}	27.5 ± 0.62^{ab}	5.19 ± 0.21^{c}	7.82 ± 0.44^{b}
Tilapia	8.27 ± 0.24^{b}	50.02 ± 0.93^{ab}	27.3 ± 0.61^{ab}	6.42 ± 0.16^{d}	8.46 ± 0.57^{bc}
Silver carp	7.66 ± 0.31^{a}	52.88 ± 0.82^{c}	26.6 ± 0.82^{a}	4.36 ± 0.20^{b}	8.73 ± 0.21^{bc}
Acid silage	12.48 ± 0.42^{d}	43.1 ± 0.54^{a}	28.6 ± 0.66^{ab}	6.01 ± 0.24^{d}	9.84 ± 0.34^{d}

Results are presented as mean ± standard deviation (SD) of 3 replications .^{a,b,c,d}, mean in a column with the same superscript values are not significantly different ($p<0.05$).

Table 2. Amino acid composition (g 100 g^{-1}) of feed used for rat study prepared from silage of different species.

	Control	Jew fish	Tilapia	Silver carp	Acid silage
Asp	7.07	9.81	9.33	10.06	8.82
Thr	3.73	4.17	4.21	4.47	4.32
Ser	7.28	7.28	5.91	6.51	6.21
Glu	18.92	18.40	17.02	17.37	17.08
Pro	0.00	1.30	1.53	1.42	1.41
Gly	4.10	9.45	8.60	10.97	10.15
Ala	3.03	6.93	5.77	7.36	5.98
Cys	0.00	0.61	0.00	0.36	0.39
Val	7.29	5.85	4.62	5.03	7.44
Met	1.80	2.86	1.67	1.60	1.73
Ile	5.07	5.52	5.88	4.89	5.43
Leu	8.50	8.37	8.70	8.48	8.63
Tyr	1.26	1.25	3.27	1.91	1.65
Phe	3.64	3.36	4.97	4.04	3.52
Try	1.61	2.38	1.83	1.06	0.98
His	12.84	3.87	4.76	3.16	4.44
Lys	1.62	1.15	1.13	1.23	1.05
Arg	5.87	1.87	3.64	2.14	1.77

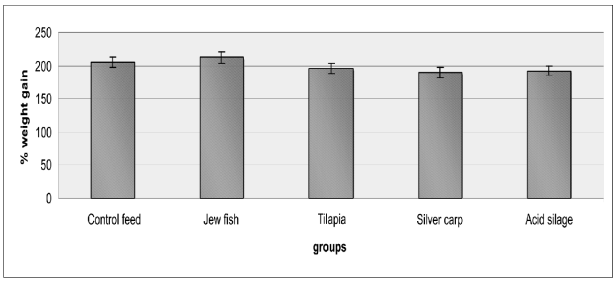

Fig. 1. Weight gain (%) in normal and experimental groups of rats

Table 4 depicts the results of PER, TD, AND & NPR. PER of all the samples showed almost a similar trend except silver carp silage supplemented groups which showed significantly low PER. This could be due to the low nutritional quality of silver carp meat. Neethiselvan et al. (2001) reported a higher PER for *E. suratensis* fed with fermented fish silage based diets than the control diets based on plant protein. According to Gonclaves et al. (1989), eel fingerlings showed better FCR and PER with silage diets compared to control. Rodriquez et al. (1990) reported similar PER in rats fed with acid fish silage when compared to casein diet. Kompiang et al. (1977) reported a better feed conversion ratio in all the five silage incorporated feeds at different levels with the highest value for 29% silage in the feed.

True digestibility of all the experimental samples showed significantly higher values when compared to the control. Acid fish silage showed the highest value of more than 65%, which could be due to the presence of higher levels of hydrolysed proteins in the acid silage. Among the fermented silages, Jew fish silage supplemented rats showed higher value indicating that the protein of this species is of better quality. According to Marit et al. (1989) true digestibility of acid silages stored for different periods does not show significant difference even after storage for

Table 3. Chemical score for important amino acid in silages prepared from different raw materials

Amino acid	FAO/WHO (1985) standards	Tilapia silage	Silver carp silage	Jew fish silage
Tryptophan	1	3.15	1.23	1.53
Lysine	5.5	0.16	0.22	0.21
Arginine	5	1.03	0.39	0.23
Valine	5	0.54	0.99	1.16
Methionine	3.5	0.81	0.57	0.68
Isoleucine	4	1.39	1.29	1.31
Leucine	7	1.13	1.14	1.15

Chemical score = (g amino acid 100 g^{-1} test protein)/(g amino acid 100 g^{-1} standard protein).
Results are presented as mean±standard deviation (SD) of 3 replications .^{a,b} mean the bars with the same superscript values are not significantly different ($p<0.05$).

Table 4. PER, TD, AND and NPR of rats fed with fermented fish silage incorporated diet.

	PER	TD (%)	AND (%)	NPR
Control	1.63 ±0.07^{b}	51.59 ±3.2^{a}	48.40 ±1.8^{e}	0.52 ±0.02^{b}
Jew fish	1.78 ±0.06^{c}	64.09 ±1.4^{c}	35.91 ±1.8^{b}	0.61 ±0.04^{c}
Tilapia	1.72 ±0.21^{c}	56.47 ± 2.1^{b}	43.53 ±2.4^{d}	0.52 ±0.1^{ab}
Silver Carp	1.38 ±.01^{a}	63.19 ±2.4^{c}	36.81±1.4^{c}	0.49 ±0.15^{a}
Acid silage	1.85±0.12^{d}	65.88±3.1^{e}	34.12 ±1.3^{a}	0.50±0.03^{ab}

Results are presented as mean±standard deviation (SD) of 3 replications .^{a,b,c}, mean in a column with the same superscript values are not significantly different ($p<0.05$).

180 days. However, decreased utilization of protein in stored silage has been reported in experiments with rats by Stone & Hardy (1986).

Apparent Nitrogen Digestibility of all the experimental groups fed with silage were found to be significantly less than the control groups. This could be due to absorption of excess of free amino acids present in the silage samples by the animals, thus directing more of the amino acids from the diet into the catabolic pathway (Yamada et al. 1981). According to Raa & Gildberg (1982), the nutritional difference of different fish silage products reported may be related to the quality of the raw material used. Luiz et al. (2006) suggested that the use of fermented fish silage incorporated feed is linked to the characteristics of its protein fractions and may be supplied as a source of less soluble proteins.

Significantly lower NPR of silver carp silage fed group might be related to the comparatively lesser availability of essential amino acid concentration in the species (Table 2) as observed by Luiz et al (2003). But the Jew fish silage fed groups had significantly higher NPR, which confirms the higher quality of the protein, supported by other indices studied.

Feeding studies using diets supplemented with fermented fish silage indicated better growth performance for jew fish silage-based feed in experimental animals. Utilisation of trash fish and fish processing waste for the production of silage by fermentation is an environmental friendly method. Considering the probiotic impact of lactobacillus fermented silage, use of this product as feed supplement is a better option for the utilisation of fish waste.

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