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Characterization and Nutritional Quality of Formic Acid Silage Developed from Marine Fishery Waste and their Potential Utilization as Feed Stuff for Common Carp *Cyprinus carpio* Fingerlings

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Abstract

The present study was undertaken to bring out a cost effective feed from fish silage for promoting growth in common carp, *Cyprinus carpio.* For this, acid silages were prepared using fishery wastes supplemented with three different concentrations (2, 2.5 and 3%) of formic acid and fermented for 30 days. During fermentation, the physicochemical parameters and aerobic plate count of silage products were estimated for 30 days at an interval of 10 days. Results indicated that pH, dry matter, ash, protein contents and aerobic plate count showed a declining trend during ensilaging day's interval. However, lipid and mineral contents were gradually increased up to 30th day. After 30 days of fermentation, the amino acids and vitamins were quantitatively as well as qualitatively ascertained in all silages. Further, the individual silage products were used as one of the major ingredients to prepare formulated diets (E1: 2%; E2: 2.5% and E3: 3%). The prepared diets were fed to *C. carpio* fingerlings for 45 days and the growth promoting efficacy of the diets was tested. From the results it was understood that, 2% acid silage diet had higher weight gain (2.38g), SGR (1.49%) and significant increase in biochemical constituents than other diets fed fish

Keywords: Fish waste; formic acid; silage; *C. Carpio.*

Introduction

Fish wastes resulting from industrial fish processing operations often consists of offal of flesh, skins, bones, entrails, shells etc. Generally industries that want to be effectively clean must use fish processing wastes in the manufacture of new products, contributing not only to environmental preservation, but also in increasing their own revenues. Fish waste represents half of the raw material volume of the industry and is a source of low – cost nutrients (Oetterer, 2002). The best alternative solution is to utilize the waste material for the production of by – products and also to avoid waste production. This could be achieved through production of cost effective fish silage products. The product has a good nutritive quality and can be sufficient for animal feeding.

Fish waste can be advantageously upgraded into fish feed through fish silage conversion. This procedure is safe, cost effective and eco-friendly too (Hanafy and Ibrahim, 2004). Fish silage is a brown, stable liquid stock feed prepared by acidifying fish waste by direct addition of acids (acid fish silage). Fish silage preparation usually depends on the locally available raw materials and conditions (Hasan, 2003). Formic acid (organic acid) is the best choice for the preparation of chemical silage, the silages made using formic acid are not excessively acidic and therefore do not require neutralization before being used (Oetterer, 2002).

The increasing demand and progressive scarcity of fishmeal in the international market boosted its price and launched the quest for reduction of fishmeal in fish diets and the consequent search for alternative, acceptable and digestible protein sources (Pereira and Oliva-Teles, 2003). Fish silage is an excellent alternative to fish meal in utilizing waste/trash fish (accounted for about 5% of the annual farm production) as protein feed stuff for Tilapia (Wassef *et al*., 2003). Silage production acquires potential importance compared to fish meal with the following advantages: the process is virtually independent from the scale; the technology is simple and the investment is little. Due to the similarity of the protein source with the raw material and low cost, especially when compared to fish meal, silage has a potential use in aquaculture (Vidotti *et al*., 2003).

Many authors have evidenced the nutritional benefits and economic feasibility of using fish silage as good feed stuff for aqua feeds. For instance, it was found that acid fish silage based diets supplied to Nile tilapia (*Oreochromis niloticus*) and blue tilapia (*O. aureus*) had good growth performance equivalent to that of fish meal diet (Wassef *et al*., 2003). Likewise, fermented fish silage based diets supplied to Nile tilapia *O. niloticus* and African cat fish *C. garipinus* evidenced 25 to 50% successful replacement of fishmeal (Hanafy and Ibrahim, 2004). Feeding digestibility and growth studies on pacu *Piaractus mesapotamicus* have shown that fish silage is highly digestible and recorded effective replacement for up to 75% of fish meal in aqua-feeds (Vidotti *et al*., 2002). Considering the importance of the mentioned topics, the present study was undertaken to prepare acid silage using fish processing wastes and to investigate the effect of prepared silages by replacing dietary ingredients in the feed to determine the growth promotion efficiency of cultivable fresh water fish *Cyprinus carpio* fingerlings.

Materials and Methods

Silage Preparation

Processing wastes (Fish head, viscera, frames and bones) of grouper *Epinephelus malabaricus* were aseptically collected from a fish processing industry (Oceanic fisheries Pvt. Ltd) of Kanyakumari District, South India. The collected fish wastes were washed in potable water and were chopped, minced using a wet grinder into paste for silage preparation. Acid silage was prepared by acidifying 5kg of minced fish paste with three different concentrations (2, 2.5 and 3% (w/v)) of formic acid. Simultaneously triplicates were maintained in each concentration.

Ensiling process was then aided by incubating the materials in the air tight bitumen coated containers (5L capacity) at ambient room temperature (28±2ºC) up to 30 days. The silages were stirred daily in the morning aseptically and samples of known volume were drawn from the respective containers at definite intervals of 0, 10, 20 and 30 days duration. During each sampling interval, ensilages were evaluated for physicochemical parameters like ash content, dry matter (DM), pH and biochemical constituents like protein and lipid (AOAC, 1990). The minerals such as total nitrogen, total phosphorus and total potassium were also determined (AOAC, 1990). Subsequently microbiological analysis of aerobic plate count was determined using standard culture medium. At the end of the ensiling process (30^{th} day), the vitamins and amino acid (AOAC, 1990; Vidotti *et al*., 2003) contents of silages were determined. Finally the individual silage products were dried in an oven (KEMI, KOS.1, Mumbai, India) at 40ºC for 12h to a weight consistency and packed in containers.

Fish Feed Preparation

A basic diet which contains 49% anchovy meal, 16% soy meal, 6% rice bran, 19% ground nut oil cake, 4% seaweed powder, 3% tapioca powder was prepared. The diet was then steam cooked in a closed aluminum container (<100ºC) for 20 min and then cooled rapidly to room temperature (28±1 ºC). To this basic diet 2% cod liver oil and 1% Nutrisan (vitamin and mineral mix tablets, Sadam India Ltd, Mumbai, India), 2ml stickon (binder) and 4% gelatin solution were added to make the control diet (C). Subsequently three different experimental diets with replacement of basal feed ingredients of 43% fish meal, 15% soy meal, 18% ground nut oil cake and along with 8% individual formic acid silage such as 2% (E1), 2.5% (E2) and 3% (E3) were added. All these diets containing 40% protein were prepared separately. The diets were made into noodles using a noodle – making machine having a perforation diameter of 2.5mm in the die. The noodles were dried at room temperature till the moisture content was reduced to <10% and were broken manually into small pieces. The dried pellets were packed in individual air tight plastic containers and used for further study. The proximate biochemical constituents such as protein, carbohydrate and lipid contents of prepared diets were determined (AOAC,1990).

Experimental Fish and Feeding Trials

The experimental fish, common carp (*Cyprinus carpio*) fingerlings were procured from a local fish breeding centre of Putheri, Kanyakumari District, South India. After 10 days of acclimatization in culture tanks, healthy carps with uniform size (2.42 to 2.50g) were divided into four groups (C – control, E1 - 2.0%, E2 - 2.5% and E3 - 3% of acid silage groups), each group contained 50 individuals in experimental re-circulatory water tanks (1000L capacity), and three replicates were arranged randomly for each group. The fish were starved for 24h before the commencement of the feeding experiment. During the experimental period, the water quality variables such as temperature (28±1ºC), pH (7.3±0.2), and DO (>5 mgL^{-1}) were maintained. Fish were fed with the respective diets at the rate of 10% of their body weight/day until the end of experiment. The daily food amount was adjusted twice a day (09.00 and 17.00h) @ 50 and 50%. The unconsumed food remains were removed 30 min before each feeding. Everyday, faecal pellets and other unwanted materials were siphoned off from the tanks; the feeding experiment was carried out for 45 days. Finally at the end of rearing experiment, body mass of fish reared in experimental diets were measured to determine the growth performance including specific growth rate (Immanuel *et al*., 2009) and finally the biochemical constituents of experimental fish were analysed (AOAC,1990).

Statistical Analysis

The data obtained in the present study were expressed as Mean±SD and were analyzed using One Way ANOVA test at 5% significant level. Further a multiple comparison test (SNK) was conducted to compare the significant differences amongst the parameters using computer software Statistica 6.0 (Statosoft, U.K).

Results and Discussion

Physicochemical Parameters of Ensilages

Maintenance of acidity in fish silage has the added advantage of keeping the product more hygiene and safety by inhibiting the growth of pathogenic organisms. In the present study, the pH of the acid ensilages decreased significantly ($P<0.05$) from the initial pH of 5.23±0.03 to 4.60±0.04 with respect to progressive increase in concentration of 2 to 3% formic acid. At the end of the experimental duration (30^{th} day), the pH level of silages was considerably reduced and recorded as 3.98±0.40, 3.60±0.30 and 3.42±0.04 at 2, 2.5 and 3% formic acid ensilages, respectively (Table 1). The value of pH obtained in the present study was in consonance with Hasan (2003), who observed the reduction of pH from 6.5 to 3.38 in 3% formic acid acidified fish mince of *Rastreliger brachysoma*. According to Yeoh (1979), the result on pH profile obtained in the present study is within the recommended range of successful fermentation.

In the present study, the dry matter composition of silages decreased gradually from the first phase of experiment. It was noticed that at the beginning of the experiment, the dry matter recorded in 2% formic acid ensilage was 19.15±0.30%, but it decreased to 9.33±0.30% at the end of the experiment. Maximum dry matter of 23.33±0.03% was observed in 2.5% acid ensilage during initial day, but it was 21.23±0.80% in 3% acid ensilage. When the ensilage process duration increased, a constant decrease in dry matter was noticed till the end of the experiment and attained 17.02 ±0.05 and 19.14±0.32% in 2.5 and 3% acid ensilages, respectively (Table 1). In agreement with the present findings, Hammoumi *et al*. (1998) stated that decrease in dry matter in the silage product relative to the initial raw material and also it may be due to hydrolysis of protein content by enzyme or microorganisms. In contrary, Hasan (2003) found only 6.7% reduction of dry matter content in 3% formic acid ensilage of mackerel *R. brachysoma* at the end of 30 days of ensilation process.

Result of ash content in the present experiment showed no significant ($P>0.05$) variation with respect to increase in experimental duration as well as increase in concentrations of acid. For instance, the initial ash content of the three groups of acid ensilage was between 14.23±0.07 and 14.60±0.02% (Table 1). In agreement with the present study, Martin and Benzerra (2004) also emphasized that formic acid ensilage made from minced tilapia *O. niloticus* has attributed 14.10% ash content after 30 days of experimental duration. A similar phenomenon of decreasing lower level of ash content was reported during formic acid ensilation of shrimp head silage

Table 1. Physico – chemical parameters of different concentrations of formic acid silages at various ensilaging days interval.

Parameters	Ensilation Days	Acid Silage (%)		
		2	2.5	3
pH	0	5.23 ± 0.03^{a}	4.80 ± 0.10^{a}	4.60 ± 0.04^{a}
	10	4.84 ± 0.50^{b}	4.05 ± 0.40^{b}	3.75 ± 0.30^{b}
	20	4.26 ± 0.30^{c}	3.85 ± 0.04^{c}	3.62 ± 0.30^{b}
	30	3.98 ± 0.40^{d}	3.60 ± 0.30^{d}	3.42 ± 0.04^{c}
Dry matter (%)	0	19.15 ± 0.30^{a}	23.33 ± 0.03^{a}	21.23 ± 0.80^{a}
	10	13.30 ± 0.70^{b}	19.29 ± 0.04^{b}	20.93 ± 0.30^{a}
	20	10.73 ± 0.20^{c}	17.91 ± 0.15^{c}	20.11 ± 0.21^{b}
	30	9.33 ± 0.30^{d}	17.02 ± 0.05^{d}	19.14 ± 0.32^{bc}
Ash (%) DM	0	14.60 ± 0.02^{a}	14.52 ± 0.04^{a}	14.23 ± 0.07^{a}
	10	14.41 ± 0.20^{a}	14.32 ± 0.02^{a}	14.18 ± 0.08^{a}
	20	14.32 ± 0.05^{a}	14.26 ± 0.05^{a}	14.11 ± 0.05^{a}
	30	14.24 ± 0.06^{a}	14.22 ± 0.01^{a}	14.04 ± 0.04^{a}
Protein (%) DM	0	40.62 ± 0.12^{a}	40.45 ± 0.06^{a}	40.38 ± 0.06^{a}
	10	40.13 ± 0.13^{a}	39.82 ± 0.06^{a}	38.72 ± 0.05^{a}
	20	39.46 ± 0.11^{a}	37.78 ± 0.07^{b}	37.44 ± 0.05^{b}
	30	38.40 ± 0.09^{b}	36.66 ± 0.06^{b}	36.06 ± 0.05^{b}
Lipid (%) DM	0	8.08 ± 0.07^{a}	8.19 ± 0.10^{a}	8.27 ± 0.12^{a}
	10	9.06 ± 0.03^{b}	9.35 ± 0.06^{b}	9.78 ± 0.08^{b}
	20	9.92 ± 0.06^{c}	10.08 ± 0.02^{c}	11.04 ± 0.07^{c}
	30	10.66 ± 0.04^{d}	11.19 ± 0.01^{d}	12.24 ± 0.05^{d}

DM = Dry matter; Each value is a mean ± SD of three replicate samples: values in the same column with different superscripts for each group are statistically significant (One–way ANOVA test $p<0.05$ and subsequent *post hoc* multiple comparisons with SNK test).

meal (Fox *et al*., 1994). This little reduction of ash content in the present study may be due to deprivation of inorganic constituents within the waste materials used for silage preparation.

The proximate biochemical constituents such as protein and lipid contents of the ensilages were studied (Table 1). Result displayed a significant (P<0.05) decrease of crude protein content of silages with respect to increase in experimental duration as well as increase in concentration of acid level. For instance, during the initial day (0 day) of the experiment, the crude protein content recorded with 40.62±0.12, 40.45±0.06 and 40.38±0.06% in 2, 2.5 and 3% formic acid ensilages, respectively. But at the end of the experiment, a fall in protein level was noticed with considerable level of 38.40±0.09, 36.66±0.06 and 36.06±0.05% in 2, 2.5 and 3% formic acid ensilages, respectively. Reduction of protein content in the ensilage may be due to break down / hydrolysis of protein (FAO, 2007). Vidotti *et al.* (2002) observed a reduction in crude protein level in combined (2% each of formic acid and sulfuric acid) fermented silage of tilapia filleting residue when compared to non-fermented tilapia filleting residue. Similarly decrease of crude protein level in the combined (1:1 ratio of formic and sulfuric acids) ensilage of tilapia filleting residue was noticed after 180 days of storage (Geron *et al.,* 2007).

The lipid content in the present study revealed that at the beginning of the experiment, it was between 8.08±0.07 and 8.27±0.12% in all the tested concentrations of formic acid silages. But when the experimental days prolonged, the lipid content significantly (P<0.05) increased and at the end of the experiment, the increase in lipid content was in between 10.66±0.04 and 12.24±0.05%, respectively in the three silages. The finding of lipid profile in the present study was supported by the work of Hasan (2003), who found a continuous increase (11.2 to 19.23%) of lipid content in 3% formic acid silage of *R. brachysoma* during the ensilation process upto 60 days. In the present study the continuous increase in lipid content from 2.58 to 3.97% in 2 -3% formic acid silage with respect to increase in storage period may be due to release of fats from the viscera of raw material used. In accordance to the present study, Dapkevicius *et al*. (1998) reported 3.6% increase in lipid content from 11.3 to 14.9% from initial to final stage (15 days) of storage of 3% formic acid ensilage of blue whiting.

Total Nitrogen, Mineral and Vitamin Contents of Ensilages

Results of total nitrogen in the present study did not show much variation (P>0.05) with respect to experimental duration and increase in formic acid concentrations. For instance, the initial nitrogen content recorded was within the range between 6.45±0.04 and 6.49±0.30% in 2 to 3% formic acid ensilages, but it reached from 5.76±0.03 to 6.13±0.01% in the above respective silages during the end of the experiment (Table 2). Here the marginal decrease in nitrogen content at the end of ensilation was due to breakdown and reduction of protein content. In support of this Raa and Gildberg (1982) reported the reduction of nitrogen content due to breakdown of proteins in acid digested silages. Analysis of minerals such as phosphorus, potassium and sodium revealed a substantial increase with respect to increase in experimental duration, whereas it decreased significantly (P<0.05) with increase in level of formic acid concentrations. Followed by nitrogen, a same trend was observed in total phosphorus, potassium and sodium from initial day of ensiling process with 0.90±0.04 to 1.03±0.03%

Table 2. Total Nitrogen and mineral contents of different concentrations of formic acid silages at various ensilaging days interval

Parameters	Ensilaging Days	Acid Silage (%)		
		2	2.5	3
Total Nitrogen (%) DM	0	6.49±0.30^{a}	6.48±0.05^{a}	6.45±0.04^{a}
	10	6.42±0.05^{a}	6.37±0.03^{a}	6.19±0.04^{a}
	20	6.30±0.08^{a}	6.04±0.06^{a}	5.98±0.05^{b}
	30	6.13±0.01^{a}	5.85±0.05^{a}	5.76±0.03^{b}
Total Phosphorus (%) DM	0	1.03±0.03^{a}	0.96±0.05^{b}	0.90±0.04^{b}
	10	1.41±0.02^{a}	1.05±0.02^{b}	0.98±0.10^{c}
	20	1.73±0.03^{a}	1.21±0.01^{b}	1.09±0.03^{c}
	30	1.88±0.08^{a}	1.39±0.04^{b}	1.27±0.03^{c}
Total Potassium (%) DM	0	0.30±0.05^{a}	0.29±0.02^{a}	0.26±0.04^{b}
	10	0.42±0.02^{a}	0.34±0.04^{b}	0.42±0.02^{a}
	20	0.68±0.05^{a}	0.59±0.04^{b}	0.60±0.05^{b}
	30	0.71±0.04^{a}	0.62±0.02^{b}	0.61±0.03^{b}
Total Sodium (%) DM	0	0.05±0.007^{a}	0.05±0.002^{a}	0.05±0.003^{b}
	10	0.14±0.01^{a}	0.13±0.02^{b}	0.12±0.02^{c}
	20	0.17±0.03^{a}	0.18±0.02^{b}	0.17±0.03^{ac}
	30	0.21±0.03^{a}	0.19±0.01^{b}	0.18±0.02^{c}

DM= Dry matter; Each value is a mean±SD of three replicate samples: values in the same row with different superscripts for each group are statistically significant (One way ANOVA test P<0.05 and subsequent *post hoc* multiple comparison with SNK test).

phosphorus, 0.26±0.04 to 0.30±0.05% potassium and 0.05±0.002 to 0.05±0.007% sodium in the respective formic acid silages. But with progressive increase in time duration, all the above contents considerably increased and recorded with 1.27±0.03 to 1.88±0.08% phosphorus, 0.61±0.03 to 0.71±0.04% potassium and 0.18±0.02 to 0.21±0.03% sodium at the end of the experiment (Table 2). The result of potassium content observed in the present study was higher than the result of Delgado *et al.* (2008), who reported only 0.1320 mg/kg (0.13%) potassium content in 200 ppm of THBQ Spanish mackerel silage. However, the sodium and phosphorus contents observed in the present study were consistently similar to the results of Delgado *et al.* (2008) in which they found to be 1895 mg/kg (0.18%) sodium and 24470 mg/kg (2.44%) phosphorous. In the present study, in addition to the mineral contents, an attempt was also made to analyze the vitamin contents in all the acid silages. Vitamin analysis inferred a better progression of Thiamine, Riboflavin, Pyridoxine, Cyano-cobalamine and Niacinamide with 323.56, 87.62, 189.74, 87.83 and 786.45 mcg/100g in 2% acid ensilage. Whereas in 3% acid silage Folic acid, Vitamin C, Vitamin A and Vitamin D recorded maximum with 133.28, 189.04, 386.82 and 6.34 mcg/100g, respectively. But in 2.5% acid silage the Vitamin E content recorded maximum with 3.60 mcg/100g (Table 3). At the end of 30 days of ensilation process, results of vitamin content showed significant (P<0.05) variation with respect to increase in concentrations of acid.

Amino Acid Composition of Silage

The nutritive value of protein of any ingredient depends mainly on the protein's capacity to fulfill the needs of organism with respect to essential amino acids (Vidotti *et al*., 2003). In the present study the proximate amino acid profile of the silages at the end of the ensiling process evidenced a significant (P < 0.05) increase in level of amino acids in 2% acid ensilage than the other respective ensilages. Among the identified amino acids, glycine (3.56g/100g), threonine (3.23g/100g), arginine (2.45g/100g), serine (2.34g/100g) and tyrosine (2.34g) recorded with higher level. Geron *et al.* (2007) showed a similar trend of higher level of amino acids such as arginine, glycine, tyrosine, threonine and serine in combined acid silage of tilapia filleting residue. Likewise, Vidotti *et al.* (2003) have identified higher concentration of glycine, arginine and lysine in 2% formic and sulfuric acid silage of tilapia residue. In the present study, considering the aminoacid profile of the ensilages, cystine and tryptophan levels were lower than the other identified aminoacids (Table 4). In accordance with these, Dapkevicius *et al.* (1998) also stated that acid hydrolysis method used for silage preparation lead to partial destruction of cystine. Similarly Arason (1994) indicated relatively a similar report that tryptophan is unstable in acid medium; therefore it becomes the first limiting aminoacid in fish acid silage. In the present study, followed to tryptophan, taurine, aspargine, methionine, valine, proline and histidine recorded lower level in all the acid silages. Dapkevicius *et al.* (1998) recorded lower levels of methionine, histidine and proline in 3% formic acid ensilage of Blue whiting (*M. poutassou* Risso). Thus the reduction of amino acid contents noticed in increasing concentrations of acid ensilages in the present study might have occurred due to some chemical reactions between α amino and aldehyde groups present in the amino acid during ensilation process (Johnson *et al*., 1985).

Microbiological Analysis of Ensilages

The result on aerobic plate count of different silages is presented in Figure 1. In the first day of ensiling process, the aerobic plate count load observed was 19 to 26 x 10^{-4} cfu ml^{-1} in 2-3% formic acid ensilages, but with subsequent increase in ensiling process, a decrease in microbial population was observed in all the silages and at the end of the experimental period, the microbial load recorded was 3 to 8 x 10^{-4} cfu ml^{-1} in 2-3% acid ensilages, respectively. This may be due to reduction of pH with respect to increase in acidity and increase in ensilaging duration. A similar result of reduction of

Table 3. Vitamin content (mcg/100g) of different concentrations of formic acid silage after 30 days of ensilation.

Vitamins (mcg/100g)	Acid silage (%)		
	2.0	2.5	3.0
B1 – Thiamine	323.56±4.56^{a}	125.86±2.23^{b}	112.35±2.46^{c}
B2 – Riboflavin	87.62±1.68^{a}	76.53±1.38^{b}	56.40±1.22^{c}
B6 – Pyridoxine	189.74±2.12^{a}	178.63±2.74^{b}	177.65±1.88^{b}
B12- Cyanocobalamine	87.83±1.48^{a}	45.64±1.14^{b}	32.30±0.84^{c}
Niacinamide	786.45±4.84^{a}	567.85±3.76^{b}	332.47±2.66^{c}
Folic acid	87.85±1.40^{a}	121.20±1.85^{b}	133.28±1.34^{c}
Vitamin C	98.30±0.82^{a}	125.53±1.20^{b}	189.04±1.65^{c}
Vitamin A (Retinol)	364.10±2.90^{a}	381.10±2.86^{a}	386.82±3.02^{ab}
Vitamin D	2.95±0.02^{a}	3.20±0.01^{b}	6.34±0.04^{c}
Vitamin E	2.30±0.03^{a}	3.60±0.04^{b}	3.53±0.01^{b}
Vitamin K	BDL	BDL	BDL

BDL - Below Detectable Level. Each value is a mean±SD of three replicate samples: values in the same row with different superscripts for each group are statistically significant (One way ANOVA test P<0.05 and subsequent *post hoc* multiple comparison with SNK test).

Table 4. Aminoacid content (g/100g) of different concentrations of formic acid silages after 30 days of ensilation

Amino acids (g/100g)	Acid silages (%)		
	2.0	2.5	3.0
Aspartic acid	1.32 ±0.01[a]	1.123±0.02[b]	1.120±0.01[b]
Glutamic acid	1.67±0.01[a]	1.498±0.01[b]	1.323±0.02[c]
Asparagine	0.565±0.002[a]	0.756±0.004[b]	0.7897±0.003[b]
Serine	2.34±0.02[a]	0.112±0.003[b]	0.321±0.001[c]
Glutamine	1.57±0.06[a]	0.434±0.005[b]	0.321±0.004[c]
Glycine	3.56±0.01[a]	0.323±0.003[b]	0.1121±0.008[c]
Threonine	3.23±0.06[a]	0.112±0.005[b]	0.2012±0.002[c]
Alanine	1.676±0.02[a]	0.304±0.004[b]	0.0543±0.0002[c]
Arginine	2.456±0.02[a]	0.432±0.006[b]	0.1123±0.004[c]
Cystine	0.280±0.004[a]	0.232±0.002[b]	0.1034±0.001[c]
Tyrosine	2.345±0.003[a]	0.443±0.001[b]	0.1146±0.003[c]
Histidine	0.787±0.002[a]	0.332±0.004[b]	0.2106±0.002[c]
Valine	0.786±0.004[a]	0.121±0.004[b]	0.1021±0.003[c]
Methionine	0.765±0.001[a]	0.312±0.002[b]	0.11221±0.001[c]
Isoleucine	1.786±0.02[a]	0.331±0.003[b]	0.1043±0.005[c]
Phenylalanine	1.45±0.02 [a]	0.1014±0.003[b]	0.1212±0.006[c]
Leucine	0.896±0.006[a]	0.1042±0.002[b]	0.1043±0.002[b]
Lysine	2.12±0.03[a]	0.43±0.008[b]	0.1212±0.004[c]
Proline	0.786±0.002[a]	0.113±0.006[b]	0.1045±0.004[c]
Tryptophan	0.456±0.005[a]	0.1212±0.004[b]	0.1033±0.006[c]
Taurine	0.475±0.002[a]	0.221±0.006[b]	0.1121±0.008[c]

Each value is a mean±SD of three replicate samples; values in the same row with different superscripts for each group are statistically significant (One way ANOVA test $P < 0.05$ and subsequent *post hoc* multiple comparison with SNK test).

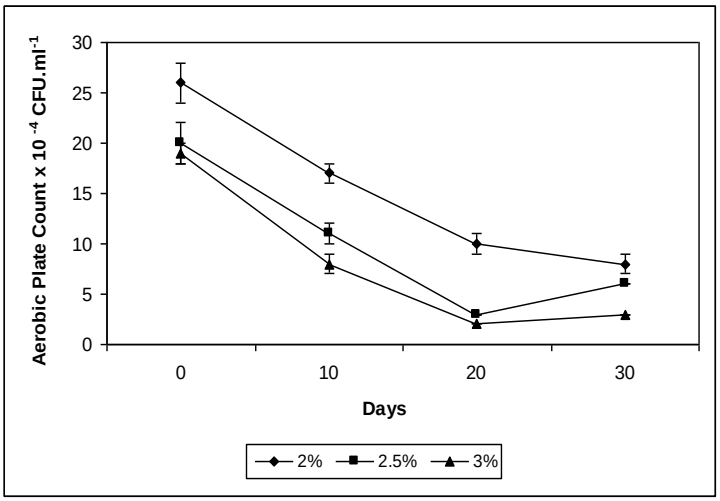

Figure 1. Aerobic plate count of different concentrations of formic acid silages at various fermentation days interval.

aerobic mesophiles and coliforms in the Spanish mackerel silage was observed due to low pH maintenance during the process (Delgado *et al*., 2008).

Proximate Biochemical Composition of Experimental Diets

Proximate biochemical composition of experimental diets supplemented with different concentrations of formic acid silages is given in Table 5. Crude protein level of the control and experimental diets was around 40%. Nwanna *et al.* (2004) observed a similar range of protein in diet made with combined acids (2 % each of formic and ethanoic acids) ensiled shrimp head meal. In the present study, the lipid content of the experimental diets was significantly ($P<0.05$) varied between 5.02±0.02 and 5.25±0.04%, whereas in the control diet, it was 4.15±0.05%. Wassef (2005) also reported a similar composition of lipid content in diet prepared with lactic acid silage of gilthead sea bream fry. The carbohydrate (10.86±0.20 to 10.94±0.05%) and ash content (13.90±0.50 to 14.61±0.02%) in both the control and experimental diets did not vary significantly. Inconsistent to the present results, Nwanna *et al.* (2004) pointed out a higher ash content of about 15 to 16.75% in the series of diets prepared with chemically preserved shrimp

head meal.

Growth Performance of Fish *C. carpio* Fingerlings

Growth responses of *C. carpio* fingerlings fed on experimental diets showed significantly (P<0.05) higher weight gain than the control (Table 6). This result was in accordance with Srinivasan *et al.* (1985), who observed a double weight gain in fish *C. carpio* fry that fed upon silage based experimental diets as compared to control. In the present study fish fed on 2% acid silage supplemented experimental diet had the highest weight gain (2.38±0.018 g) and SGR (1.49±0.036%). For instance, the growth performance of 2.5 and 3% acid silages incorporated diets fed *C. carpio* displayed 2.01 and 1.87g weight gain and 1.31 and 1.26% SGR, respectively. At the same time, the control diet fed carp had the weight gain of 1.49g and the SGR of 1.06%. The better growth performance of the silage based diets may be due to the presence of comparatively higher amount of free amino acids and active hydrolytic enzymes than the control diet with fish meal (Gallagher, 1993). Thus in the present study, the better weight gain and SGR obtained with 2% formic acid diet may also attributed to its higher content of aminoacids and appropriate vitamin profile.

Comparatively less growth performance of tilapia fed on 2.5 and 3% silage incorporated diets shown high level of acidity in diets, high proportion of free aminoacids and hydrolyzed proteins (Stone *et al*., 1989). On the other hand loss of essential aminoacids might also contribute poor protein utilization and digestibility and eventually results in poor growth. This observation supports the work of Fagbenro and Jauncey (1998), who reported that low amount of available aminoacid, would cause a lower protein utilization and digestibility resulting in less fish growth.

Biochemical Composition of *C. carpio* after Feeding Experiment

Biochemical analysis at the end of the experiment is frequently used to determine the influence of feed on fish carcass biochemical composition. Results obtained from the present study showed, that the fish fed on experimental diets had significantly (P<0.05) higher protein, and lipid contents than the control diet fed fish. Maximum carcass protein (48.25%) and lipid (3.62%) contents were recorded in 2% acid silage diet fed fish. At the same time, the carbohydrate (3.93%) and ash (11.86%) contents were relatively less than control diet fed fishes. The control diet fed fish showed comparatively lower level of protein (40.44±0.01%) and lipid (2.72±0.02%) contents, but carbohydrate and ash contents were high (4.23 and 12.25%) (Figure 2). Carcass biochemical composition of *C. carpio* obtained in the present study supported the results of Nwanna *et al*. (2004), who inferred that African cat fish *C. gariepinus* fingerlings that fed on chemically preserved shrimp head waste silage diets had significant increase in weight gain, specific growth rate and mineral deposition. Based on the results obtained in the present study, it could be attributed that inclusion of 2% formic acid diet had better growth performance and nutrient utilization.

Conclusion

The present findings clearly pointed out the successful replacement of basal diet with 2% formic acid silage product significantly augmented weight gain and SGR of the experimental fish *C. carpio*

Table 5. Proximate biochemical composition of control and experimental diets fed to common carp fingerlings

Biochemical Components	Control diet (C)	Experimental diets 2% (E1)	2.5% (E2)	3% (E3)
Protein (%)	40.04 ± 0.02^{a}	40.13 ± 0.05^{a}	39.98 ± 0.01^{a}	40.08 ± 0.07^{a}
Lipid (%)	4.15 ± 0.05^{a}	5.02 ± 0.02^{b}	5.14 ± 0.02^{bc}	5.25 ± 0.04^{bcd}
Carbohydrate (%)	10.91 ± 0.01^{a}	10.94 ± 0.05^{a}	10.90 ± 0.04^{a}	10.86 ± 0.20^{a}
Ash (%)	13.90 ± 0.50^{a}	14.53 ± 0.03^{a}	14.58 ± 0.50^{a}	14.61 ± 0.02^{a}

Each value is a mean ± SD of three replicate samples: values in the same row with different superscripts are statistically significant (One way ANOVA test P<0.05 and subsequent *post hoc* multiple comparison with SNK test).

Table 6. Growth performance of *C. carpio* fingerlings fed on control and experimental diets

Growth Parameters	Control diet (C)	Experimental diets 2%	2.5%	3%
Initial weight (g)	2.42 ± 0.038	2.47 ± 0.046	2.50 ± 0.024	2.45 ± 0.018
Initial length (cm)	5.49 ± 0.046	5.51 ± 0.062	5.54 ± 0.060	5.48 ± 0.042
Final weight (g)	3.91 ± 0.052^{a}	4.85 ± 0.026^{b}	4.51 ± 0.034^{c}	4.32 ± 0.072^{cd}
Weight gain (g)	1.49 ± 0.024^{a}	2.38 ± 0.018^{b}	2.01 ± 0.026^{c}	1.87 ± 0.014^{d}
Final length (cm)	5.77 ± 0.034^{a}	6.28 ± 0.026^{b}	5.95 ± 0.068^{c}	5.85 ± 0.056^{cd}
SGR (%)	1.06 ± 0.012^{a}	1.49 ± 0.036^{b}	1.31 ± 0.044^{c}	1.26 ± 0.028^{c}

Each value is a mean ± SD of three replicate samples: values in the same row with different superscripts are statistically significant (One way ANOVA test P <0.05 and subsequent *post hoc* multiple comparison with SNK test). Values in parenthesis indicate the weight gain (g) of fish fed on respective diets.

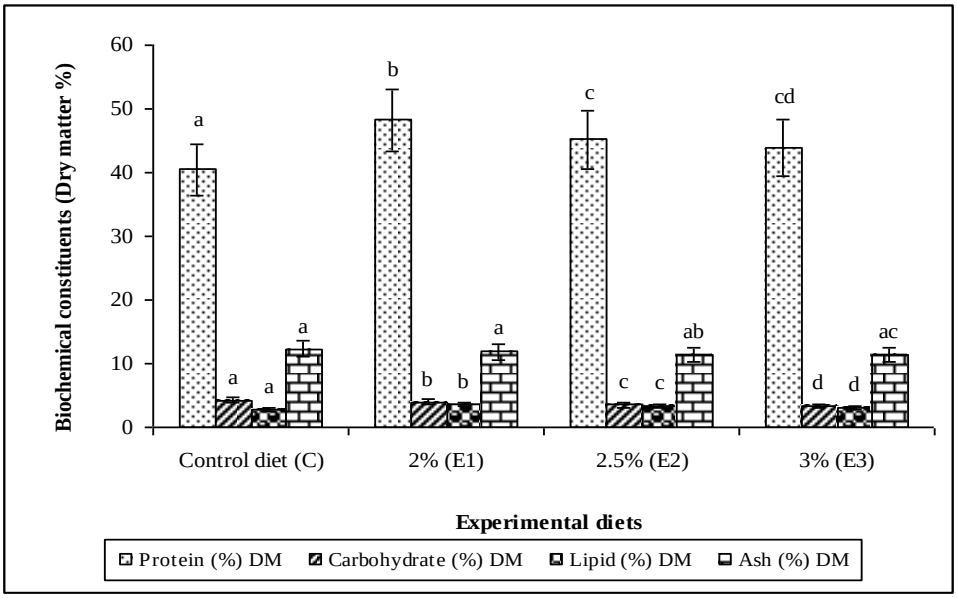

Figure 2. Biochemical composition of fish *C. carpio* fingerlings fed on control and experimental diets. Each value is the mean ± SD of three replicates samples; In each biochemical constituents bars with different alphabets are statistically different from each other (One way ANOVA test $p < 0.05$ and subsequent *post hoc* multiple comparison with SNK test).

fingerlings. Moreover it concludes that acid fish silage prepared from the processing wastes could effectively be utilized as fish feed stuff and indicates its potential means of minimizing fishmeal and reducing possible environmental pollution.

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