EFFECTS OF USING FISH BIOSILAGE AS FISH MEAL REPLACER ON FEEDING, GROWTH AND GUT HISTOLOGY IN COMMON CARP *Cyprinus carpio* L. FINGERLINGS

Basim M. Jasim Sajed S. Al-Noor Salah M. Najim

Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Basrah, Iraq.

(Received June 2014 ,Accepted 22 June 2014)

Keywords; Biosilage, fingerlings, protein.

ABSTRACT

This study was carried out to evaluate the effects of locally produced fish biosilage as fish meal alternative on feeding, growth efficiency and gut histology in common carp *C. Carpio* fingerlings. Biosilage was prepared by fermenting marine by-catch fish with date fruit residues, domestic vinegar and citric acid. The produced biosilage was incorporated in feeds to replace 0, 25, 50 or 75% of fish meal protein. Fish were fed for 14 weeks and feeding and growth parameters were close in the four feed groups so as fish survival rate during the experiment (88.9-93.3%). Histological examination of intestine and liver sections has showed improvements when fish fed on the four different feeds. Initial fish group showed signs of nutritional deficiency through limited size of intestinal villi and hepatocytes. However, the histological structure of gut was improved after fish were fed on the experimental feeds without significant differences between fish meal or fish biosilage feeds. The study concluded that fish silage could replace fish meal without adverse effects on feeding, growth efficiency and gut histology.

INTRODUCTION

The cost of feed represents an important proportion of fish farming operational costs. Protein sources are the major contributors in fish feed cost. Fish meal is the preferred dietary protein source for many farmed fish and shrimp species because of its amino acid balance, vitamin content,palatability and unidentified growth factors (Majumdar *et al.*, 2014). However, fish meal supplies witnessed significant fluctuations in supplies and thus in prices during the last decade. This encouraged the search for fish meal alternatives from plant and animal sources. Plant materials suffer from low digestibility, high fibre content and antinutritional factors which limit their use effectively in aquaculture feeds [3, 12]. Animal protein concentrates like blood meal and meat and bone meal were banned due to the outbreaks of BSE disease [5].

All the above mentioned reasons make fish biosilage a viable alternative for fish meal in aquaculture. Fish biosilage is defined as a liquid product produced from the whole fish or parts of it, to which acids or lactic acid-producing bacteria are added, with the liquefaction of the mass provoked by the action of enzymes from the fish [2]. It is characterized by similar or even better proximate composition in comparison with fish meal. The high quality content of fish oil rich in PUFA fatty acids makes fish silage an excellent source of essential fatty acids. It could be made easily even at farm level from different raw materials like by-catch fish or fish wastes without need for advanced technology [10, 11].

Histological analysis of the digestive system is considered a good indicator of the nutritional status of fish. The intestine and liver are the most important organs in digestion and absorption of nutrients from food, and therefore monitoring of these organs is considered necessary.For this, various methods of histological analysis are used, most often semi-quantitative scoring system, histochemical and immunohistochemical method, while stereological methods are rarely used [19, 21]. This study was carried out to investigate feeding, growth and general histological structures of intestine and liver in common carp fingerlings in response for different partial replacements of fish meal by fish biosilage produced from local raw materials.

MATERIALS AND METHODS

Fish biosilage was produced using marine by-catch fish obtained from marine shrimp fisheries at Al-Fao city southern Basrah. Ensiling fermentation process was carried out by adding date fruit residues (10%) as carbohydrate substrate, domestic vinegar (20%) as an acidulant and inoculant and citric acid as starting acidifying agent. Ensiling mixture was incubated at $35^{0}C$ for 10 days. Fish meal was produced by the standard method from the same fish sample for comparison purposes [3].

Fingerlings of the common carp (5.81± 0.29 gm.) were obtained from fish farm at Marine Science Center, University of Basrah. Upon arrival to the fish hatchery at the Department of Fisheries and Marine Resources fish were distributed in culture system of 12 glass aquariums (60 x 30 x 42 cm) each containing about 57 liters of dechlorinated tab water, acclimatized to the laboratory conditions for 3 days and to the experimental diets for 2 other days then redistributed on aquariums at 15 fish/ aquarium. The experiment included 4 treatments with 3 replicated aquariums for each. Feeds A, B, C and D were designed according to [15] criteria to be isonitrogenous (35% crude protein) and isocaloric (4400 Kcal/kg) replacing 0, 25, 50 and 75% of fish meal protein content by fish biosilage (table 1). Each aquarium was equipped with air flow and thermostat controlled heater fixed at 28± 1^{0}C. Fish were fed 5% of body weight daily twice daily (8 am and 2 pm) sex days a week. About 30% of aquarium water was changed daily before morning feeding. Fish were weighed biweekly and feed ration was adjusted accordingly. Specific growth rate (SGR) and feed conversion efficiency (FCR) were calculated according to [6]. Fish survival was monitored also during this experiment which lasted for 14 weeks from 6 October 2013 to 12 January 2014. Water quality parameters (temperature, oxygen, salinity, pH and nitrate and ammonia concentrations) were monitored daily and maintained within the suitable ranges for this species [14, 16].

Table 1. Feed formulation and proximate composition using fish biosilage as a partial replacement for fish meal.

Feedstuff %	Feed formulation			
	A	B	C	D
Fishmeal	34	25.5	17	8.5
Fish silage	0	10.1	20.2	30.3
Soybean meal	15	15	15	15
Corn meal	15	15	15	15
Barley flour	18	18	18	18
Wheat bran	11	11	11	11.2
Corn oil	5	3.4	1.8	0
Premix*	2	2	2	2
Proximate composition, %				
Moisture	6.50±0.52	6.88±0.64	6.91±0.49	7.15±0.81
Protein	36.63±1.17	35.41±1.79	35.24±1.58	34.42±0.92
Lipid	10.90±0.91	11.28±0.85	11.15±1.04	12.09±1.11
NFE	35.76±2.69	34.84±1.71	35.58±2.15	34.41±2.17
Ash	11.21±0.83	11.59±1.21	11.12±1.50	11.93±1.43
Energy, Kcal/kg	4473 ± 33	4402 ± 45	4411 ± 38	4404 ± 39
P/E Ratio, mg/Kcal	81.90±1.54	80.43±1.71	79.88±1.66	78.15±1.67

*Vapcomix, VAPCO Veterinary and agricultural product manufacturing Co., Amman, Jordan.

Histological study was carried out on fish before and after feeding on the four experimental feeds. Three specimens of liver and mid intestine were fixed in Bouin's fluid for 72 hours. Thereafter, specimens were washed, dehydrated in ascending series of ethanol, cleared and imbedded in paraffin overnight. Paraffin blocks were sectioned at 7μm by a rotary microtome and stained with hematoxylin-eosin (HE) stain [23].Stained sections were observed and images were captured using Leica EZ4 HD stereomicroscope provided with LAS EZ 2.0.0 software for Windows® (Leica Microsystems, GmbH, Wetzlar, Germany).

The data were analyzed by one-way analysis of variance (ANOVA, F test) using IBM® SPSS® version 19. The differences between means were tested by least significant difference LSD test on SPSS with significance level of $p \leq 0.05$. All means with standard deviations are produced from at least three replicates

RESULTS AND DISCUSSION

Table (2)presents feeding and growth performance parameters for common carp fingerlings fed the four experimental diets with different replacement ratios of fish meal by fish silage (0, 25, 50 and 75% of fish meal protein). Weight gain was ranged from 20.42 gm. in feed C group to 22.30 gm. in feed B group with significant differences ($p < 0.05$) between the four feed treatments.

Table 2. Feeding and growth performance of common carp *C. Carpio* fingerlings feed different experimental feeds.

Parameter	Experimental feed			
	A	B	C	D
Initial wt., gm.	5.98± 0.286	5.96± 0.309	5.46± 0.311	6.11± 0.374
Final wt., gm.	27.71± 1.47	28.26± 1.80	25.88± 1.88	27.12± 2.03
Weight gain, gm.	21.73± 1.12^{a}	22.30± 1.07^{b}	20.42± 1.30^{c}	21.01± 1.65^{d}
SGR	1.825± 0.105^{a}	1.853± 0.151^{b}	1.852± 0.148^{bc}	1.774± 0.217^{d}
FCR	2.40± 0.088^{a}	2.42± 0.067^{a}	2.49± 0.087^{a}	2.51± 0.089^{b}
Survival rate, %	93.3± 2.11^{a}	91.1± 3.17^{b}	93.3± 2.11^{a}	88.9± 4.33^{c}

SGR, specific growth rate; FCR, feed conversion ratio. Values in the same raw which carry different superscript letters are significantly ($p \leq 0.05$) different.

Values of SGR ranged between 1.774 and 1.853 in feed D and B groups, respectively. Significant differences ($p < 0.05$) were found between the various treatments except B and C ($p > 0.05$). Feed conversion ratio FCR showed values between 2.40 in feed A and 2.51 in feed D groups which was the only treatment that differed significantly ($p < 0.05$) from others. Fish survival rate was differed significantly ($p < 0.05$) from 88.9% in feed D to 93.3% in both feed A and C groups.

Al-Faraje [1] reported SGR values of 0.788-1.098 and FCR 4.5-7.8 with total replacement of animal protein by lactic acid fermented fish silage (Khishni *Liza abu*) in feeds for common carp fingerlings. These values are lower than those reported in the current study. The potential reasons for these differences are lower protein (13.99-28.86), lipid (3.86-6.92) and thus caloric (3800-3900 Kcal/kg) contents in his feeds in comparison with the current study. Several previous studies indicated that replacement ratio of fish meal by fish silage in fish feed should not exceed 50% of dietary protein to obtain better growth [4, 7]. Ramasubburayan [17] reported SGR values of 1.06-1.49 for common carp fingerlings fed diets with different fish silage contents (0-3%). They concluded that addition of fish silage had improved fingerling growth and that fish silage prepared from the processing wastes could be utilized as feed stuff with a potential of minimizing fishmeal and reducing possible environmental pollution. These values are lower than those reported by the current study. This may be ascribed to the lower inclusion levels of fish silage (maximum 3% in comparison with 10-30% in the current study) and fish silage type (formic acid silage vs. biosilage in the present study).

Dapkevicius [4] and Fagbenro [7] indicated that fish silage may become more advantageous for fish feeding if it replace between 25-50% of dietary protein. The same authors pointed out that biological fish silage has better digestibility of protein, higher quality of fish oil and the activity probiotic LAB bacteria with its metabolites that improve digestion, immunity and general health of fish. This rapprochement may be explained by using fish as a main component in fish meal replacing materials i.e. biosilage and ribotricin, in both studies. The results of feeding and growth efficiency in the current study are in line with several previous studies which indicated that fish biosilage is a suitable alternative for fish meal in fish feeds without negative impacts on feeding and growth efficiency if it added with the proper ratio [4, 7, 22].

Figures (1A and B) show intestine section in initial fish and feed A group. It can be observed that villi were shorter (average height 42 vs. 137 µm, respectively) and narrower (average width 37 vs. 49 µm, respectively) in initial fish in comparison with feed A group. This means lower absorptive area in initial fish intestine. All four feed groups (A, B, C and D) showed similar developed villi structures in comparison with initial fish as shown in figure 2 for feed D group. Differences between initial fish and feed groups may be attributed initially to the age of fish but more likely to the nutritional status. Fingerlings in hatchery stocking ponds did not often receive enough nutrition for economic reasons. However, significant improvements were occurred in intestine villi histology when fish fed on different experimental diets. Fish silage addition did not affect adversely on intestine villi histology which is agree with several previous studies [6, 7,20].

Histological examination of hepatic tissue of initial and feed A fish groups (figures 3A and 3B) showed significant differences in hepatocyte diameter (5.91-9.39 µm, respectively) and hepatocyte nucleus diameter (2.33-3.11 µm, respectively). No adverse alterations were observed in hepatic tissue of fish feed different silage feeds (B, C and D). Liver histology in initial fish group, although normal in general, reflect poor growth and the smaller hepatocytes may indicate low reserves of glycogen and fat as it improved significantly when fish were fed the different diets.

Liver histopathological examination is one of the powerful tools that could be used effectively to monitor fish health in general [19]. As liver is the main gland associated with the digestive system in fish, liver histological changes can reveal any adverse effects of feed components especially the novel or innovated ingredients [18]. Many previous studies showed that replacing fish meal with fish silage did not lead to any adverse effects on liver histology in several cultured fish species [6, 8, 13]. This agrees with the results of the current study where replacing up to 75% of fish meal protein with fish biosilage in common carp fingerling feeds did not result in any adverse alterations or abnormalities in fish liver structure (Figure 4). The nutritional adequacy of fish biosilage, as indicated by the histological examination of fish intestine and liver in the current study, may be ascribed partially to its content of fish oil rich in polyunsaturated fatty acids that reported to enhance general metabolism, tissue structural integrity, nutritional and health status of fish [9, 24, 25].

In conclusion, locally prepared fish biosilageis a good candidate as a partial fish meal alternative in common carp feeds based on feeding and growth efficiency parameters as well as histological study of intestine and liver in fish.

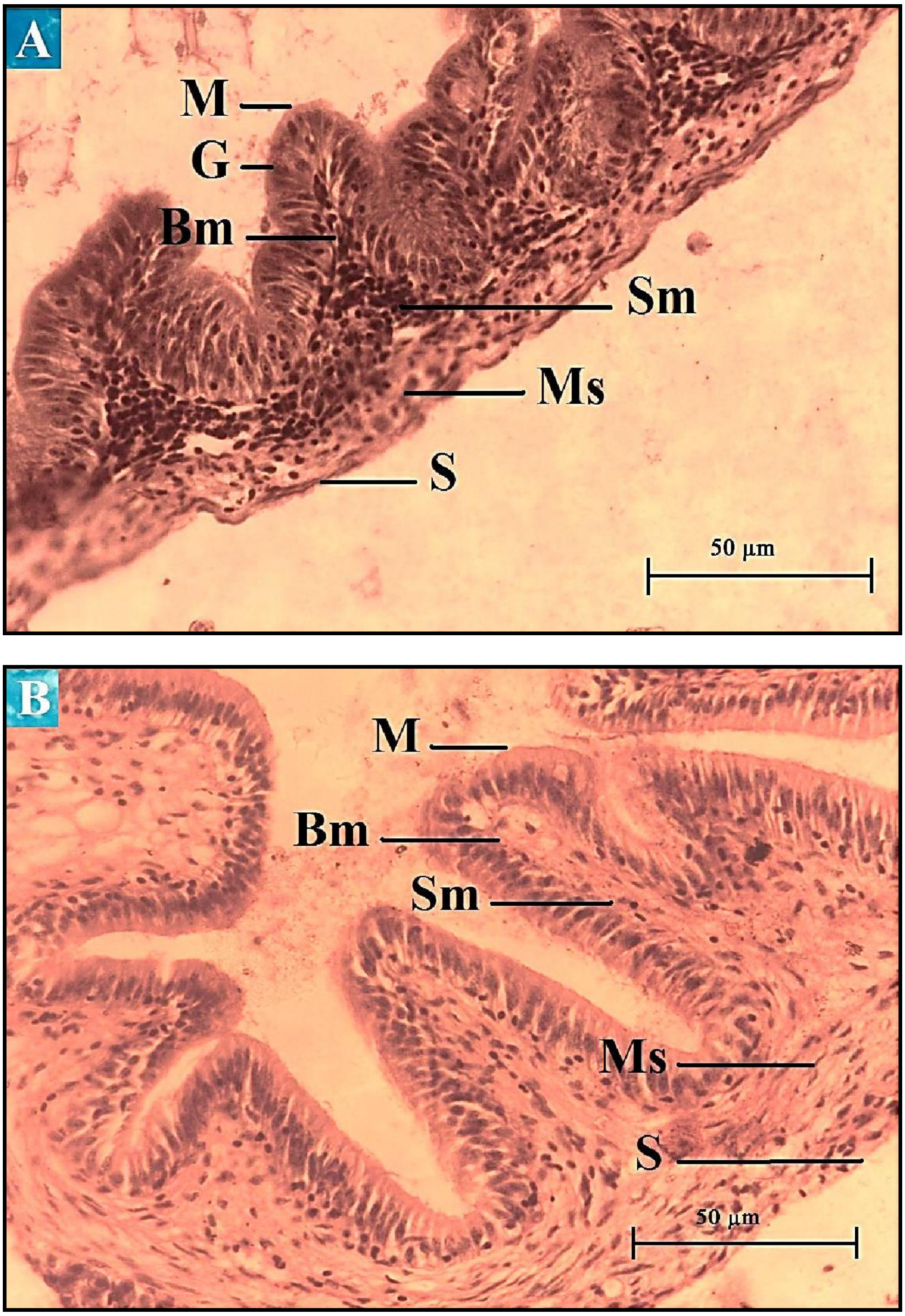

Figure 1. Cross section in fish intestine showing normal structures and size variation in fold size.(A). initial fish group. (B) Feed A fish group. M, mucosa; Bm, basement membrane; G, goblet cell; Sm, submucosa; Ms, muscularis; S, serosa (HE, 400X).

Figure 2. Cross section showing normal structures in intestine of fish in feed D fish group (75% fish biosilage). M, mucosa; Bm, basement membrane; Sm, submucosa; Ms, muscularis; S, serosa (HE, 400X).

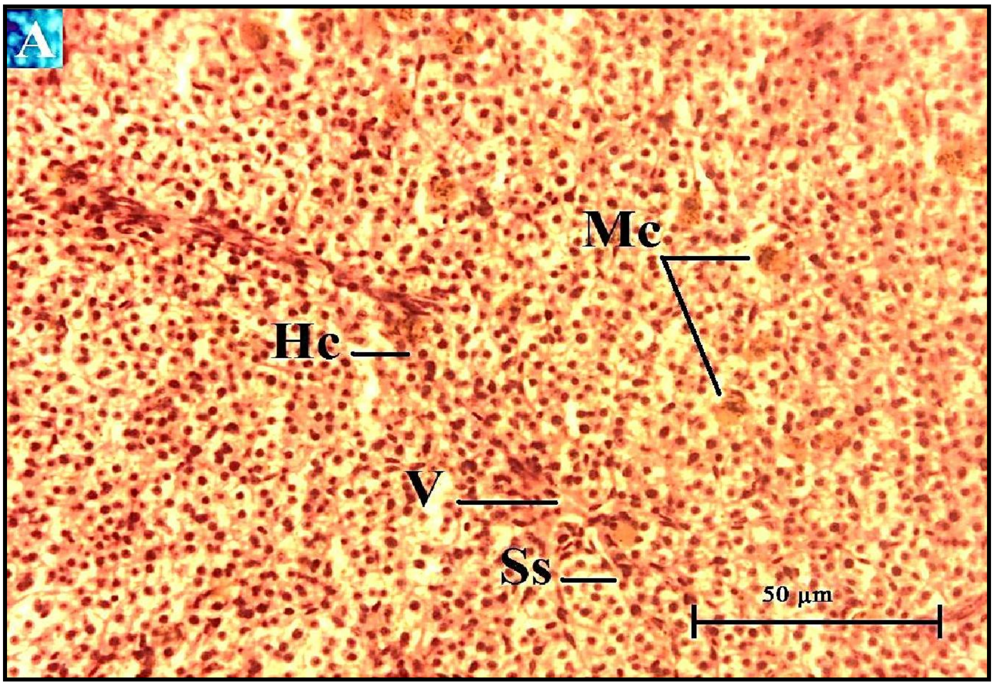

Figure 3A. Fish liver histology showing normal structures and hepatocyte size variation in initial fish group. C, Capsule; Hc, hepatocyte; Hp, hepatopancreas; Mc, macrophage centre; Ss, sinusoid; V, vein. (HE, 400X).

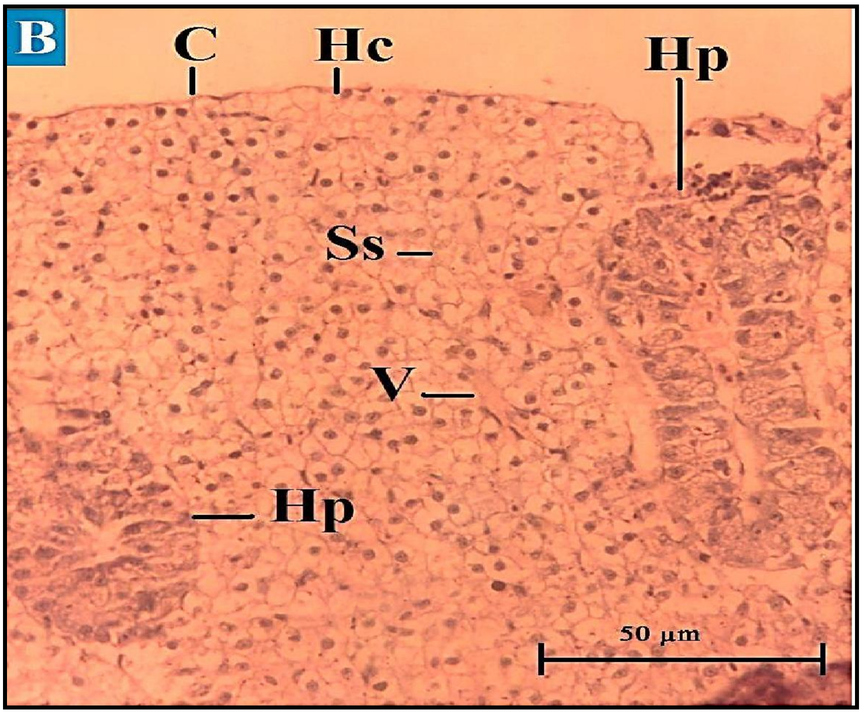

Figure 3B. Fish liver histology showing normal structures and hepatocyte size variation in feed A fish group. C, Capsule; Hc, hepatocyte; Hp, hepatopancreas; Mc, macrophage centre; Ss, sinusoid; V, vein. (HE, 400X).

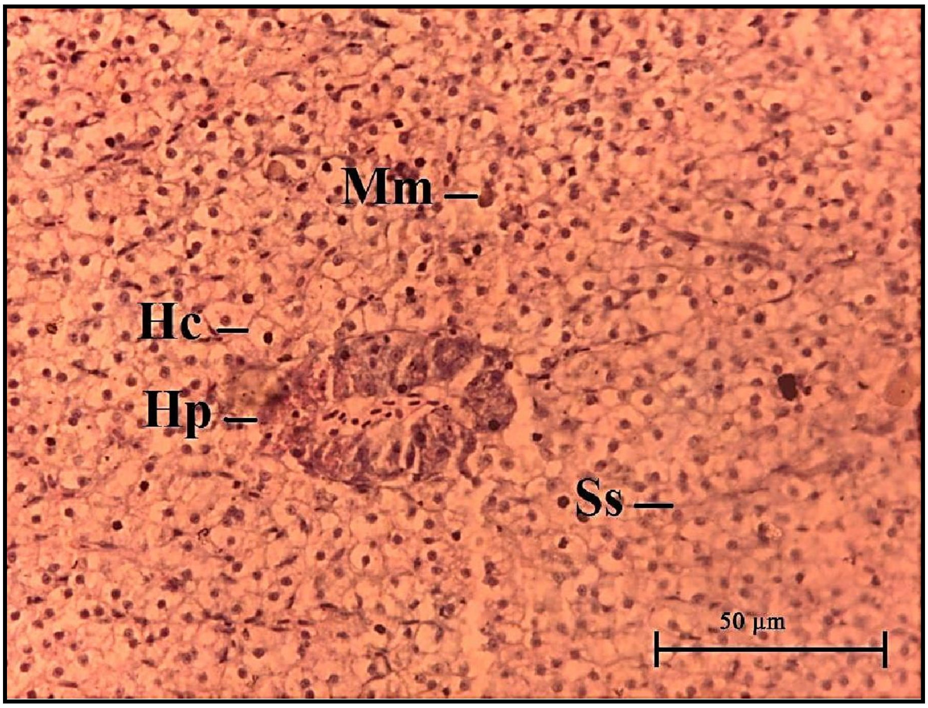

Figure 4. Fish liver histology showing normal structure of hepatocytes in Feed D fish group (75% fish biosilage). Hc, hepatocyte; Hp, hepatopancreas; Mm, melanomacrophage; Ss, sinusoid; (HE, 400X).

تأثير استخدام السيلاج السمكي الحيوي كبديل لمسحوق السمك على التغذية والنمو والتركيب النسجي للقناة الهضمية في أصبعيات الكارب العادي *Cyprinus carpio L.*†

باسم محمد جاسم ساجد سعد النور صلاح مهدي نجم*

قسم الأسماك والثروة البحرية، كلية الزراعة، جامعة البصرة، البصرة، العراق

الخلاصة

أجريت الدراسة الحالية لتقييم تأثيرات استبدال مسحوق السمك بالسيلاج السمكي المنتج محليا على كفاءة التغذية والنمو والتركيب النسيجي للأمعاء في إصبعيات الكارب العادي *C. carpio* . حضر السيلاج الحيوي بتخمير أسماك الصيد الثانوي البحري مع ثفل التمر والخل المنزلي وحامض الستريك.أضيف السيلاج الحيوي المنتج إلى الأعلاف لاستبدال 0 أو 25 أو 50 أو 75% من بروتين مسحوق السمك. غذيت الأسماك لمدة 14 أسبوعاوكانت مقاييس التغذية والنمو متقاربة جدا في مجموعات العلف الاربعة وكذلك معدل بقاء الأسماك أثناء التجربة (88.9-93.3 %). أظهر الفحص النسيجي لمقاطع الأمعاء والكبد تحسنا حين غذيت الأسماك على الأعلاف الأربعة المختلفة. أظهرت مجموعة أسماك البداية علامات نقص التغذية من خلال الحجم المحدود للطيات المعوية والخلايا الكبدية، ومع ذلك، تحسن التركيب النسيجي للقناة الهضمية بعد أن غذيت الأسماك على الأعلاف التجريبية بدون اختلافات معنوية بين علف مسحوق السمك وأعلاف السيلاج السمكي الحيوي. استنتجت الدراسة الحالية أن السيلاج السمكي الحيوي يمكن أن يكون بديلا لمسحوق السمك دون تأثيرات سلبية على كفاءة التغذية والنمو والتركيب النسجي للقناة الهضمية.

REFERENCES

1 Al-Faraje, J.K.A. (2000). Production of dried fish silage by lactic acid fermentation and its nutritional performance in the growth of common carp *Cyprinus carpio* fingerlings. M.Sc. Thesis. Coll. Agric., Univ. Baghdad. 79p.

2 Arruda, L.F.; Borghesi, R. and Oetterer, M. (2007). Use of fish waste as silage – A Review. Brazil. Arch. Biol. Technol., 50(5): 879-886.

3 Atanasoff, A.P. (2014). Replacement of fish meal by ribotricin indiets of carp *Cyprinus carpio*. Mac. Vet. Rev., 37 (1): 55-59.

4 Dapkevicius, M.L.N.E. (2002). Biological ensilage of fish. Optimization of stability, safety and functionality. PhD Thesis. Wageningen Univ., Wageningen. 169p.

5 De Vos, C. J. and Heres, L. (2009). The BSE risk of processing meat and bone meal in non-ruminant feed: a quantitative assessment for the Netherlands. Risk Anal., 29(4): 541-557.

6. El-Ajnaf, S.M. (2009). Development of fermented fish silage derived from pelagic sardine and apple pomace by-product for inclusion in diets for the European seabass (*Dicentrarchus labrax*). PhD. Dissertation. Univ. Plymouth. 201p.

7. Fagbenro, O. (1994). Studies on the use of fermented fish silage in diets for juvenile tilapia (*Orechromis niloticus*) and catfish (*Clarias gariepinus*). PhD thesis. Univ. Stirling, Scotland. 200p.

8. Fagbenro, O. and Jauncey, K. (1995b) Growth and protein utilization by juvenile catfish fed dry diets containing co-dried lactic-acid-fermented fish-silage and protein feedstuffs. Bioreso. Technol., 51(1): 29-35.

9. Fard, E.R.; Kamarudin, M.S.; Ehteshami, F.; Zadeh, S.S.; Saad, C.R. and Zokaeifar, H. (2014). Effect of dietary Linolenic acid (18:3*n*–3)/Linoleic acid (18:2*n*–6) ratio on growth performance, tissue fatty acid profile and histological alterations in the liver of juvenile *Tor tambroides.* Iran. J. Fish. Sci., 13(1): 185-200.

10. Ghaly, A.E.; Ramakrishnan, V.V.; Brooks, M.S.; Budge, S.M. and Dave, D. (2013). Fish processing wastes as a potential source of proteins, amino acids and oils: A critical review. J. Microbiol. Biochem. Technol., 5: 107-129.

11. Goosen, N.J.; de Wetb, L.F.; Gorgensa, J.F.; Jacobs, K. and de Bruyn, A. (2014). Fish silage oil from rainbow trout processing waste as alternative to conventional fish oil in formulated diets for Mozambique tilapia *Oreochromis mossambicus*. Animal Feed Sci. Technol., 188: 74-84.

12. Lall S.P. and Anderson S. (2005). Amino acid nutrition of salmonids: Dietary requirements and bioavailability. In: Montero, D.; Basurco, B.; Nengas, I.; Alexis, M. and Izquierdo, M. (Eds.). Mediterranean fish nutrition. Zaragoza : CIHEAM, Cah. Opt.Mediter., 63: 73- 90.

13. Majumdar, R.K.; Deb, S. and Nath, K.B. (2014). Effect of co-dried silage from fish market waste as substitute for fish meal on the growth of the Indian major carp *Labeo rohita* (Hamilton, 1822) fingerlings. Indian J. Fish., 61(4): 63-68.

14. Markovic, Z.; Dulic, Z.; Zivic, I. and Mitrovic-Tutundzic, V. (2009). Influence of abiotic and biotic environmental factors on weight gain of cultured carp on a carp farm. Arch. Biol. Sci., 61: 113–121.

15. National Research Council (NRC). (2011). Nutrient Requirements of Fish and Shrimp. The National Academy Press, Washington, D.C. 376p.

16. Rahman, M.M.; Jo, Q.; Gong, Y.G.; Miller S.A. and Hossain M.Y. (2008). A comparative study of common carp (*Cyprinus carpio* L.) and calbasu (*Labeo calbasu* Hamilton) on bottom soil resuspension, water quality, nutrient accumulations, food intake and growth of fish in simulated rohu (*Labeo rohita* Hamilton) ponds. Aquaculture , 285 (1-4): 78–83.
17. Ramasubburayan, R.; Iyapparaj, P.; Subhashini, K.J.; Chandran, M.N.; Palavesam, A. and Immanuel, G. (2013). 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. Turk. J. Fish. Aquat. Sci., 13:281-289.
18. Raskovic, B.S.; Stankovic, M.B.; Markovic, Z.Z. and Poleksic, V.D. (2011). Histological methods in the assessment of different feed effects on liver and intestine of fish. J. Agric. Sci., 56 (1): 87-100.
19. Raskovic, B.; Jaric, I.; Koko, V.; Spasic, M.; Dulic, Z.; Markovic, Z. and Poleksic, V. (2013). Histopathological indicators: a useful fish health monitoring tool in common carp (*Cyprinus carpio* Linnaeus, 1758) culture. Cent. Eur. J. Biol., 8(10): 975-985.
20. Reyes-Becerril, M.; Ascencio-Valle, F.; Macias, M.E.; Maldonado, M.; Rojas, M. and Esteban, M.A. (2012). Effects of marine silages enriched with *Lactobacillus sakei* 5-4 on haematoimmunological and growth response in Pacific red snapper (*Lutjanus per*) exposed to *Aeromonas veronii*. Fish Shellfish Immunol., 33(4): 984-992.
21. Segner, H.; Sundh, H.; Buchmann, K.; Douxfils, J.; Sundell ,K.S.; Mathieu , C.; Ruane, N.; Jutfelt, F.; Toften, H. and Vaughan, L. (2012). Health of farmed fish: its relation to fish welfare and its utility as welfare indicator. Fish Physiol. Biochem., 38: 85-105.
22. Soltan, M.A. and El-Laithy, S.M. (2008). Evaluation of fermented silage made from fish, tomato and potato by-products as a feed ingredient for Nile tilapia, *Oreochromis niloticus*. Egypt. J. Aquat. Bio. Fish., 12 (1): 25 – 41.
23. Spencer, L.T. and Bancroft, J.D. (2013). Microtomy: Paraffin and frozen. In: Suvarna, S.K.; Layton, C. and Bancroft, J.D. (Eds.). Bancroft's theory and practice of histological techniques. 7th ed. Churchill Livingstone, Elsevier Ltd. London, pp: 125-138.

24. Torstensen, B.E. and Tocher, D.R. (2010). The effects of fish oil replacement on lipid metabolism of fish. In: Turchini, G.M; W.-K., Ng and Tocher, D.R. (Eds.). Fish oil replacement and alternative lipid sources in aquaculture feeds. CRC press, Boca Raton, FL, pp: 405-437.

25. Turchini, G.M.; Torstensen, B.E. and Ng, W.-K. (2009). Fish oil replacement in finfish nutrition. Rev. Aquacult., 1(1): 10-57.