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A Mathematical Relationship between Dietary Fumaric Acid Level and the Fatty Acids Composition of Common Carp *(Cyprinus Carpio* L.).

Osama M El-Husseiny, Alaa I Obayes*, Rania S Mabroke, and Ashraf Suloma.

Fish Nutrition Lab, Animal production Department, Fac. Agriculture, Cairo University, Egypt.

ABSTRACT

This investigation was designed to study the mathematical relationship between dietary fumaric acid level and the fatty acids composition of common carp *(Cyprinuscarpio*L.). The study was designed in 2x3 factorial experiment arrangement of two lipid levels (2%; LL and 9%; HL) and three levels of fumaric acid $HO_2CCH=CHCO_2H$ (0.0, 0.5 and 1.0 %). F^0LL, F^0HL, $F^{0.5}$LL, $F^{0.5}$HL, $F^{1.0}$LL, $F^{1.0}$HL. The relationships between the whole body fatty acids composition and fumaric acid levels in experimental diets were evaluated by applying linear and polynomial regression analysis.ARA profile increased with elevation of dietary fumaric (R^2 =0.881) under conditions of low dietary lipid. Where a low relation were noticed for fish fed on low dietary lipid (R^2 = 0.111).weak relation recognized between dietary fumaric and DHA profile was recognized for fish fed on low dietary lipid (R2= 0.283).On the other hand, A significant positive effect was observed (R^2 = 0.935) in the presence of high dietary lipid condition. With elevation of dietary lipid, more incubation of dietary fumaric acid up to 1% negatively affected sum of saturated fatty acid (R^2= 0.407) and sum of mono saturated fatty acid profile (R^2 = 0.601).High inclusion of dietary fumaric in the presence of high dietary lipid encourage the improvement in DHA profile and decrease the presence of saturated and mono saturated fatty acids.

Keywords: Common carp, Fumaric acid, Fatty acids and DHA.

Corresponding author

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INTRODUCTION

Most developing countries are located in tropical or sub-tropical areas, and fish is a vital component of food security for these countries [1,2]. The production and consumption of freshwater fish, has increased during recent. Fish provided more than 2.9 billion people with almost 20 percent of their intake of animal protein [3]. Therefore effort is needed to improve the output performances and quality of the most important tropical freshwater fish [4].

Lipids are an important component in fish and human diets, both as energy and fatty acids (FA) sources. Among the FA, particular emphasis has been placed on the n-3 and n-6 polyunsaturated fatty acids (PUFA). Polyunsaturated omega-3 (n-3) fatty acids, eicosapentaenoic acid (EPA, C-20:5) and docosahexaenoic acid (DHA, C-22:6), are of interest because their benifical effect on prevention and treat of hard diseases , hypertention , diabetes , arthritis , inflammatory , autoimmune disorder rand cancer [5]. Since these fatty acids composition may vary among fish species, it is necessary to determine both the lipid content and the PUFA distribution. The polyunsaturated FA (PUFA) showed to be around 17.55%. in carp which was the lowest in comparsion with other fishes as Mullet (*Mugil cephalus*) and trout (*Oncorhynchus mykiss*), 29.1 % , 43.13 % respectively [5]. Freshwater fish have higher levels of w 6 fatty acids than marine species due to the composition of the food chain [6]. As a result, many studies were established to improve the fatty acid profile of carp by trying to elevate the percentage of ω_3. Some studies suggested that the finishing feeding strategy is a viable way to reduce the use of fish oil in feed and lipid quality of farmed fish could be maintained at a high level. Meanwhile that exploiting the ability of carp to biosynthesise n-3 HUFA de novo. These sources can be rapeseed mouldings, rapeseed oil or cake, linseed and hempseed [6]. Meanwhile feed manufacturing process could effectively affect fatty acid profile of carp, where, the extruded feed diet led to 69% greater n-3, and 53% lower n-6 fatty acid contents in comparison with grains and pelted feed [7]. Using feed additive to manipulate fatty acid profile is another direction which was followed in our study.

Acidifier is a term that describes the organic acids and their salts. The use of organic acid salts or blends is an interesting option to promote the growth performance and health of a wide variety of aquaculture species worldwide. Organic acids and their salts may be promising feed additives that can indirectly improve the utilization of plant protein diet. The mode of action of acidifier's acts in two directions: on one hand they reduce the bacterial growth and mold in feedstuff to preserve hygienic quality [8]. On the other hand acidifiers affect the media conditions of gastrointestinal tract and reduce the PH in the stomach which positivity affects the pepsin activity and improving protein digestion. Positive effects of organic acids on protein hydrolysis have been demonstrated. Meanwhile, acid and accumulation of salts anions inhibit bacteria growth [9]. There is some evidence that acidifiers improve the bioavailability of minerals and improve the absorption of calcium and phosphorus [10]. It was indicated that dimethylfumarate have the ability to activate nuclear factor (erythroid-derived 2)-related factor (NrF2). That further augments the natural antioxidant responses in multiple sclerosis tissue [11]. Nrf2 inhibits lipid accumulation and oxidative stress in mouse liver after feeding a high- fat diet, probably by interfering with lipogenic and cholesterologenic pathways [12]. This investigation was designed to study the mathematical relationship between dietary fumaric acid level and the fatty acids composition of common carp *(Cyprinus carpio* L.)

MATERIALS AND METHODS

The present study was carried out at the Fish Nutrition Lab. (FNL), Department of Animal Production Faculty of Agriculture, Cairo University, Egypt.

Rearing techniques

Common carp with an initial body weight of 0.43 g were obtained from Kafr El-Sheik governorate, Egypt. Prior to the start of the experiment, fish was acclimatized to laboratory conditions for 10 days and fed basal diet of 30 % CP. The fingerlings were stocked into 18 cylindrical plastic tanks (with water capacity of 50 L each) at a rate of 15 fish $tank^{-1}$, representing six experimental treatments. The tanks were supplied with well water source. Aeration was continuously provided using an air blower. The experiment was lasted for 63 days. Six days $week^{-1}$ fish was fed the experimental diets 40% CP ad lip. Fish in each replicate aquarium was weighed every 15 days.

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Experimental diets

The proximate composition of the experimental diets is tabulated in Table (1). The study was designed in 2x3 factorial experiment arrangement of two lipid levels (2%; LL and 9%; HL) and three levels of fumaric acid $HO_2CCH=CHCO_2H$ (0.0, 0.5 and 1.0 %). F^0LL, F^0HL, $F^{0.5}$LL, $F^{0.5}$HL, $F^{1.0}$LL, $F^{1.0}$HL.

Table 1: Formulation and chemical composition of the experimental diets.

Ingredients	F^0LL	F^0HL	$F^{0.5}$LL	$F^{0.5}$HL	$F^{1.0}$LL	$F^{1.0}$HL
FM	20	20	20	20	20	20
SBM	50	50	48	48	46	46
Corn	26	19	27.5	20.5	29	22
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Oil	2	9	2	9	2	9
BHT	0.04	0.04	0.04	0.04	0.04	0.04
Premix[1]	1	1	1	1	1	1
Vit.c	0.06	0.06	0.06	0.06	0.06	0.06
CMC [2]	0.4	0.4	0.4	0.4	0.4	0.4
Fumaric[3]	0	0	0.5	0.5	1	1
Total %	100	100	100	100	100	100
Moisture	6.04	6.90	6.94	5.36	7.62	5.68
% Protein	43.06	43.69	46.75	42.90	45.73	41.84
Fat	10.12	15.33	12.45	15.91	10.79	15.01
Ash	7.60	7.51	7.71	7.64	7.65	7.25
NFE[4]	32.04	31.35	26.15	28.19	28.20	30.22
Energy[5] **(kcal/kg)**	4708.67	5204.25	4886.44	5081.74	4759.62	5023.16

Where 1; Premix supplied the following minerals (g kg-1 of diet) and vitamins (IU or mg kg-1 of diet): $CuSO_4$-$5H_2O$, 2.0 g; $FeSO_4$-$7H_2O$, 25 g; $ZnSO_4$-$7H_2O$, 22 g; $MnSO_4$-4H2O, 7 g; Na_2SeO_3, 0.04 g; KI, 0.026 g; $CoCl_2$-$6H_2O$, 0.1 g; Vitamin A, 900,000 IU; Vitamin D, 200,000 IU; Vitamin E, 4,500 mg; Vitamin K3, 220 mg; Vitamin B1, 320 mg; Vitamin B2, 1,090 mg; Vitamin B5, 2,000 mg; Vitamin B6, 500 mg; Vitamin B12, 1.6 mg; Vitamin C, 5,000 mg; Pantothenate, 1,000 mg; Folic acid, 165 mg; Choline, 60,000 mg.
2 Carboxy methyl cellules; 3 Fumaric acid: WINLAB laboratory chemicals leicestershire,le16 9ej.U.K; 4NFE= 100 – (Protein+ Lipid + Ash + Moisture); 5Calculated based on the standard physiological fuel values: 5.6 kcal g-1 for protein, 9.4 kcal g-1 for lipid and 4.2 kcal g-1 for carbohydrate.

Fatty acid analysis

Lipid Extraction

By the end of the experiment the smallest eight fish from all treatments were sacrificed and freeze dried far fatty acid analysis weigh 2-20g of the sample into a 250 ml centrifuge bottle, add sufficient water to bring total water present to 16 ml together with 40 ml methanol and 20 ml chloroform. Macerate for 2 min; add further 20 ml chloroform and macerate for 30 sec; add 20 ml water and macerate again for 30 sec. centrifuge the mixture for 10 min at 2000-2500 rpm. Draw off the lower chloroform layer and filter through a coarse filter paper into a dry weighed flask or beaker. Evaporate the chloroform to dryness [13].

Methylation of lipid

In a tube weigh 50 mg of lipid, add 5 ml of methanolic sulphuric acid (1 ml conc. sulphuric acid and 100 ml methanol) and 2 ml of benzene, close the tube well and place in water bath at 90c for an hour and half. Cool, add 8 ml water and 5 ml petroleum ether skake strongly and separate out the ethereal layer in a dry tube. Evaporate to dryness [14].

Statistical analysis

The relationships between the whole body fatty acids composition and fumaric acid levels in experimental diets were evaluated by applying:

- Linear regression analysis as described by [15]. The analysis was based upon standard simple linear regression taking Y as the response variable and x as a sole explanatory variable.

The used model Y=a+bx, Where a; is the intercept and b; is the parameter of interest.

- 2) Nonlinear regression using a second degree polynomial resulting in the following relationship:

$$Y=ax^2+bx+c$$

The linear and polynomial regressions were calculated using Excel 2007 software (Microsoft, Seattle, WA).

RESULTS AND DISCUSSION

Body fatty acid composition of common carp

Regression between dietary fumaric level and fatty acid profile of common carp are summarized in Table (2 and 3).

Polynominal regression model had best fitted the relation between dietary fumaric level and fatty acid profile of common carp fed on high and/or low dietary lipid.

Most saturated fatty acid decreased with increasing level of dietary fumaric up to (1%). The opposite trend was noted for fish fed high dietary lipid except for C8:0 fatty acid. The regression data did not vary between dietary lipid levels.

Table 2: The relationship between fatty acids composition of common carp fed low lipid diets with dietary fumaric acid level of the experimental diets calculated by linear and polynomial regressions.

	POLYNOMIAL	LINEAR
C8:0	$y = 0.813 x^2 - 0.573x + 0.21 (R^2 = 0.781)$	$y = 0.24x + 0.142 (R^2 = 0.399)$
C10:0	$y = 0.006 x^2 - 0.13x + 0.62 (R^2 = 0.065)$	$y = -0.123x + 0.619 (R^2 = 0.065)$
C11:0	$y = -1.353 x^2 + 1.13x + 0.76 (R^2 = 0.539)$	$y = -0.223x + 0.872 (R^2 = 0.133)$
C12:0	$y = -1.5 x^2 + 1.31x + 2.216 (R^2 = 0.122)$	$y = -0.19x + 2.341 (R^2 = 0.019)$
C14:1	$y = 0.033 x^2 + 0.103x + 0.186 (R^2 = 0.305)$	$y = 0.136x + 0.183 (R^2 = 0.304)$
C14:0	$y = -0.02 x^2 - 0.57x + 2.173 (R^2 = 0.468)$	$y = -0.59x + 2.175 (R^2 = 0.468)$
C16:1	$y = 1.486 x^2 - 1.716x + 3.423 (R^2 = 0.408)$	$y = -0.23x + 3.299 (R^2 = 0.091)$
C16:0	$y = -2.926 x^2 + 0.903x + 19.19 (R^2 = 0.741)$	$y = -2.023x + 19.44 (R^2 = 0.631)$
C18:2C	$y = 0.533 x^2 - 1.006x + 23.89 (R^2 = 0.039)$	$y = -0.473x + 23.84 (R^2 = 0.035)$
C18:1	$y = 1.413 x^2 + 0.9x + 24.07 (R^2 = 0.349)$	$y = 2.313x + 23.95 (R^2 = 0.338)$
C18:0	$y = -1.213 x^2 + 3.053x + 8.116 (R^2 = 0.188)$	$y = 1.84x + 8.217 (R^2 = 0.181)$
C20:4	$y = 1.853 x^2 - 1.306x + 3.113 (R^2 = 0.881)$	$y = 0.546x + 2.958 (R^2 = 0.450)$
C21:0	$y = -0.086 x^2 + 0.11x + 0.62 (R^2 = 0.011)$	$y = 0.023x + 0.627 (R^2 = 0.005)$
C22:6	$y = 0.126 x^2 + 0.256x + 1.113 (R^2 = 0.283)$	$y = 0.383x + 1.102 (R^2 = 0.280)$
C22:2	$y = 1.566 x^2 - 1.896x + 3.596 (R^2 = 0.198)$	$y = -0.33x + 3.466 (R^2 = 0.069)$
Sum sat	$y = 0.866 x^2 - 0.72x + 4.736 (R^2 = 0.091)$	$y = 0.146x + 4.664 (R^2 = 0.023)$
Sum mono	$y = 2.966 x^2 - 0.803x + 28.23 (R^2 = 0.333)$	$y = 2.163x + 27.98 (R^2 = 0.288)$

Table 3: The relationship between fatty acids composition of common carp fed high lipid diets with dietary fumaric acid level of the experimental diets calculated by linear and polynomial regressions.

	POLYNOMIAL	LINEAR
C8:0	$y = 1.246 x^2 - 1.463x + 0.503 (R^2 = 0.764)$	$y = -0.216x + 0.399 (R^2 = 0.203)$
C10:0	$y = 1.233 x^2 - 1.583x + 0.806 (R^2 = 0.693)$	$y = -0.35x + 0.703 (R^2 = 0.340)$
C11:0	$y = 2.353 x^2 - 2.943x + 1.51 (R^2 = 0.626)$	$y = -0.59x + 1.313 (R^2 = 0.269)$
C12:0	$y = 1.653 x^2 - 1.866x + 2.446 (R^2 = 0.558)$	$y = -0.213x + 2.308 (R^2 = 0.093)$
C14:1	$y = -0.273 x^2 + 0.263x + 0.206 (R^2 = 0.179)$	$y = -0.01x + 0.229 (R^2 = 0.002)$
C14:0	$y = 1.546 x^2 - 2.053x + 2.17 (R^2 = 0.957)$	$y = -0.506x + 2.041 (R^2 = 0.539)$
C16:1	$y = 1.446 x^2 - 1.783x + 2.616 (R^2 = 0.510)$	$y = -0.336x + 2.496 (R^2 = 0.201)$
C16:0	$y = 6.946 x^2 - 9.073x + 18.55 (R^2 = 0.932)$	$y = -2.126x + 17.97 (R^2 = 0.493)$
C18:2C	$y = -17.08 x^2 + 14.93x + 24.90 (R^2 = 0.711)$	$y = -2.146x + 26.32 (R^2 = 0.113)$
C18:1	$y = -5.306 x^2 + 9.933x + 23.68 (R^2 = 0.652)$	$y = 4.626x + 24.12 (R^2 = 0.588)$
C18:0	$y = 0.946 x^2 + 0.486x + 8.14 (R^2 = 0.614)$	$y = 1.433x + 8.061 (R^2 = 0.592)$
C20:4	$y = -0.266 x^2 + 0.326x + 2.486 (R^2 = 0.111)$	$y = 0.06x + 2.508 (R^2 = 0.042)$
C21:0	$y = -0.44 x^2 + 0.606x + 0.506 (R^2 = 0.735)$	$y = 0.166x + 0.543 (R^2 = 0.465)$
C22:6	$y = -0.893 x^2 + 1.68x + 1.056 (R^2 = 0.935)$	$y = 0.786x + 1.131 (R^2 = 0.845)$
C22:2	$y = -0.966 x^2 + 1.236x + 3.72 (R^2 = 0.218)$	$y = 0.27x + 3.800 (R^2 = 0.105)$
Sum sat	$y = -0.993 x^2 + 1.55x + 4.383 (R^2 = 0.407)$	$y = 0.556x + 4.466 (R^2 = 0.321)$
Sum mono	$y = -4.593 x^2 + 9.143x + 26.87 (R^2 = 0.601)$	$y = 4.55x + 27.25 (R^2 = 0.554)$

Arachidonic acid (ARA) profile increased with elevation of dietary fumaric acid (R^2 =0.881) at low dietary lipid. A little relation was observed for fish fed on slow dietary lipid (R^2 = 0.111). Little relation between dietary fumaric and Decosahexaenoic Acid (DHA) profile was recognized for fish fed on low dietary lipid (R2= 0.283). A significant positive effect was observed (R^2 = 0.935) in the presence of high dietary lipid condition. Elevation of dietary lipid, more incubation of dietary fumaric acid up to 1% negatively affected sum of saturated fatty acid (R^2= 0.407) and sum of mono saturated fatty acid profile (R^2 = 0.601).

Dietary acidification may reduce the rate of gastric emptying [16], insequence, improve the digestion and feed utilization, even in the presence of high dietary lipid. The authors suggested that, as carp fish is a stomach less fish, digestive enzymes lipase and pancreatic enzymes are active under PH of around 7. Acidity of fumaric acid under condition of low dietary lipid may lead to suppression of lipase activity, subsequently digestion and absorption of fatty acids. The opposite may hypothesized under high dietary lipid where the effect of fumaric acid could be modulated by high dietary lipid. Coated acidifiers are an innovative, where product is coated and protected by a matrix of fatty acids. It is rather stable and slow-released so that an acidic condition is expectedly maintained along the gastrointestinal tract. Particularly, protected acidifiers can reach the hind gut and inhibite pathogenic bacteria [17]. According to [18], the technology of microencapsulation and coating is exploited to help avoid loss of feed palatability. Regarding our results, to produce common carp to act as functional feed, a modification in fatty acid profile and elevation the ω_3 level is needed. Fumaric acid in presence of high dietary lipid may be a promising tool for produce highly ω_3 common carp.

CONCLUSION

From regression datd, high inclusion of dietary fumaric acid in the presence of high dietary lipid encourage the improvement in DHA profile and decrease the presence of saturated and mono saturated fatty acids.

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