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Full Length Research Paper

Functional alteration of breast muscle fatty acid profile by manipulation of dietary *n-6:n-3* ratios in broiler chickens

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Breast muscle fatty acid (FA) profile was studied in broiler chickens fed at different levels of *n-6:n-3* polyunsaturated fatty acid (PUFA) ratios in 4 treatment groups; very high level of *n-6:n-3* ratios (VH), high level of *n-6:n-3* ratios (H), low level of *n-6:n-3* ratios (L), very low level of *n-6:n-3* ratios (VL) and control, respectively. All the birds were slaughtered at 42 days of age and breast muscle were collected. FA profile of breast muscles was determined by gas liquid chromatography. Increased levels of fish oil significantly (P < 0.05) increased the long chain PUFA (LC-PUFA) *n-3* level, mainly because of the docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) content in the VL and L groups. *n-6*: *n-3* ratios were effectively changed by the experimental diets. The LC- PUFA n-3 content of the VL and L groups of the breast muscle was significantly (P < 0.05) lower than in the VH, H and control groups. In conclusion, an increase in PUFAs *n-3* associated with the dietary supplementation of fish oil maybe consider as a functional approach to elevate the nutritional value of chicken meat with regards to human nutrition.

Key words: Fatty acid, LC-PUFA *n-3*, chicken, fish oil.

INTRODUCTION

The fast growing demand of omega-3 enriched meat and meat products, lead producers to include more vegetable and marine oil sources in animal diets. Several studies have shown that, increasing monounsaturated fatty acid (MUFA) of diet can prevent cardiovascular disease by lowering the low density lipoprotein cholesterol content of the blood plasma and reducing the susceptibility of low-density lipoprotein to oxidation (Kris-Etherton et al., 1999). Researchers showed that, saturated fatty acid (SFA) and cholestrol are closely correlated to occurrence of cardiovascular and heart diseases (Sacks and Katana, 2002; Mozaffarian et al., 2010). It is widely reported that, there is an urgent need to return to a balanced fatty acid diet by decreasing the consumption of saturated fats (Evans et al., 2002; Mozaffarian et al., 2010). In the last decades, many studies were focused to reduce fat,3 cholesterol and SFA contents of poultry meat by dietary supplementation with garlic (Konjufca et al., 1997), copper (Pesti and Bakalli, 1996), α-tocopherol acetate (Ashgar et al., 1989) and *n-3* fatty acid (Ayerza et al., 2002). Polyunsaturated fatty acids, in particular long-chain PUFA *n-3* have favourable effects on human health (Sacks and Katana, 2002; Simopoulos, 1999). Vegetable sources, such as linseed oils and rapeseed oil, may increase the *n-3* FA content of diet as linolenic acid, but this elevation did not proceed efficiently to increase the LC-PUFA *n-3* content in chicken meat (Ajuyah et al., 1993; Scaife et al., 1994). Therefore, marine source of fat with high level of *n-3* fatty acid specially long chain *n-3* polyunsaturated fatty acid came out into view as a potential functional source to manipulate *n-3* fatty acid in chicken meat (Hargis and Van Elswyk, 1993).

Supplementation of fish oil in human diets were

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Table 1. Ingredient and nutrient composition of diets.

Ingredient (%)	Starter (1 to 21 days)					Finisher (22 to 42 days)				
	Control	VL	L	H	VH	Control	VL	L	H	VH
Corn	44.91	45.86	45.75	45.55	45.42	49.90	51.55	51.3	51.1	50.97
Soybean meal	43.95	43.47	43.49	43.53	43.55	38.67	38.34	38.39	38.43	38.46
Palm oil	6.58	0.00	0.00	0.00	0.00	7.31	0.00	0.00	0.00	0.00
sunflower oil	0.00	0.50	2.00	3.50	4.50	0.00	0.50	2.00	3.50	4.50
Tuna oil	0.00	5.50	4.00	2.50	1.50	0.00	5.50	4.00	2.50	1.50
DCP	1.91	1.91	1.91	1.91	1.91	1.77	1.77	1.77	1.77	1.77
Limestone	1.20	1.20	1.20	1.20	1.20	1.06	1.06	1.06	1.06	1.06
Common salt	0.44	0.44	0.44	0.44	0.44	0.31	0.31	0.31	0.31	0.31
Vitamine permix[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Mineral permix[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.25	0.25	0.25	0.25	0.25	0.23	0.23	0.23	0.23	0.23
L-lysine	0.26	0.26	0.26	0.26	0.26	0.15	0.15	0.15	0.15	0.15
Calculated analysis[2]										
Crude protein	22.00	22.00	22.00	22.00	22.00	20.50	20.50	20.50	20.50	20.50
ME (Cal/Kg)	3080.00	3080.00	3080.00	3080.00	3080.00	3150.00	3150.00	3150.00	3150.00	3150.00
Available P.	0.45	0.45	0.45	0.45	0.45	0.42	0.42	0.42	0.42	0.42
Ca	1.00	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90	0.90
Methionine	0.55	0.55	0.55	0.55	0.55	0.50	0.50	0.50	0.50	0.50
Lysine	1.20	1.20	1.20	1.20	1.20	1.00	1.00	1.00	1.00	1.00
Na	0.20	0.20	0.20	0.20	0.20	0.15	0.15	0.15	0.15	0.15

[1]Mineral and vitamin premix provided the following per kilogram of feed; vitamin A, 9,000 IU; vitamin D3, 2,100 IU; α-tocopherol, 20 mg; nicotinic acid, 30 mg; vitamin B12, 0.12 mg; calcium pantothenate, 10 mg; vitamin K3, 2 mg; thiamin, 1 mg; riboflavin, 4.2 mg; vitamin B6, 1.7 mg; folic acid, 0.5 mg; biotin, 0.5 mg; Fe, 80 mg; Cu, 10 mg; Mn, 100 mg; Zn, 80 mg; Co, 0.2 mg; I, 1.0 mg; Se, 0.3 mg; monensin, 100 mg/kg. [2]Based on NRC (1994) feed composition; CON, basal diet; VL, basal diet containing 5.5% tuna oil + 0.5% sunflower oil; L, basal diet containing 4% tuna oil + 2% sunflower oil; H, basal diet containing 2.5% Tuna oil + 3.5% sunflower oil; VH, basal diet containing 1.5% tuna oil + 4.5% sunflower oil.

showed to increased LC-PUFA *n-3* in human plasma and tissue (Harris, 1989), rats (Billiar et al., 1988), mice (German et al., 1987; Whelan et al., 1991) and poultry (Chanmugam et al., 1992; Friedman and Sklan 1995; Fritsche et al., 1991; Qi et al., 2010). Although, most research on dietary oil supplementation has used high levels (> 5 g/100 g) of inclusion in the diet, in mice, the ratio of *n-3*: *n-6* PUFA appears to be more important in modulating eicosanoid biosynthesis than the absolute concentration of (*n-3*) PUFA in the diet (Boudreau et al., 1991; Broughton et al., 1991). The ratio among total *n-6* fatty acid to total *n-3* fatty acid is also very important for the determination of the physiological balance among the *n-6* and *n-3* derived eicosanoids in the rat (Anding and Hwang, 1986; Watkins and German, 1998). In an attempt to modify this ratio, Lopez-Ferrer et al. (1999) found a gradual linolenic acid carcase accretion in birds given flaxseed oil over a 5 weeks period. The same trend was observed for LC *n-3* when marine *n-3* sources were fed to broilers.

The main objective of this study was to evaluate the *n-6*:*n-3* ratios in broiler chickens breast muscle as modified by dietary supplementation of fish oil, sunflower oil and palm oil.

MATERIALS AND METHODS

Birds and housing environment

A total of 375 male broiler chicks of one day age (Cobb 500) were obtained from a local hatchery. The chicks were wing-banded, individually weighed and housed. The birds were raised in groups of 15 in 25 pens in a conventional open-sided deep litter house with cyclic temperatures (minimum, 24°C; maximum, 34°C). The relative humidity was between 80 to 90%. Feed and water were provided *ad libitium* and lighting was continuous.

Commencing from day 1, five pens were randomly assigned to each of the five dietary treatment groups as follows: (1) basal diet (CON) (NRC, 1994); (2) basal diet containing 5.5% tuna oil + 0.5% sunflower oil (VL); (3) basal diet containing 4% tuna oil + 2% sunflower oil (L); (4) basal diet containing 2.5% Tuna oil + 3.5% sunflower oil (H); (5) basal diet containing 1.5% tuna oil + 4.5% sunflower oil (VH). The diets (mash form) were formulated to meet or exceed the requirements by the NRC (1994) for broiler chickens (Table 1). A control diet containing no sunflower oil and fish oil was provided and two nutritional phases (starter and finisher) were implemented. All diets were formulated to be isocaloric and iso nitrogenous. No antimicrobial, anticoccidial drugs or feed enzymes were included in the diets. The experimental procedure was approved by the Animal Care And Use Committee (ACUC) of the University of Putra, Malaysia.

At 42 days of age, all the birds were killed by cervix dislocation for meat evaluation. The viscera were dissected out manually and

the breast skin of birds were removed and the pectoralis profundus muscles on the right side of the breast were carefully excised, wrapped in aluminum foil and stored at -80°C until fatty acid analysis.

Fatty acid extraction and identification

The total fatty acids were extracted from the diets and breast muscle samples using chloroform:methanol (2:1) (v/v) based on the method of Folch e t al. (1957) with an antioxidant for preventing the oxidation during sample preparation. The experimental diets and breast meat were homogenized in 40 ml chloroform: methanol [2:1 (v/v)] using an ultra-turrax T5 FU homogenizer (IKA Analysentechnik GmBH, Germany) in a 50 ml stoppered ground-glass extraction tubes. The mixture containing the extracted fatty acids was filtered through a No. 1 Whatman paper (Whatman International Ltd., Maidstone, England) into a 250 ml separatory funnel using a laboratory funnel. The paper was then washed with 10 ml of chloroform-methanol [2:1 (v/v)]. 12 ml of normal saline solution were added to facilitate phase separation. The mixture was then shaken vigorously for one minute and then left to stand for 4 h. After complete separation at the end of the fourth hour, the upper phase was discarded and lower phase was collected in a round-bottom flask and was evaporated by Heidolph vacuum rotary evaporation (Laborota 4000-efficient, Heidolph, Germany) at 70°C. The total lipid extracts was then immediatley transferred to a capped methylation tube by re-diluting it with 5 ml fresh chloroform-methanol [2:1 (v/v)]. Transmethylation of the extracted fat to fatty acid methyl esters to their fatty acid methyl esters (FAME) were carried out using KOH in methanol and 14% methanolic boron triflouride (BF_3) (Sigma Chemical Co. St. Louis, Missouri, USA) according to methods of AOAC (1990). The internal standard, heneicosanoic acid (C21:0) (Sigma Chemical Co., St. Louis, Missouri, USA) was added to each sample prior to transmethylation to determine the individual fatty acid concentrations within the samples. The methyl esters were quantified by GC (Agilent 7890N) using a 30 m x 0.25 mm ID (0.20 µm film thickness) Supelco SP-2330 capillary column (Supelco, Inc., Bellefonte, PA, USA). One microliter was injected by an auto sampler into the chromatograph, equipped with a split/splitless injector and a flame ionisation detector (FID). The injector temperature was programmed at 250°C and the detector temperature was 300°C. The column temperature program initiated runs at 100°C for 2 min, warmed to 170°C at 10°C /min, held for 2 min, warmed to 220°C at 7.5°C /min and then, was held for 20 min to facilitate optimal separation. All results of the fatty acid were presented as the percentage of total fatty acids.

Statistical analysis

A completely randomized design (CRD) with five replicates was employed. Statistical analyses were conducted using the ANOVA general linear model procedure (GLM) of SAS software (SAS Institute, 2005). When ANOVA revealed significant effects, means were separated by Duncan's multiple range test. The results were expressed as mean ± standard error of mean. Statistical significance was considered at $P < 0.05$.

RESULTS

Fatty acid profile of feed

The fatty acid profile of the experimental diets is shown in Table 2. Linoleic acid (C18:2 *n-6*) was the main PUFA *n-6* in the diets. It decreased as the fish oil was increased in the diets. An opposite trend was seen in the EPA and DHA contents, while α-linolenic acid (C18:3*n-3*) remained unchanged. Therefore, as expected, the total PUFA *n-6* decreased and the total PUFA *n-3* was increased as the level of fish oil increased in the diets. By increasing the sunflower oil, the PUFA *n-6* was increased mainly because of the high linoleic acid content of sunflower oil.

Fatty acid profile of breast muscle

The FA composition of breast muscle sample for all the treatments are presented in Table 3.

It can be generalized that, the percentage of the total SFA was not significantly different in all the treatment groups ($P > 0.05$). The major fatty acid contributor for the total saturated fat content was palmitic and (C16:0) and stearic acids (18:0) in all the treatment groups. The VL and L diets had the least MUFA; increased levels of fish oil in the diet clearly decreased the MUFA contents in VL and L diets significantly in the breast samples ($P < 0.05$). Oleic acid (C18:1 *n-9*) always had the highest among all the MUFA.

The *n-3* content of the diets reflected the ingredients used (Table 2). Dietary LC-PUFA *n-3* increased when fish oil was increased in the diet. In the birds given the highest amount of fish oil, there was a major increase in LC-PUFA *n-3* ($P < 0.05$). The high level fish oil treatments produced a wide variation in *n-3* profiles in the breast muscle. The extent to which either linoleic acid or LC-PUFA *n-3* predominated in the carcass depended on the dietary oil content.

In general, the VL and L animals had significantly more total LC- PUFA *n 3* in their muscles compared with the other groups ($P< 0.05$). The CON group showed significantly lower *n-3* fatty acids ($P < 0.05$) in the breast muscle (Table 3). The main LC-PUFA *n-3* was in the form of DHA and EPA in the treatment diets. The long chain 22-carbon *n-3* series PUFA was the major contributors to the overall PUFA *n-3* make-up in the breast muscle in the treatment diets. The response to the increase in PUFA *n-3* in tissues followed from that of diets. The trend of *n-3* in breast muscle followed VL>L>H>VH>CON. There was a 50-fold variation in the *n-6:n-3* ratios among the diets and 50-fold variation in the breast meat among the dietary treatment groups.

DISCUSSION

In this study, the inclusion of fish oil in the diet increased the accumulation of LC-PUFA *n-3* in the breast muscle, especially that of EPA and DHA, when compared with the other two FA sources (soybean and palm oil). Therefore, the *n-6:n-3* ratios in muscle was changed accordingly. The effects of dietary LC-PUFA *n-3* on fatty

Table 2. Fatty acid composition of the experimental diet.

Fatty acid	Treatment				
	CON	VL	L	H	VH
:0	0.04	0.08	0.07	0.12	0.06
10:0	0.02	0.29	0.13	0.14	0.38
12:0	0.34	0.06	0.05	0.05	0.07
14:0	0.78	2.46	1.75	0.93	0.76
15:0	0.03	0.65	0.44	0.25	0.17
15:1	0.06	0.14	0.06	0.05	0.09
16:0	32.69	19.54	17.34	15.72	12.20
16:1	0.17	3.32	2.41	1.35	1.03
17:0	0.10	1.39	1.04	0.85	0.42
17:1	0.06	0.77	0.55	0.26	0.35
18:0	4.14	5.59	4.97	4.89	3.99
18:1	39.21	23.31	26.93	27.83	31.19
18:2 *n-6*	21.92	24.79	31.91	38.82	45.12
18:3 *n-3*	0.43	0.58	0.55	0.51	0.45
20:4 *n-6*	ND	1.07	0.72	0.55	0.21
20:5 *n-3*	ND	3.23	2.26	1.49	0.75
22:5 *n-3*	ND	0.82	0.56	0.36	0.19
22:6 *n-3*	ND	11.93	8.17	5.60	2.61
Total saturated	38.17	30.04	25.88	23.18	18.02
Total unsaturated	61.83	69.96	74.12	76.82	81.98
Total MUFA	39.49	27.54	29.95	29.50	32.65
Total PUFA *n-3*	0.43	16.56	11.54	7.96	4.00
Total PUFA *n-6*	21.92	25.86	32.63	39.37	45.33
n-6 : *n-3* Ratio	51.37	1.57	2.83	5.12	11.34
Unsat: Sat	1.62	2.33	2.87	3.46	4.55
Poly: Sat ratio	0.59	1.41	1.71	2.11	2.74

[a,b]Values in the same row with no common superscript were significantly different. Total saturated = sum of 8:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0; total unsaturated= sum of 15:1, 16:1, 17:1, 18:1, 18:2 *n-6*, 18:3 *n-3,* 20:4 *n-6,* 20:5 *n-3,* 22:5 *n-3,* 22:6 *n-3;* total MUFA = sum of 15:1, 16:1, 17:1, 18:1; total PUFA *n-3* = sum of 18:3*n-3*, 20:5*n-3*, 22:5*n-3*, and 22:6*n-3*; *n-6*; total PUFA *n-6* = sum of 18:2*n-6* and 20:4*n-6*.
ND, not detected; CON, basal diet; VL, basal diet containing 5.5% tuna oil + 0.5% sunflower oil; L, basal diet containing 4% tuna oil + 2% sunflower oil; H, basal diet containing 2.5% Tuna oil + 3.5% sunflower oil; VH, basal diet containing 1.5% tuna oil + 4.5% sunflower oil.

acid profile of broiler meat were presented in several works using fish oil or fish meal (Miller and Robisch, 1969; Hulan et al., 1988; Phetteplace and Watkins, 1989; Nash et al., 1995; Lopez-Ferrer et al., 1999). In agreement to our results, they clearly showed that, PUFA *n-3* rich diets modified and increased these fatty acids in the breast muscle of chicken at 42 days of age.

Moreover, in agreement to Yau et al. (1991) and Scaife et al. (1994), we demonestrated that, lack of unsaturated fatty acid supplementation in CON birds resulted in the highest MUFA level of the birds among the treatment groups (39.80%). The high level of palmitic and stearic acids in the CON group probably accounted for such a high level of oleic acid in the meat, due to elongation and Δ^9 desaturation. The unsaturated fatty acid content in meat was the same for all treatment groups ($P > 0.05$) as reported also by Ajuyah et al. (1991, 1993). All the LC-PUFA *n-3* (EPA and DHA) had the highest values ($P < 0.05$) when fish oil was increased in the diet. The same phenomenon was reported by Olomu and Baracos (1991) and Whelan et al. (1991) when they supplemented the broiler diet with linseed oil or purified linolenic acid. High levels of LC-PUFA *n-3* may have decreased the desaturation and elongation of linoliec acid to its derivatives, as reported also by Bezard et al. (1994) in mammals.

It can be concluded that, suplementation of fish oil in broiler diet may be consider as a functional practice to

Table 3. Fatty acid composition of breast muscle of chicks according to different *n-6:n-3* ratio in diets.

Fatty acid	Treatment				
	CON	VL	L	H	VH
8:0	0.36±0.02	0.44±0.05	0.40±0.05	0.41±0.04	0.34±0.03
10:0	0.65±0.06^{bc}	0.98±0.04^{ab}	1.05±0.02^{ab}	1.19±0.02^{a}	0.34±0.03c
14:0	0.66±0.04^{c}	1.07±0.06^{a}	0.90±0.05^{ab}	0.79±0.03^{bc}	0.96±0.04^{ab}
14:1	0.25±0.03	0.39±0.08	0.31±0.05	0.35±0.07	0.43±0.07
15:0	0.45±0.08	0.56±0.06	0.53±0.09	0.42±0.04	0.44±0.06
15:1	1.05±0.01^{ab}	1.21±0.07^{a}	1.23±0.04^{a}	1.32±0.01^{a}	0.71±0.02^{b}
16:0	23.50±0.51^{ab}	21.64±1.48^{b}	22.03±0.35^{b}	21.82±0.46^{b}	24.92±0.16^{a}
16:1	2.81±0.45^{b}	2.51±0.28b^{c}	1.63±0.26^{dc}	1.29±0.14^{d}	4.16±0.61^{a}
17:0	0.37±0.03	0.71±0.03	0.66±0.07	0.60±0.02	0.52±0.05
17:1	0.58±0.07^{ab}	0.41±0.03^{bc}	0.53±0.05^{ab}	0.69±0.12^{a}	0.29±0.05^{c}
18:0	10.01±0.51	11.92±0.89	11.71±0.68	11.81±0.36	9.65±1.10
18:1	35.11±1.00^{a}	19.53±1.57^{b}	20.96±1.39^{b}	21.26±0.78^{b}	31.88±1.41^{a}
18:2 *n-6*	16.47±0.41^{b}	14.77±1.49^{b}	16.24±1.06^{b}	20.57±0.90^{a}	16.08±0.29^{b}
18:3 *n-3*	0.45±0.03^{ab}	0.59±0.08^{a}	0.42±0.08^{ab}	0.42±0.03^{ab}	0.39±0.04^{b}
20:3 *n-6*	1.62±0.04	1.65±0.04	0.93±0.04	1.43±0.08	1.04±0.04
20:4 *n-6*	5.67±0.70^{ab}	3.95±0.61^{b}	5.11±0.77^{ab}	6.17±0.41^{a}	3.75±0.97^{b}
20:5 *n-3*	ND	2.83±0.14^{a}	2.24±0.37^{a}	1.20±0.16^{b}	0.72±0.09^{b}
22:6 *n-3*	ND	14.84±1.18^{a}	13.13±0.82^{a}	8.26±0.74^{b}	3.38±0.43^{c}
Total saturated	35.99±0.52	37.32±1.10	37.27±1.01	37.04±0.90	37.17±2.06
Total unsaturated	64.01±0.52	62.68±1.10	62.73±1.01	62.96±0.90	62.83±2.06
Total MUFA	39.80±1.23^{a}	24.04±1.39^{b}	24.66±1.51^{b}	24.91±0.73^{b}	37.48±3.63^{a}
Total PUFA *n-3*	0.45±0.03d	18.26±1.19^{a}	15.79±0.96^{a}	9.88±0.82^{b}	4.48±0.49^{c}
Total PUFA *n-6*	23.76±0.96^{b}	20.38±1.67^{b}	22.28±0.86^{b}	28.17±0.81^{a}	20.87±1.24^{b}
n-6 : *n-3* Ratio	54.29±4.79^{a}	1.14±0.12^{b}	1.45±0.15^{b}	2.96±0.29^{b}	4.75±0.33^{b}
Unsat:Sat	1.78±0.04	1.69±0.08	1.69±0.08	1.71±0.07	1.71±0.05
Poly:Sat ratio	0.67±0.02^{b}	1.05±0.09^{a}	1.02±0.03^{a}	1.03±0.05^{a}	0.68±0.01^{b}

^{a,b,c}Values in the same row with no common superscript were significantly different($P < 0.05$). nd, not detected; total saturated = saturated fatty acids; total MUFA = total monounsaturated fatty acids; sum of the 14:1, 15:1, 17:1, 18:1; total PUFA = polyunsaturated fatty acids; *n-3* = sum of 18:3*n-3*, 20:5*n-3*, 22:5*n-3*, and 22:6*n-3*; *n-6* = sum of 18:2*n-6* and 20:4*n-6*; unsat:sat ratio = sum 15:1, 16:1, 17:1, 18:1, 18:2*n-6*, 18:3*n-3*, 20:4*n-6*, 20:5*n-3*, 22:2*n-6*, 22:5*n-3*, and 22:6*n-3*: sum of 14:0, 15:0, 16:0, 17:0, 18:0; *n-6*/*n-3* ratio = sum of (18:2*n-6*, 18:3*n-6*, 20:4*n-6*) /sum of (18:3*n-3*, 20:5*n-3*, 22:5*n-3*, and 22:6*n-3*).

ND, not detected; CON, basal diet; VL, basal diet containing 5.5% tuna oil + 0.5% sunflower oil; L, basal diet containing 4% tuna oil + 2% sunflower oil; H, basal diet containing 2.5% Tuna oil + 3.5% sunflower oil; VH, basal diet containing 1.5% tuna oil + 4.5% sunflower oil.

produce PUFA *n-3* enriched meat with optimum *n-6:n-3* ratio.

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