บทความวิจัย

การใช้มันเทศเป็นแหล่งสารสีในอาหารปลากัด

Utilization of Sweet Potato as Pigment Source in Siamese Fighting Fish (*Betta splendens*) Feed

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บทคัดย่อ

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาการใช้มันเทศเป็นสารเร่งสีในอาหารปลากัด (*Betta splendens*) โดยวางแผนการทดลองแบบ Completely Randomized Design แบ่งเป็น 4 ชุดการทดลองๆ ละ 3 ซ้ำ ประกอบด้วย ชุดควบคุมไม่เสริมมันเทศ (Control), อาหารเสริมมันเทศสีส้ม 5 % (OrSP), อาหารเสริมมันเทศสีเหลือง 5 % (YeSP) และอาหารเสริมมันเทศสีม่วง 5 % (PuSP) อาหารทดลองมีโปรตีน 31.18-32.01 % ไขมัน 6.73 - 7.04 % และมีปริมาณแคโรทีนอยด์ใน Control, OrSP, YeSP และ PuSP เท่ากับ 15.63 42.61 38.79 และ 32.32 mg/kg ตามลำดับ โดยนำปลากัดเพศผู้อายุประมาณ 2 เดือน มาปรับสภาพแวดล้อมก่อนทำการทดลอง หลังทดลอง 8 สัปดาห์ พบว่าประสิทธิภาพการเจริญเติบโตไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p > 0.05$) แต่ส่งผลต่อการแสดงออกของสีของปลา โดยค่าความสว่าง (L*) ของปลาชุดควบคุมมีค่าสูงกว่าชุดการทดลองอื่น ($p \leq 0.05$) ค่าความเข้มสีแดง (a*) ของปลาชุดการทดลอง OrSP และ YeSP มีค่าสูงกว่าปลาชุดควบคุมและปลาชุดการทดลอง PuSP ($p \leq 0.05$) ปลาชุดการทดลอง OrSP มีค่าความเข้มสีเหลือง (b*) สูงที่สุด ($p \leq 0.05$) การสะสมแคโรทีนอยด์ในอวัยวะของปลาหลังได้รับอาหารทดลองมีการสะสมแคโรทีนอยด์สูงขึ้นทุกชุดการทดลอง โดยปลาชุดการทดลอง OrSP มีการสะสมแคโรทีนอยด์ในผิวหนังและเกล็ด ครีบหลัง ครีบหาง และครีบก้นสูงกว่าชุดการทดลองอื่น ($p \leq 0.05$)

คำสำคัญ: ปลากัด มันเทศ สารสี การเจริญเติบโต การแสดงออกของสี

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(Received: October 8, 2020; Revised: February 10, 2021; Accepted: February 17, 2021)

Abstract

This study investigated the effect of different sweet potato varieties as dietary pigment sources on the coloration of Siamese Fighting Fish (*Betta splendens*). The experiment was conducted as a completely randomized design (CRD) with four treatments and three replicates. The experimental feed consisted of; control feed, 5% orange sweet potato (OrSP), 5% yellow sweet potato (YeSP) and 5% purple sweet potato (PuSP). The experimental feeds were formulated with 31.18-32.01 % crude protein, 6.73 - 7.04 % crude fat and total carotenoids in control, OrSP, YeSP and PuSP were 15.63, 42.61, 38.79 and 32.32 mg/kg, respectively. Two-month male fishes were obtained from a reliable commercial farm and acclimated to the rearing environment. After the 8 weeks rearing period, the results showed that the final average weight, final average length, average daily gain, weight gain, specific growth rate and feed conversion ratio (FCR) were not significantly different between treatment groups ($p > 0.05$). However, dietary supplementation with sweet potato affected the color expression and carotenoid accumulation by *Betta splendens*. The lightness (L*) of fish fed with the control feed showed the highest value ($p \leq 0.05$). The redness (a*) of fish fed with 5% OrSP and 5% YeSP was higher than in the other treatments ($p \leq 0.05$) and the yellowness (b*) of fish fed with 5% OrSP showed higher values than in the other groups ($p \leq 0.05$). The results of carotenoid accumulation in each organ increased in all treatment groups. After 8 weeks, the fish fed with 5% OrSP showed the highest carotenoid accumulation in skin and scale, dorsal fin, anal fin and caudal fin as compared to the other groups ($p \leq 0.05$).

Keywords: *Betta splendens*, Sweet Potato, Pigment, Growth Performance, Color Expression

Introduction

Ornamental fish culture generates huge income for Thailand and in April 2018, the export value of ornamental aquatic animals via Suvarnabhumi airport was approximately 55 million baht. The Siamese fighting fish has the highest export volume of 1,654,577 units worth 14,629,993.24 baht [1]. The Siamese fighting fish (*Betta splendens* Regan, 1910) is a freshwater fish that is distributed in all regions of Thailand and is especially heavily cultured in the Nakhon Pathom province. The fish are easy to raise, can tolerate rapid diurnal temperature changes and live in habitats with low oxygen availability [2]. Therefore, it has continued to gain popularity. Siamese fighting fish are sexually dimorphic in both weight and size with males being heavier and bigger than females. Male' fins are long and colorful, while the female fish are usually smaller, shorter and the color is fierce than males [2, 3]. The size of the fish and their color expression are important factors in determining the purchase price of ornamental fish. Color expression in the skin, scales and fins, especially red coloration is caused by carotenoids and depends on many factors such as age, phenotype and interaction between age and phenotype [3] Nowadays, dietary pigments are added as a source of pigment stimulation in ornamental fish. Amongst the dietary additives are synthetic and natural raw materials such as *Spirulina* sp. and *Chorococcum* sp. in Siamese fighting fish feed, synthetic astaxanthin in blood parrot cichlid feed, synthetic carotenoids in Spine-cheek anemone fish feed and ripe papaya, carrot, sweet potato and tomato in Goldfish feed [3 - 8].

Sweet potato (*Ipomoea batatas* L.) is the seventh most important crop in the world. It has been consumed as a secondary plant food after rice, corn, potatoes and cassava and was found to have high nutritional value such as protein, carbohydrates, fiber, as well as iron and potassium, vitamins A and C. Later, it has been used in the animal feed industry [9] In addition to the above nutrients, sweet potatoes are a source of pigments which vary depending on the type of sweet potato such as carotenoid, flavonoid and chlorophyll. Carotenoids consist of carotene and xanthophyll derivatives. The flavonoids contain many types of pigments such as anthocyanin, flavone, catechin and flavonols. Yellow and orange sweet potato varieties are found to contain large amounts of carotene. While, red and purple sweet potatoes are found to be high in anthocyanin [10] Therefore, the objective of this study was to investigate the effect of different sweet potato varieties that may be suitable for stimulating color expression, carotenoids accumulation, growth performance, feed efficiency and survival rate of *Betta splendens*.

Materials and Methodology

Experimental Design and Treatments

Two-month old male fish were obtained from a reliable commercial farm and acclimatized to the rearing environment for a week prior to feeding the experimental feeds. Fish were randomly distributed, 1 fish to each clear plastic bottle. The experiment was designed as a Completely randomized design (CRD) with 4 treatments and 3 replicates (25 fishes per replication). The 4 treatments were T1: commercial feed (32.01 %protein, control), T2: commercial feed with 5% orange sweet potato (OrSP), T3: commercial feed with 5% yellow sweet potato (YeSP) and T4: commercial feed with 5% purple sweet potato (PuSP). The fish were fed until full 3 times/day and the duration of this experiment was 8 weeks.

Experimental Feed Preparation

Sweet potatoes were washed, cleaned and cut into small pieces measuring 0.3-0.5 cm, then dried in a hot air oven at 65 °C for 12 hr. After cooling, sweet potatoes were ground and keep at 4 °C until use in feed production. The commercial feed (30 % protein) was ground and mixed with sweet potatoes according to the experimental design. The experimental feeds were pelleted and dried in a hot air oven at 65 °C for 12 hr. The feeds were analyzed for chemical composition using proximate analysis [11] and total carotenoids (mg/kg) [13] with the results presented in Table 1.

Table 1 Proximate composition (%) and total carotenoid (mg/kg) of experimental feeds (as dry basis)

Composition (%)	Control Feed	OrSP 5%	YeSP 5%	PuSP 5%
Crude Protein	32.01 ± 0.08	31.25 ± 0.14	31.18 ± 0.02	31.30 ± 0.04
Fat	7.04 ± 0.03	6.73 ± 0.02	6.81 ± 0.02	6.85 ± 0.02
Ash	9.51 ± 0.02	9.68 ± 0.04	9.62 ± 0.02	9.54 ± 0.03
Fiber	4.47 ± 0.04	4.54 ± 0.01	4.58 ± 0.01	4.58 ± 0.03
Moisture	6.65 ± 0.02	6.60 ± 0.03	6.57 ± 0.01	6.58 ± 0.02
Total Carotenoid (mg/kg)	15.63 ± 0.03	42.61 ± 0.05	38.79 ± 0.04	32.32 ± 0.03

Data represent as mean ± SD.

Data Collection

Growth performance parameters were recorded and calculated according to the method of Goddard [12]. The experimental fish were weighed every two weeks until the end of experiment to determine growth performance, survival rate and feed utilization following: weight gain, average daily gain, specific growth rate, and feed conversion ratio. Color expression of fishes were assessed with the Color Reader Model, CR10 by CIE system (to measure values for lightness (L*), redness (a*) and yellowness (b*)). Carotenoid accumulation in scale and skin, dorsal fin, anal fin and caudal fin were conducted follow the method of Britton *et al*. [13]

Statistical Analysis

Mean values and standard deviations (S.D.) were calculated from the results. One-way analysis of variance (ANOVA) was applied for comparison of the mean values. Duncan's New Multiple Range Test (DMRT) was used to compare means. Differences between means were considered to be significant at a level of $p \leq 0.05$, indicated by different indices in the results' table.

Results and Discussion

Growth Performance

Growth performance of *Betta splendens* fed with experimental feeds consisting of; control feed, 5% orange sweet potato (OrSP), 5% yellow sweet potato (YeSP) and 5% purple sweet potato (PuSP) for 8 weeks are shown in Table 2. The results show that final weight, final length, weight grain, average daily gain and specific growth rate were not significantly different between treatments ($p > 0.05$). Feed utilization in term of feed conversion ratio (FCR) were not significantly different between treatments ($p > 0.05$). Excellent survival, at 100 %, was observed in all dietary treatments. This may be due to the enhancement of colorants for the purpose of accelerating the skin color of fishes not significantly affecting the nutritional level of the feed. Therefore, all experimental feed formulae have the same nutritional levels. This is consistent with the enhancement of colorants from other pigment sources in aquaculture feeds such as ripe papaya, carrot, orange sweet potato, purple sweet potato and beetroot in goldfish (*Carassius auratus*) feed [5, 8], astaxanthin in blood parrot cichlid feed [6], supplementation of *Phafia rhodozyma*, *Paracoccus* sp. and *Haematococcus pluviallis* in fancy carp [14] and supplementation with synthetic carotenoids in spine-cheek anemone fish (*Premnas biaculeatus*) and clownfish (*Amphiprion ocellaris*) [7, 15].

Table 2 Growth performance of *Betta splendens* fed with experimental feeds for 8 weeks

Performance	Control Feed (T1)	5%OrSP (T2)	5%YeSP (T3)	5%PuSP (T4)	p-value
Initial Weight (g/fish)	0.68 ± 0.02	0.70 ± 0.01	0.68 ± 0.01	0.66 ± 0.04	0.366
Initial Length (cm/fish)	5.68 ± 0.07	5.70 ± 0.08	5.66 ± 0.03	5.61 ± 0.03	0.336
Final Weight (g/fish)	1.25 ± 0.02	1.27 ± 0.01	1.26 ± 0.02	1.26 ± 0.03	0.604
Final Length (cm/fish)	6.37 ± 0.20	6.08 ± 0.27	6.37 ± 0.20	6.11 ± 0.29	0.351
Average Daily Gain (g/fish/day)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.751
Weight Gain (g/fish)	1.52 ± 0.13	1.46 ± 0.08	1.52 ± 0.02	1.61 ± 0.17	0.503
Specific Growth Rate (%/day)	1.10 ± 0.07	1.07 ± 0.04	1.10 ± 0.01	1.14 ± 0.09	0.516
Feed Conversion Ratio (FCR)	1.94 ± 0.08	2.15 ± 0.16	2.09 ± 0.12	2.10 ± 0.09	0.205
Survival Rate (%)	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	

Data represent as mean ± SD. Different superscripts in the same row indicate significant differences at $p \le 0.05$.

Color Expression

The result of color expression of fish is shown in Table 3. The initial of lightness (L*), redness (a*) and yellowness (b*) were not significantly different between treatment groups ($p > 0.05$). However, variation in the color expression was detected in week 4 of the experiment and increased during the rearing period. By 8 weeks of the experiment, the fish fed with control feed had higher L* than the other groups ($p \le 0.05$). While, the fish fed with 5% OrSP and 5% YeSP showed a higher a* than the other groups ($p \le 0.05$). Further, the b* of fishes fed 5% OrSP was higher than all treatment groups ($p \le 0.05$). These results are in accordance with previous studies carried out with supplementation of ripe papaya in goldfish (*Carassius auratus*) feed. Ponza *et al*. (2018) [5] reported that the a* and b* of goldfish fed with ripe papaya was increased after 6 weeks of experiment diets and it was higher than the control group ($p \le 0.05$), while the control group showed a higher L* than the treatment group supplemented with ripe papaya ($p \le 0.05$). According to the study of Sornsupharp (2018) [8], goldfish fed with pumpkin and purple sweet potato had chroma in goldfish skin higher than goldfish fed with a control feed ($p \le 0.05$).

Table 3 Lightness, redness and yellowness on skin of *Betta splendens* fed with different experimental feeds

Rearing Period (weeks)	Parameters	Control Feed (T1)	5% OrSP (T2)	5% YeSP (T3)	5% PuSP (T4)	p-value
0	Lightness (L*)	24.41 ± 0.30	24.52 ± 0.22	24.74 ± 0.42	24.46 ± 0.46	0.714
	Redness (a*)	9.48 ± 0.16	9.32 ± 0.34	9.35 ± 0.12	9.42 ± 0.25	0.858
	Yellowness (b*)	3.60 ± 0.39	3.61 ± 0.17	3.65 ± 0.18	3.43 ± 0.30	0.778
4	Lightness (L*)	25.46 ± 0.42^{b}	25.75 ± 0.34^{b}	25.84 ± 0.28^{b}	24.23 ± 0.28^{a}	0.001
	Redness (a*)	10.20 ± 0.10^{a}	11.63 ± 0.27^{c}	11.13 ± 0.05^{b}	10.05 ± 0.10^{a}	< 0.001
	Yellowness (b*)	3.56 ± 0.27^{b}	4.72 ± 0.14^{c}	4.89 ± 0.05^{c}	3.17 ± 0.15^{a}	< 0.001
8	Lightness (L*)	25.95 ± 0.26^{b}	24.86 ± 0.20^{a}	24.76 ± 0.20^{a}	24.42 ± 0.48^{a}	< 0.001
	Redness (a*)	11.15 ± 0.07^{a}	12.96 ± 0.45^{b}	12.50 ± 0.17^{b}	11.24 ± 0.06^{a}	< 0.001
	Yellowness (b*)	4.32 ± 0.20^{a}	7.55 ± 0.47^{c}	5.41 ± 0.40^{b}	4.57 ± 0.27^{a}	< 0.001

Data represent as mean ± SD. Different superscripts in the same row indicate significant differences at $p \leq 0.05$.

Carotenoid Accumulation

The results of carotenoid accumulation show that total carotenoid (mg/kg) of fish at the start of the trial was not significantly different between treatments ($p > 0.05$). After 4 weeks of the rearing period, carotenoid accumulation in each organ was increased in all treatment groups. The fish fed with 5% OrSP showed the higher carotenoid accumulation in skin and scale, dorsal fin, anal fin and caudal fin than the other groups ($p \leq 0.05$). Carotenoid accumulation in skin and scale showed the highest value of 25.97 ± 0.22 mg/kg. After 8 weeks of experiments, the results for carotenoid accumulation in each organ increased in all treatment groups. The fish fed with 5% OrSP showed the higher carotenoid accumulation in skin and scale, dorsal fin, anal fin and caudal fin compared to the other groups ($p \leq 0.05$). The results showed highest values in skin and scale followed by anal fin, caudal fin and dorsal fin with values of 34.93 ± 0.29, 29.95 ± 0.43, 29.87 ± 0.39 and 26.53 ± 0.55 mg/kg respectively (Table 4). A similar result to this study has been reported by Thongprajukaew *et al.* (2012) [4], who observed that *Betta splendens* had the highest carotenoid accumulation in muscle (44-51%) followed by skin, caudal fin, anal fin, dorsal fin, pelvic fins and pectoral fins, respectively. The present results show that *Betta splendens* can use and accumulate the carotenoids in orange and yellow sweet potatoes. The carotenoids found in fruits and vegetables have orange, yellow, red and green pigments. This includes algae and photosynthetic microorganisms [16, 17]. While the purple sweet potato is high in anthocyanin pigment which gives red, purple and blue colors and the expression of red, violet or blue varies with pH and temperature. It is unstable and easy to change in structure [10]. According to Clotfelter *et al.* (2007) [18], who reported that the red coloration of *Betta splendens* is due to carotenoids and the color concentration in the fins has a higher red phenotype than the blue phenotype. Additionally, fish color expression from exposure to carotenoids is often associated with other factors such as genetics, age (reproductive age) and sex. These factors control the color expression, color distribution and pigment density of fish [19, 4]. Wallbrunn (1957) [20] reported that the color on skin and scale of *Betta splendens* was controlled by an iridescence pigment gene, pigment density gene and the genes that define the melanin. However, each fish species is unique and has the ability to utilize carotenoids as well as accumulate in organs depending on the species digestion and absorption capacity [21]

Table 4 Carotenoid accumulation (mg/kg) in each organ of *Betta splendens* fed with different experimental feeds

Rearing Period (weeks)	Organs	Control Feed (T1)	5% OrSP (T2)	5% YeSP (T3)	5% PuSP (T4)	p-value
0	Skin and Scale	13.41 ± 0.18	13.53 ± 0.13	13.41 ± 0.12	13.45 ± 0.27	0.851
	Dorsal Fin	11.50 ± 0.23	11.44 ± 0.18	11.57 ± 0.04	11.49 ± 0.18	0.847
	Anal Fin	10.37 ± 0.20	10.36 ± 0.23	10.43 ± 0.20	10.38 ± 0.13	0.968
	Caudal Fin	9.02 ± 0.06	9.20 ± 0.06	9.29 ± 0.11	9.19 ± 0.05	0.367
4	Skin and Scale	15.78 ± 0.30^{a}	25.97 ± 0.22^{d}	21.27 ± 0.35^{c}	17.02 ± 0.29^{b}	< 0.001
	Dorsal Fin	14.05 ± 0.33^{a}	20.60 ± 0.44^{d}	16.90 ± 0.66^{c}	16.00 ± 0.19^{b}	< 0.001
	Anal Fin	12.59 ± 0.42^{a}	22.18 ± 0.38^{c}	16.60 ± 0.16^{b}	12.81 ± 0.17^{a}	< 0.001
	Caudal Fin	11.37 ± 0.05^{a}	21.93 ± 0.32^{d}	18.46 ± 0.45^{c}	12.47 ± 0.13^{b}	< 0.001
8	Skin and Scale	22.64 ± 0.68^{a}	34.93 ± 0.29^{d}	29.63 ± 0.96^{c}	24.71 ± 0.36^{b}	< 0.001
	Dorsal Fin	14.60 ± 0.32^{a}	26.53 ± 0.55^{c}	23.01 ± 0.70^{b}	22.37 ± 0.30^{b}	< 0.001
	Anal Fin	14.61 ± 0.27^{a}	29.95 ± 0.43^{d}	25.42 ± 0.47^{c}	19.41 ± 0.95^{b}	< 0.001
	Caudal Fin	13.52 ± 0.27^{a}	29.87 ± 0.39^{d}	25.43 ± 1.26^{c}	19.03 ± 0.43^{b}	< 0.001

Data represent as mean ± SD. Different superscripts in the same row indicate significant differences at $p \leq 0.05$.

Supplementation with sweet potato in ornamental fish feed can stimulate color expression and total carotenoids accumulation in fish. It also has antioxidant properties, is a source of vitamin A, iron, calcium and phosphorus, and can improve the immune system and resist bacteria which can cause disease [22]. In addition, the color enhancer for ornamental fish can also be used for living feed and other raw materials such as *Spirulina* sp., *Chlorella vulgaris*, *Haematococcus pluvialis*, fairy shrimp (*Beanchinella thandensis*) [23, 24] and synthetic pigments such as chlorophyll, carotenoid and astaxanthin [25, 7, 26].

Conclusion

Supplementation with orange, yellow and purple sweet potato in *Betta splendens* diets showed non-significant differences in growth performance, feed conversion ratio and survival rate but affected color expression and carotenoid accumulation. The fish fed with orange sweet potato had the highest yellowness and fish fed orange and yellow sweet potato had highest redness, while the fish fed with control feed, without sweet potato supplementation had higher lightness than the treatment groups. Regarding carotenoid accumulation, the fish fed with orange sweet potato showed the highest carotenoid accumulation in skin and scale, dorsal fin, anal fin and caudal fin.

Acknowledgment

The authors would like to express our thanks for the support from the Faculty of Agriculture and Technology, Rajamangala University of Technology Isan Surin Campus, Surin, Thailand.

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