

## **EVALUATION OF SOME NON CONVENTIONAL DIETS FOR NILE TILAPIA FISH:**

### **II- CONCERNING BLOOD PROFILE AND CHEMICAL COMPOSITION OF FISH.**

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### **ABSTRACT**

The aim of this study was to evaluate some unconventional diets on blood picture and whole fish and fish muscles composition of Nile tilapia fingerlings. Glass aquaria were used. The initial body weight of the fingerlings was 7 – 8 g. The adaptation period was two weeks before the experimental period of sixteen weeks. The basal diet contained 25% crude protein. The diets were offered daily at two meals at 3% of fish body weight. Each dietary treatment was performed in two aquaria. Fish were stocked at a rate of 7 fingerlings per aquarium. The experimental diets were nearly isocaloric and isonitrogenous. The 1<sup>st</sup> diet was a control, diets No. 2 – 5 are the control diet but their fishmeal was substituted by 25, 50, 75 and 100%, respectively with duckweed meal (DW), diets No. 6 – 9 included crayfish meal (CrFi) at the same previous replacement rates, and diets No. 10 – 13 included a mixture of DW + CrFi (1:1) as a substitute for fishmeal at the same rates. The important results refer to diet No. 6 which reflected the highest values of hemoglobin, hematocrit, total protein, and globulin, but diet No. 4 gave the lowest levels. Transaminases activity increased with diet No. 5 and decreased with diet No. 7 referring to affected hepatic function. The 5<sup>th</sup> diet also increased blood levels of urea, uric acid and creatinine, referring to affected kidney function. Carcass and muscle composition of the tested fish significantly affected by dietary treatments. The highest dry matter was determined in the 5<sup>th</sup> group but the lowest in the 6<sup>th</sup> group. Diets No. 6 and 4 were responsible for the highest and the lowest protein %, respectively. Diets No. 5 and 6 reflected the highest and lowest ether extract %, respectively. The lowest ash % was estimated for the 4<sup>th</sup> and 5<sup>th</sup> groups, whereas the highest with diet No. 7. There were positive relationships between moisture and protein as well as between moisture and ash contents, but negative relationships between moisture and ether extract as well as between protein and ether extract % in whole fish body and fish muscles. Decreased silica level of the 2<sup>nd</sup> diet led to low silica content of fish body of this group. The highest silica level was found in the 9<sup>th</sup> diet; yet, the highest silica content was recorded in the 13<sup>th</sup> fish group. The highest lead content was found in diet No. 9, but in fish of the 7<sup>th</sup> and 13<sup>th</sup> groups. The lowest lead content in diet and fish was of the 2<sup>nd</sup> treatment. The 13<sup>th</sup> group reflected the highest cadmium levels in diet and fish, but the lowest in diet and fish was of the control group. From the foregoing results, it would be clear that the 6<sup>th</sup> diet (25% freshwater crayfish meal as partial replaced of dietary fish meal) was significantly the best concerning blood hemoglobin, hematocrit, total protein, and globulin besides highest protein and lowest fat in whole fish and fish muscles.

**Keywords:** Nile tilapia – Blood profile– Fish composition.

## **INTRODUCTION**

Tilapia culture is one of the fastest growing farming activities, with an average annual growth rate of 13.4%, during 1970–2002. Tilapia are widely cultured in about 100 countries in the tropical and subtropical regions. As a result, the production of farmed tilapia has increased from 383,654 mt in 1990 to 1,505,804 mt in 2002, representing about 6% of total farmed finfish. Nile tilapia is, by far, the most important farmed tilapia species in the world. The production of farmed Nile tilapia reached 1,217,055 mt representing about 81% of total production of farmed tilapia in 2002 (FAO, 2004). Nile tilapia is herbivorous by nature, consuming mainly phytoplankton, but can as well consume a variety of other natural food organisms found in ponds (Moriarty, 1973). To increase fish production, supplementary or artificial feeds may be added. However, supplementary feeds can take up to 60% of fish production costs (Green, 1992) making them unaffordable for most farmers in developing countries (Liti *et al.*, 2005). Little research was conducted on animal protein sources as alternatives for fish meal such as blood meal, earth worms, fish silage, silk worm pupae and processed meat soluble (Millamena *et al.*, 2000). The utilization of the cheaper sources such as freshwater crayfish meal or aquatic plants meal are promising and need further investigations. (El Sayed *et al.*, 2005). The objective of this study was to evaluate replacing dietary fish meal protein by plant and animal protein sources in tilapia fish diets and to investigate its effects on blood profile and chemical composition of tilapia fingerlings.

## **MATERIALS AND METHODS**

Experimental fish (7-8 g) and diets (25 % crude protein) as well as the experimental procedure are presented in the 1<sup>st</sup> part of this series of articles (Abdelhamid *et al.*, 2010). Blood samples were withdrawn for hematological and biochemical determinations in a private human pathological Lab. At AL-Hamoul, Kafr EL-Sheikh governorate. Determinations of DM, CP, EE, ash and silica in the diets and in fish body at the start and at the end of the experiment were carried out according to the method of A.O.A.C. (1990). At the end of the experiment, three fish were derived from each group (aquarium) for drying at 60°C for 48 hours and then milled through electrical mill and kept at 4°C until analysis. Heavy metals determination was carried out at Botany Department Lab of the National Research Center, Dokki using Atomic Absorption Spectrophotometer (Germany Company). Blood samples were withdrawn for hematological and biochemical determination in a private human pathological Lab. At AL-Hamoul, Kafr EL-Shikh governorate. For microbiological test, samples were taken in sterilized test tubes then prepared for total bacterial count according to AOAC (1990) and kindly examined by Dr. Manal I. EL-Barbary from the Aquatic Pathology Lab., National Institute of Oceanography and fisheries. The data were statistically analyzed by using general linear models procedure adapted by SAS (1996) for users guide. Means were separated using Duncan's multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

### Blood Analysis:

Data of blood hematological and biochemical analyses in tilapia fish as affected by the dietary treatments are given in Table (1). Diet No. 6 reflected the highest Hb and PCV values as well as total protein and globulin. While, diet No. 4 gave the lowest values of Hb, PCV, total protein, albumin and globulin. Hepatic function in terms of AST and ALT activities was highest with diet No. 5 and lowest with diet No. 7. Also, kidney functions in terms of urea, uric acid and creatinine concentrations were highest with diet No. 5. This means that 75% duckweed replacing fish meal in tilapia diets reduced the hematological parameters measured as well as blood total protein and globulin and 100% DW diet led to dysfunctions of both liver and kidney. Yet, 25% C<sub>r</sub>F<sub>i</sub> improved (duplicated) the hematological parameters and total protein (and globulin) to be better than the control. Moreover, 50% C<sub>r</sub>F<sub>i</sub> also led to hepatic dysfunction through lowering the activity of both enzymes AST and ALT to be half that of the control. The negative effects of both substitutes may be attributed to their high content of Pb, Cd, and silica as well as the level of CF and/or ash of these diets (Abdelhamid *et al.*, 2010). The blood parameters are harmonized also with growth performance parameters and feed and nutrients utilization by the fish (Abdelhamid *et al.*, 2010).

However, disturbing liver function be caused by hepatitis and/or liver injures, and kidney dysfunction may be occurred by kidney disease, hemolysis, and/or hypoproteinemia (Merck, 1974). Moreover, the low Hb concentration is an anemia symptom (Merck, 1976). Increases in blood urea may occur in a number of diseases in addition to those in which the kidneys are primarily involved. Also, the plasma creatinine increases in renal disease. Uric acid belongs to, and is the end product of the metabolism of, the group of substances known as the purines, some of which are present in nucleic acid found in the molecule of the nucleoproteins. It is accordingly formed endogenously from nucleoprotein metabolism, and exogenously from the metabolism of purines taken in the food. Uric acid estimations give little information of value to the clinician except in cases of gout. A reduction in the total protein is one of the causes of edema. In all conditions, there is a negative nitrogen balance due to increased protein breakdown, and increase in blood urea may be found. A low serum albumin is found in severe liver disease and may be a factor in causing edema in liver disease. An insufficient amount of dietary protein may also lead to a low plasma albumin, with a low total protein also. Increase in globulin occurs most commonly in advanced liver disease. Increases in both transaminases are a common finding in liver diseases (Varley, 1978).

### Chemical Composition of Fish:

Chemical composition of the whole fish body revealed that DM, CP, and EE increased at the end of the experimental period. However, the dietary treatments significantly affected all fractions of the proximate analysis of the carcass (Table 2) as well as of the muscles (Table 3). The highest DM% was recorded in both carcass and muscles with 100% DW diet but the

lowest was with 25% CrFi diet; yet, the highest CP% in both carcass and muscles was found with 25% CrFi diet, but the lowest was with 75% DW. Ether extract percentages were the highest and lowest with 100% DW and 25% CrFi, respectively in the carcass and muscles. The diets containing 75 – 100% DW reflected the lowest ash percentages and the diets containing 50% CrFi gave the highest values in both carcass and muscles.

Hassan and Edwards (1992) found that increasing *Lemma* rates in the diet (up to 3%) increased DM, CP and EE of the fish, but higher levels led to the opposite trend. Shiau and Yu (1999) reported that body lipid content of the fish reflects the general pattern of the lipid digestibility. Moreover, Eweedah *et al.* (2006) registered decreases in DM and CP of Nile tilapia carcasses at the supplementing levels (33.3 – 100%) of either CrFi meal or silage comparing with the control. Tables 2 and 3 reflect positive relationships between EE and EC of the carcass and between moisture and protein contents as well as moisture and ash, contents of both carcass and muscles, and negative relationship between moisture and EE as well as between protein and EE percentages. These relations were confirmed before by Abdelhamid *et al.* (2002 and 2008) and El-Ebiary and Zaki (2003).

Silica contents (mg/kg) of diets and fish are presented in Table (4). Diet No. 2 contained the lowest content, thus led to the lowest silica content in the fish carcass. Yet, the highest dietary silica content and fish carcass content were found in diet No. 9 and with diet No. 13, respectively. Table (5) presents lead (Pb) and cadmium (Cd) contents of both diets and fish carcasses. The highest dietary Pb content was found in diet No. 9, and the lowest in diets No. 2 and 10. Yet, the lowest Pb content of fish was registered with diet No. 2, but the highest with diets No. 7 and 13. However, the highest Cd level was found in diets No. 8, 9, 11 and 13, but the lowest was in the control diet No. 1; yet, the highest fish Cd level was given with diet No. 13, and the lowest with diets No. 1, 2, 5, 6 and 7.

However, Abd El-Rahman and Badrawy (2007) reported the lowest Pb and Cd levels in Nile tilapia fed on 50% replacement of fish meal with crayfish meal comparing with the control. They added that the concentrations of Cd and Pb in edible muscle of different freshwater fish of Egypt are often above the maximum permissible limits according to FAO standards. The child intake of fish with these levels is generally above the maximum allowable concentrations, which means human health risk with current Egyptian dietary intakes of fish. However, many heavy metals are frequently occurred in Egyptian water, earth, plants and animals including fish, whether of freshwater or saltwater, and differ in their concentrations from species to another species of fish, and from season to other as well as from location to other (Abdelhamid and Gawish, 1998 and Abdelahmid *et al.*, 2000 and 2006a).

**1+2**

Table (3): Means\* ( ± SE )of muscles chemical composition (%dry matter bases) of Nile tilapia fed on the experimental diets.

Treatments	DM %	Proximate analysis		
		CP	EE	Ash
Control	12.41±0.06b	61.17±0.01b	17.22±0.02g	18.55±0.02c
Duckweeds (25%)	13.17±0.03a	58.52±0.02h	23.01±0.08c	16.71±0.05g
Duckweeds (50%)	13.23±0.04a	56.69±0.06j	25.32±0.01b	16.28±0.01h
Duckweeds (75%)	13.25±0.03a	56.09±0.02k	26.80±0.03a	15.12±0.02i
Duckweeds (100%)	13.27±0.04a	54.30±0.00l	26.97±0.22a	15.14±0.02i
Cray fish (25%)	11.76±0.46bc	61.85±0.01a	16.64±0.19h	18.82±0.05ab
Cray fish (50%)	12.04±0.02bc	61.05±0.02bc	16.54±0.02h	18.91±0.05a
Cray fish (75%)	12.15±0.03bc	60.96±0.03c	16.75±0.02h	18.62±0.03c
Cray fish (100%)	12.25±0.03bc	60.02±0.03e	18.75±0.03f	18.73±0.04abc
Mixed (DW+CrFi) 25%	11.48±0.11c	60.18±0.01d	21.02±0.01e	18.20±0.01d
Mixed (DW+CrFi) 50%	12.13±0.04bc	59.19±0.02f	21.65±0.00d	18.66±0.01bc
Mixed (DW+CrFi) 75%	12.30±0.02b	58.68±0.01g	23.36±0.01c	17.86±0.02e
Mixed (DW+CrFi) 100%	12.23±0.02bc	57.05±0.02i	25.52±0.01b	17.50±0.05f

\*Means (in the same column) superscripted with different letters significantly ( $P \leq 0.05$ ) differ.

Table (4): Silica contents of the experimental diets and fish.

Treatments	Silica in fish, mg/kg	Silica in diets, mg/kg
Control	4.751	5.751
Duckweeds (DW) 25%	2.347	2.347
Duckweeds (DW) 50%	2.398	3.407
Duckweeds (DW) 75%	2.555	4.596
Duckweeds (DW) 100%	2.445	7.406
Cray fish (CrFi) 25%	2.445	4.750
Cray fish (CrFi) 50%	2.449	2.398
Cray fish (CrFi) 75%	4.407	7.407
Cray fish (CrFi) 100%	4.556	9.046
Mixed (DW+CrFi) 25%	2.398	4.597
Mixed (DW+CrFi) 50%	2.445	4.597
Mixed (DW+CrFi) 75%	4.568	4.751
Mixed (DW+CrFi) 100%	7.407	7.457

Table (5): Lead and cadmium contents of the experimental diets and fish.

Treatments	Fish		Diet	
	Lead, mg/kg	Cadmium, mg/kg	Lead, mg/kg	Cadmium, mg/kg
Control	20.5	25.5	20.5	25.5
Duckweeds (DW) 25%	14.0	25.5	17.5	51.0
Duckweeds (DW) 50%	17.5	51.0	24.5	67.5
Duckweeds (DW) 75%	24.5	76.5	24.5	76.5
Duckweeds (DW) 100%	24.5	25.5	28.0	102
Cray fish (CrFi) 25%	28.0	25.5	24.5	25.5
Cray fish (CrFi) 50%	31.5	25.5	28.0	51.0
Cray fish (CrFi) 75%	28.0	51.0	24.5	127.5
Cray fish (CrFi) 100%	28.0	102	31.5	127.5
Mixed (DW+CrFi) 25%	17.5	76.5	17.5	102
Mixed (DW+CrFi) 50%	21.0	76.5	21.0	127.5
Mixed (DW+CrFi) 75%	28.0	76.5	21.0	102
Mixed (DW+CrFi) 100%	31.5	127.5	24.5	127.5

#### Microbiological test:

Throughout the course of the experiment, the 25 and 50% DW diets led to changes in water quality, i.e. dark silver color with off smell. This led to

caring out the total count of microorganisms (Table 6). Yet, there were no critical counts, whether in water, diet or fish tissues. Also, there were no relations among the counts in water, diets, muscles and/or liver of the tested fish. Abdelhamid *et al.* (2006b) registered the presence of pathogenic bacteria ( $1.3 - 2.0 \times 10^5$  cfu) in samples of water, feed, sediments and fish, mainly in summer season. There was no difference between fish of natural resources and those of aquaculture concerning bacterial contamination. Also, Abdelhamid *et al.* (2006b and 2008) found pathogenic bacteria ( $\times 10^6$  cfu) in samples of water, feed, intestinal, and liver of Nile tilapia up to 113.0, 38.7, 28.33, 23.33, and 25.00, respectively.

**Table (6): Count of microorganisms obtained on nutrient agar media.**

Treatments	Microbial count (cfu*10 <sup>5</sup> /g or ml)			
	Liver	Muscles	Diet	Water
Control	5	45	-	2
Duckweeds (25%)	120	100	3	18
Duckweeds (50%)	132	98	-	1
Duckweeds (75%)	125	35	40	-
Duckweeds (100%)	118	102	22	9
Cray fish (25%)	135	120	41	80
Cray fish (50%)	110	122	70	-
Cray fish (75%)	107	125	8	9
Cray fish (100%)	60	20	120	-
Mixed (DW+CrFi) 25%	22	120	39	-
Mixed (DW+CrFi) 50%	105	117	118	-
Mixed (DW+CrFi) 75%	102	120	42	-
Mixed (DW+CrFi) 00%	105	-	21	-

Cfu: colony forming unit

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### تقييم بعض العلائق غير التقليدية لأسماك البلطي النيلي:

#### ٢- من حيث التأثير على صورة الدم وتركيب الجسم للأسماك.

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أجريت هذه التجربة الغذائية عام ٢٠٠٨م بمعمل رطب، وذلك لدراسة تقييم بعض العلائق غير التقليدية على صورة الدم و تركيب جسم وعضلات اصباغيات أسماك البلطي النيلي في أحوض زجاجية. كان الوزن الأولي للاصباغيات ٧ – ٨ جم، فترة أقلمة لأسبوعين قبل بداية التجربة التي استمرت ١٦ أسبوعاً، العليقة الأساسية احتوت على ٢٥% بروتين خام، قدمت العلائق على وجبتين يومياً بمعدل ٣% من وزن السمك يومياً، كل معاملة كررت في حوضين، معدل تخزين السمك ٧ سمكات لكل حوض، العلائق التجريبية كانت متساوية البروتين والطاقة تقريباً، العلائق رقم ١ مقارنة، وأرقام ٢ – ٥ استبدل فيها مسحوق سمك المقارنة بمعدل ٢٥، ٥٠، ٧٥، ١٠٠% بواسطة مسحوق نبات عدس الماء، والعلائق أرقام ٦ – ٩ استبدل فيها مسحوق سمك المقارنة بواسطة مسحوق استاكوزا المياه العذبة بنفس المعدلات السابقة، والعلائق أرقام ١٠ – ١٣ استبدل فيها مسحوق سمك المقارنة بنفس المعدلات السابقة لكن بمسحوق مخلوط عدس الماء + استاكوزا المياه العذبة (١/١). ولقد كانت أهم النتائج المتحصل عليها هي أن العليقة رقم ٦ قد حققت أعلى محتوى هيوجلوبيين ونسبة هيماتوكريت، وتركيزات بروتين كلى وجلوبيولين، بينما العليقة الرابعة أعطت أقل هذه القيم. ارتفعت أنشطة إنزيمات نقل الأمين مع العليقة الخامسة، بينما انخفضت مع العليقة السابعة، مما يشير لتأثير وظائف الكبد. ارتفعت تركيزات اليوريا وحمض اليوريك والكرياتينين مع العليقة الخامسة، مما يشير لتأثير وظائف الكلى. تأثر معنوياً تركيب الجسم والعضلات للسمك بالمعاملات الغذائية. أعلى مادة جافة كانت بالتغذية على العليقة رقم ٥، وأقلها كانت بالتغذية على العليقة رقم ٦. أعلى محتوى بروتيني سجل كذلك مع العليقة رقم ٦، وأقله مع العليقة رقم ٤. أعلى مستخلص إثري سجل مع العليقة الخامسة، وأقله مع العليقة السادسة. أقل نسبة رماد وجدت مع العليقتين الرابعة والخامسة، بينما أعلى نسبة رماد وجدت مع العليقة السابعة. وجدت علاقة موجبة بين كل من رطوبة وبروتين، رطوبة ورماد، وعلاقة عكسية بين رطوبة ودهن، وبروتين ودهن كل من جسم وعضلات السمك. انخفاض سليكا العليقة الثانية أدى لانخفاض محتوى سليكا جسم أسماك هذه المجموعة. أعلى محتوى سليكا وجد في العليقة التاسعة، وإن احتوت أجسام أسماك العليقة رقم ١٣ على أعلى محتوى سليكا. أعلى تركيز رصاص وجد في العليقة رقم ٩ وسمك المجموعتين رقم ٧ ورقم ١٣، وأقله في العليقة والسمك للمجموعة الثانية. أعلى تركيز كاديوم في العليقة والسمك كان في المجموعة رقم ١٣، وأقله في عليقة وسمك المجموعة المقارنة. مما سبق عرضه من نتائج يتضح أن العليقة السادسة (٢٥% مسحوق استاكوزا مياه عذبة حلت محل مسحوق السمك في عليقة المقارنة) هي الأفضل معنوياً من حيث محتوى الدم من الهيموجلوبيين والهيماتوكريت والبروتين الكلى والجلوبيولين، وكذا تحقيق أعلى نسبة بروتين في جسم وعضلات السمك مع أقل نسبة دهن.

#### قام بتحكيم البحث

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**Table (1): Data of blood hematological and biochemical analysis of tilapia fish as affected by the dietary treatments.**

Treatments	Hbg/dl	PCV%	Pg/dl	Ag/dl	Gg/dl	Umg/dl	Umg/dl	Cmg/dl	ASTu/l	ALTu/l
Control	4.36	13.95	3.13	1.43	1.70	5.69	2.11	0.375	24.16	9.26
Duckweeds (DW) 25%	2.93	9.37	2.51	1.17	1.34	6.76	2.52	0.338	26.13	12.91
Duckweeds (DW) 50%	3.01	9.63	2.62	1.21	1.41	7.15	2.73	0.361	27.09	13.29
Duckweeds(DW) 75%	2.76	8.83	2.44	1.13	1.31	7.74	2.77	0.475	29.06	17.62
Duckweeds(DW) 100%	2.78	8.89	2.48	1.16	1.32	8.77	2.79	0.477	31.91	19.31
Cray fish (CrFi) 25%	6.56	20.99	4.56	2.51	2.05	4.47	1.56	0.362	13.38	5.89
Cray fish (CrFi) 50%	6.49	20.77	4.33	2.53	1.80	4.74	1.75	0.359	12.09	5.70
Cray fish (CrFi) 75%	5.86	18.75	4.12	2.14	1.98	5.07	1.93	0.363	21.13	8.13
Cray fish (CrFi) 100%	5.51	17.63	4.16	2.22	1.94	5.17	1.97	0.359	21.19	8.61
Mixed (DW+ CrFi) 25%	6.13	19.62	4.01	2.01	2.00	4.36	1.66	0.329	16.02	6.13
Mixed (DW+ CrFi) 50%	6.32	20.22	3.96	2.16	1.80	4.35	1.73	0.336	17.03	7.17
Mixed (DW+ CrFi) 75%	5.31	16.99	3.63	1.63	2.00	5.49	2.07	0.354	22.14	8.77
Mixed (DW+ CrFi) 100%	5.22	16.70	3.72	1.72	2.00	5.39	2.06	0.349	23.06	9.01

Hb: hemoglobin , PCV: packed cell value, P: protein, A : albumin, G: globulin ,U: urea , UA:uric acid,C: creatinine ,AST: aspartate amino transaminase , and ALT : alanine amino transaminase.

**Table (2): Means\*\* (  $\pm$  SE ) of chemical composition (%dry matter bases) of Nile tilapia carcass as affected by the dietary treatments.**

Treatments	DM %	Proximate analysis			
		CP	EE	Ash	EC, kcal /100g
Control	23.23 $\pm$ 0.00h	51.26 $\pm$ 0.39abc	29.58 $\pm$ 0.02g	21.14 $\pm$ 0.15bc	568.66 $\pm$ 2.07a
Duckweeds (25%)	26.20 $\pm$ 0.06d	49.71 $\pm$ 0.62bcde	32.17 $\pm$ 0.01d	17.36 $\pm$ 0.42e	584.09 $\pm$ 3.63a
Duckweeds (50%)	26.96 $\pm$ 0.01c	48.17 $\pm$ 0.34de	32.30 $\pm$ 0.01c	15.42 $\pm$ 0.12f	576.59 $\pm$ 1.73a
Duckweeds (75%)	28.83 $\pm$ 0.02a	47.94 $\pm$ 0.41e	32.60 $\pm$ 0.03b	15.02 $\pm$ 0.02f	578.12 $\pm$ 2.57a
Duckweeds (100%)	28.86 $\pm$ 0.04a	49.28 $\pm$ 0.72cde	32.88 $\pm$ 0.02a	14.72 $\pm$ 0.08f	588.33 $\pm$ 4.15a
Cray fish (25%)	23.36 $\pm$ 0.02h	53.32 $\pm$ 0.42a	28.22 $\pm$ 0.01k	20.90 $\pm$ 0.15bc	567.10 $\pm$ 2.52a
Cray fish (50%)	23.63 $\pm$ 0.06g	52.47 $\pm$ 0.42ab	29.19 $\pm$ 0.02i	21.56 $\pm$ 0.66ab	571.42 $\pm$ 2.19a
Cray fish (75%)	25.48 $\pm$ 0.03e	51.12 $\pm$ 0.63abc	29.31 $\pm$ 0.01h	22.74 $\pm$ 0.38a	554.80 $\pm$ 9.31a
Cray fish (100%)	21.17 $\pm$ 0.02i	51.43 $\pm$ 0.36abc	29.70 $\pm$ 0.02i	21.19 $\pm$ 0.13bc	546.15 $\pm$ 15.38a
Mixed (DW+CrFi) 25%	18.70 $\pm$ 0.06j	52.15 $\pm$ 0.63ab	28.70 $\pm$ 0.01j	21.56 $\pm$ 0.29ab	565.05 $\pm$ 3.67a
Mixed (DW+CrFi) 50%	24.34 $\pm$ 0.02f	50.70 $\pm$ 0.44abcd	29.20 $\pm$ 0.00f	20.69 $\pm$ 0.08bcd	550.34 $\pm$ 7.83a
Mixed (DW+CrFi) 75%	24.40 $\pm$ 0.02f	50.56 $\pm$ 0.19abcde	31.13 $\pm$ 0.00f	19.78 $\pm$ 0.02cd	579.02 $\pm$ 1.18a
Mixed (DW+CrFi) 100%	27.35 $\pm$ 0.03b	50.87 $\pm$ 0.44abcd	31.05 $\pm$ 0.02b	19.34 $\pm$ 0.11d	583.03 $\pm$ 0.62a

\*Means (in the same column) superscripted with different letters significantly ( $P \leq 0.05$ ) differ.