VALORIZATION OF BY-PRODUCTS THROUGH FEED FORMULATION FOR *TILAPIA SP*: ZOOTECHNICAL PERFORMANCE STUDY

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Abstract. In recent years valorization of biowaste attracts lot of attention worldwide owing to its high nutritional value and low price. In this work biowaste of animal (sardines) and plant (tomato) biowaste was used to formulate a new feed for red tilapia, that showed to be competitive in its price and zootechnical performance in comparison to commercially available tilapia feeds. Mathematical modelling was used to formulate optimal feed composition with favorable chemical composition and the lowest price. Formulated feed had high protein content (40.76%) and energy value of 279.6 Kcal/100 g. Optimised feed was manufactured and compared to commercially available reference feed in respect to feed intake, feed efficiency, specific growth rate of fingerlings of Tilapia sp and, most important, zootechnical parameters. With fish survival rate of 100% calculated feed conversion index for the formulated feed was 2.7.

Keywords: conversion index, fish waste, formulated feed, tomato waste

INTRODUCTION

Fish is considered to be a wholesome nutritionally rich food highly recommended for human nutrition, since it represents a good source of proteins, vitamins (D, B3), minerals (Zn, Se, Ca, P) and polyunsaturated fatty acids (PUFAs) in the ω3 series. Each year 167 million tons of fish and crustaceans (20 kg/inhabitant) are consumed worldwide, part of which comes from fishing and part from aquaculture (F A O 2016). In future it is expected that demands for fish will increase remarkably, following the trend of population increase. This trend imposes necessities for developing new feed formulations that would be cost effective and nutritious allowing good weight gain in producing animals. Fish farmers are thus orienting towards development of new feed formulations utilizing fishery and agricultural by-products. The biowaste is often a rich source of proteins, lipids, fibers, vitamins and minerals that can be used in feed formulation. Feed price is very important factor in aquaculture, constituting ~50% of the total costs of fish farming (Guillaume et al 1999; Rana et al 2008; Slembrouck et al 1991). The final cost of the feed depends mainly on the cost of integrated proteins, and in recent years the price of fish feed has increased considerably, which encouraged fish feed industry to seek more available and cheaper protein sources (Slembrouck et al 1991; Guillaume et al 1999; Azaza et al 2005; Azaza et al 2006; Grima 2010). Several plant and animal protein sources have been tested for total or partial replacement of fishmeal in aquafeed. Animal-derived by-products such as meat and bone meal, poultry by-product meal, and tankage meal have been used as the fishmeal alternatives due to

good digestibility, good nutritional value, and low price (Galkanda-Arachchige et al 2019).

The present work was oriented towards development of new fish feed formulation, incorporating tomato and fish biowaste. The quality and feed performance of the manufactured feed, based on the least cost linear formulation, was compared with an extruded feed, imported as a control. For the estimation of the quality of the developed feed a comparative evaluation of the zootechnical performance in *Tilapia sp* fish at the juvenile stage was conducted. After optimisation of the newly-formulated feed, based on tomato and fish residues, physico-chemical properties of reference and developed feed were analysed and compared. Furthermore, two feeds were compared in respect to most important zootechnical parameters. Presented research results might have an important impact in promoting local agricultural and biowaste management offering to fish farmers solutions adapted to the context of their farms and minimizing the costs of the formulated feed while meeting the nutritional needs of specific fish species.

MATERIALS AND METHODS

Chemicals and reagents

The chemicals and reagents used are, phenol, sodium nitroprusside, trisodium citrate, sodium hydroxide, sulfanilamide, hydrochloric acid, N-Naphthylethylene diamine dichlorohydrate, ammonium chloride, sulfuric acid, ascorbic acid, potassium oxytartrate, boric acid, ethanol, methyl red were acquired from Sigma Aldrich (Madrid, Spain).

Formulation and manufacture of fish feed

Preparation of the fishmeal

The fish by-products used in our study were supplied by the "Sarl Capten", an agro-food cannery of Ténès-Chlef-Algeria. The preparation of fishmeal from fish by-products (*Sardina pilchardus*) was done in the processing unit of the "Sarl Capten" where weighing, cooking and pressing operations were carried out. After fish cleaning and chopping, the fish waste was taken to a steam cooker. Steam cooking released part of the water and fat due to proteins coagulation. To separate proteins pressing was done manually, producing a cake with strongly reduced contents of water and fat. The press cake was then put in a dryer. The drying process was done at 45°C in a duration of approximately one day until water content of 10%. The prepared meal was stored at 4°C until further milling. The obtained fishmeal, with the yield of 20%, was a fine powder, dark brown in color and with a very persistent odor. The chemical composition of the produced fishmeal is presented in "Tab.1".

Table 1

Chemical composition of the fishmeal prepared from of sardine waste

Parameter	Unit
Water content	8.07%
Ash	26.98%
Crude protein	45.88%
Fat	15.17%
Calcium	9.74%
Phosphorus	1.08%

Total sugars	0.57%
Carbohydrates	3.90%
Caloric value	338.17 Kcal/100g

Preparation of the tomato waste meal

The tomato waste consisting of skin was dried in the same way as previously described for the fish waste. Tomato meal, with caloric value of 138.61 Kcal/100g of dry matter, was produced after drying and grinding. Other chemical parameters of prepared tomato meal, are presented in "Tab. 2". Total insoluble fiber consisted mainly of cellulose.

Table 2

Chemical composition of tomato waste meal

Parameter	**Unit**
Proteins	12.78%
Fat	2.23%
Total sugars	4.78%
Starch	-
Cellulose	24.15%
Carbohydrates	4.78%
Caloric value	138.61 Kcal/100g

Formulation of the feed

The formulation of the feed was carried out on the basis of feed requirements of Tilapia fish at the alvinic stage and chemical composition of used raw materials previously determined (Guillaume et al 1999; Geoffroy et al 2019). Following ingredients were used in feed formulation: corn, soybean meal, calcium, phosphate-bi-calcite, mineral and vitamin supplements, durum wheat, previously prepared fish meal and tomato meals. As a feed binder a wheat gluten was used. The nutritional values of used ingredients were consulted in INRA tables (Guillaume et al 1999). Feed was produced following the calculation of the proximal feed composition by mathematical programming. To optimize feed formulation a linear programming using the Microsoft Excel software was applied. Mathematical programming for feed formulation allowed minimization of the production costs while finding a balance between the nutritional value, content and constraints of the ingredients used in the formulation (Al-Deseit 2009). By integrating the price, proportion, chemical composition and nutritional value of each ingredient, a linear function with eight variables under twenty-one (21) constraints (Equations 2 through 22) was obtained. Excel Solver 2013 (Office Excel) was used for calculation of respective proportions of the ingredients chosen in the formulation. The feed formulated at the lowest cost was chosen by linear programming.

The centesimal composition of the formulated feed obtained on the basis of the lowest price is reported in "Tab.3". Mathematical model predicts the highest proportion of sardine waste meal (32.60%), followed by soybean meal (30.00%), durum wheat (19.80%) and tomato meal (10%). The remaining fraction is

compensated by other ingredients (corn, calcium, phosphate, CMV, wheat gluten). The estimated price of the optimized feed was 1.01 USD/kg.

Table 3

Centesimal composition of the formulated fish feed

Ingredients	%
Sardine waste meal	32.60
Soybean cake	30.00
Durum wheat	19.80
Crude calcium	0.99
Bi-calcite phosphate	0.99
Sunflower oil	2.63
Wheat gluten	1.38
CMV	1.61
Tomato waste meal	10.00

Physico-chemical characterisation of formulated feed

Chemical composition of feed, previously formulated by mathematical modelling, was carried out in the laboratory ONAB (national cattle feed office) ford of Constantine-Algiers. "Tab. 4" lists basic chemical and nutritional parameters of both the formulated (T_0) and the reference (T_1) feeds. Developed and manufactured feed met the requirements of Red Tilapia fish at the alvin stage in crude protein (40.76%), fat (8.82%), total sugars (0.49%), starch (3.26%), moisture (7.57%), fiber (6.79%), calcium (5.6%), phosphorus (0.99%) and carbohydrate (5.9%). In comparison to reference diet, developed feed had significantly different content of crude protein, dry matter, cellulose, lipids and calcium.

The physical quality of the formulated and prepared feeds included evaluation of buoyancy (%) (Misra et al 2002), bulk density (g/cm^3) (Misra et al 2002), expansion rate (%) (Misra et al 2002), relative absorption rate (%) (Fagbenro and Jauncey, 1995) and stability in water (%) (Fagbenro and Jauncey 1995). The degree of feed spoilage was evaluated by the determination of total volatile nitrogen compounds (TVBN) by the method of Uriarte-Montoya et al 2010 (Uriarte-Montoya et al 2010).

Table 4

Proximal composition of formulated and reference feeds.

Parameter	T_1			T_0
	Content	Standard	Alvin Tilapia requirements (Guillaume et al	

			1999)	
Crude protein (%)	40.76	NA 652-1992	>=25	30
Fat (%)	8.82	NA 654-1992	- <=4	-
Total sugars (%)	0.49	BERTRAND	-	-
Starch (%)	3.26	AFNOR	-	-
Moisture (%)	7.57	NA1291-1992	<=5 <=8	-
Cellulose (%)	6,79	DE	-	3.4
Calcium (%)	5.6	AFNOR		1.2
Phosphorus (%)	0.99	NA652-1992		0.94
Lipids (%)	2.96	-		6
Carbohydrates (%)	5.9	DE		-

NA: Algerian standard; AFNOR: French Association for Standardization; DE: European Directive;

Experimental design

The zootechnical performance of the formulated feed (T_1) was compared with the imported extruded feed as a control (T_0). In all experiments alvine-phase red Tilapia fish with initial mean weight of 7.36±2.90 g (T_0) and 5.50±1.33 g (T_1) was used. The alvins were provided by a private fish farm (Garden, Algiers). A total 60 fishes were individually weighed and randomly distributed in tanks of the total capacity of 150 l, 30 fish per aquarium, thus forming two groups each corresponding to one feed. The aquariums were equipped with an air pump for oxygenation of the water, a hose for siphoning and a thermostat to regulate the water temperature. The physico-chemical parameters of the water (temperature, pH, conductivity, salinity and dissolved oxygen) were measured using a Salinometer-Oxymeter (WTW), once a day in order to assess their impact on fish growth. Nitrite, ammonia and phosphorus were measured weekly by spectrophotometric methods (JASCO V-630) on the same day of water sampling (Aminot and Chaussepied 1983). In order to maintain a certain level of cleanliness in the rearing circuit and to avoid the development of bacteria on food remains and feces, every 24 hours, one hour before the first feeding, the bottom of the aquariums was siphoned off. The edges were cleaned and rinsed well to limit the deposits of any fat left by the feed.

The fishes were acclimatized 15 days before the start of the experiment. Feeding was carried out manually, during 6 weeks, at the rate of two meals per day (9:00 am and 3:30 pm) according to the formula of the feed ratio (Fa):

Fa=Wa* N* Fr

where Wa is the average weight of fish in g; N is the total number of individuals and Fr is the feeding rate (5%)

The aquarium water was renewed three times a week to minimize toxic effects of nitrogen compounds.

Zootechnical Control

During the experiment, growth control was carried out every two weeks, using a scale and an ichthyometer, and the feed ratio was adjusted according to biomass. The following zootechnical parameters were monitored: survival rate (%), relative weight gain (%), weight gain (g/d/ind), relative length gain (%), length gain (cm/d/ind), specific growth rate (%), feed conversion rate, biomass produced (g), feed intake (g), condition factor (K) and allometry coefficient (b) following the protocols described earlier (Azaza et al 2006; Moshood et al 2014; Inabanza et al 2016; Brah et al 2019).

Statistical analysis

The analysis of variance (single-factor ANOVA) was performed for two feeds distributed in two aquariums during 45 days, in order to study their influence on the weight and linear growth of the fish.

When the variances were homogeneous, statistical analysis was performed in a parametric test of one-factor analysis of variance (ANOVA1). When the variances were not homogeneous, the statistical test included a non-parametric Kruskal-Wallis test. To determine whether the differences between the means obtained were significant, the Fisher's test (LSD) was used in the case of ANOVA 1, whereas the Mann-Whitney's test was used in the case of Kruskal-Wallis test. The probabilities $p \leq 0.05$ were considered statistically significant and $p \leq 0.01$ very significant.

RESULTS AND DISCUSSION

Quality evaluation of the formulated feed

The quality of the formulated feed was evaluated by the water absorption test "Tab.5" and stability in water "Tab.6". Observed physical properties of the produced feed were compared with the reference, commercial feed. The present study shows a difference of expansion rates ($p<0.05$) between commercial (46.87±12.5%) and formulated pellets (153.33±61.13%), as well as in terms of bulk densities. Formulated feed granules had significantly lower bulk density in comparison to the commercial ones (Table 4). Better water adsorption rates were noted in the extruded, reference feed (266.66±0.00 %) compared to the formulated pellets (83.33±23.57 %). Also, a very significant deference of flotation ($p < 0.05$) between commercial (100±0.00 %) and formulated granules (33.33 ± 5.77 %) were noted during the first five minutes. There were differences in pellets stability ($p < 0.05$) of the two compared feeds. The stability of the reference feed pellets was 100±0.00% and 83.33±0.00% for 30 and 60 min respectively, and 66.66±0.00% and 49.99±23.56% for 30 and 60 min respectively, for the formulated feed. Also, the results show that the rate of disintegration in water increased proportionally with the time for both feed types.

Table 5

Physical properties of the feeds

Parameter	**T_0**	**T_1**
Apparent density (g/cm^3)	1.05±0.42	1.42±0.08

Rate of expansion (%)	46.87±12.5**	153.33±61.13**
Relative absorption rate (%)	266.66±0.00***	83.33±23.57***

*p > 0.05 ; **p < 0.05 ; ***p < 0.01

Table 6

Percentage of flotation and stability in water.

Parameter	Time (min)	T_0	T_1
Flotation (%)	1-5	100±0.00*	33.33±5.77**
	6-10	100±0.00*	16.67±5.77***
	11-15	100±0.00*	13.33±5.77***
	16-20	100±0.00*	13.33±5.77***
	21-30	100±0.00*	6.66±5.77***
	30-50	100±0.00*	3.33±0.00***
	>50	100±0.00*	0.00±0.00***
Stability in water (%)	30	100±0.00*	66.66±0.00**
	60	83.33±0.00**	49.99±23.56***

*: for p > 0.05; **: for p < 0.05; ***: for p < 0.01;

Zootechnical experiment

The physico-chemical parameters of the rearing environment during the experiment are shown in "Tab.7". The mean values of basic physico-chemical parameters of the rearing water (temperature, pH, $N\text{-}NH_{3,4}$, $N\text{-}NO_2$ and $P\text{-}PO_4$) in general, showed little variation throughout the study period for both feeds (T_0 and T_1).

Table 7

Basic physico-chemical parameters of the water during the experiment

Parameters	T_1	T_0
Temperature (°C)	27.14±1.61*	26.61±2.43*
Dissolved oxygen (mg/l)	4.8±0.90*	4.4±1.20*
pH	8.42±0.21*	8.41±0.23*
$N\text{-}NH_{3,4}$ (mg/l)	0.081±0.12*	0.031±0.042*
N-NO2	0.53±0.74*	0.25±0.61*
$P\text{-}PO_4$	2.14±0.76*	1.73±0.40*

*: for p > 0.05; **: for p < 0.05; ***: for p < 0.01;

The tested feed demonstrated excellent physical cohesion of pellets and has shown to be safe for fish, not exhibiting any toxicity. Throughout experiment, the fish showed no pathological signs and did not suffer any mortality. The mean survival rates at the end of the experiment were 100% for both tested and reference feeds. Statistical analysis showed that there were no significant differences (p>0.05)

between survival rates of red tilapia alvines fed with formulated and commercial feeds (Fig. 1).

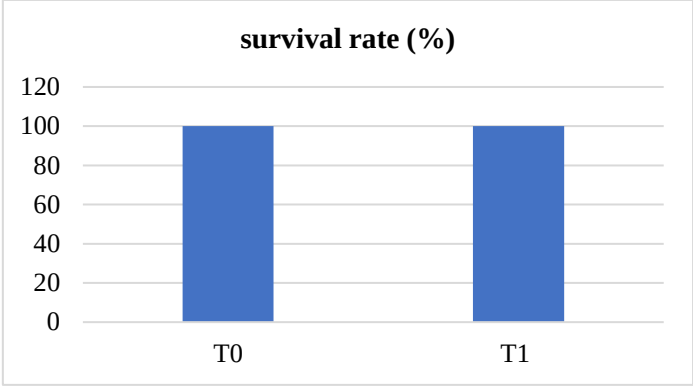

Figure 1. Survival rate at the end of the experimental cycle: T_0 – reference feed; T_1 – formulated feed; $p > 0.05$.

Fig. 2 shows the growth histograms for the two compared feeds. Formulated and reference feeds showed significantly different ($p<0.01$) impact on the growth rate of red tilapia alvines. At the end of the experimental cycle (45 days) calculated fish growth for formulated feed was 18.85±7.90 g, whereas for commercial feed the measured growth was 28.07±10.64 g. Also, the ANOVA test showed significant differences ($p<0.05$) between the mean fish weights at the beginning of the cycle, which were 5.50±1.33 g and 7.36±2.90g for the formulated and reference feed, respectively. Obtained results clearly indicate that both types of feed have similar nutritional values that meet the nutritional requirements of red tilapia at the alvinic stage during 6 weeks of trials.

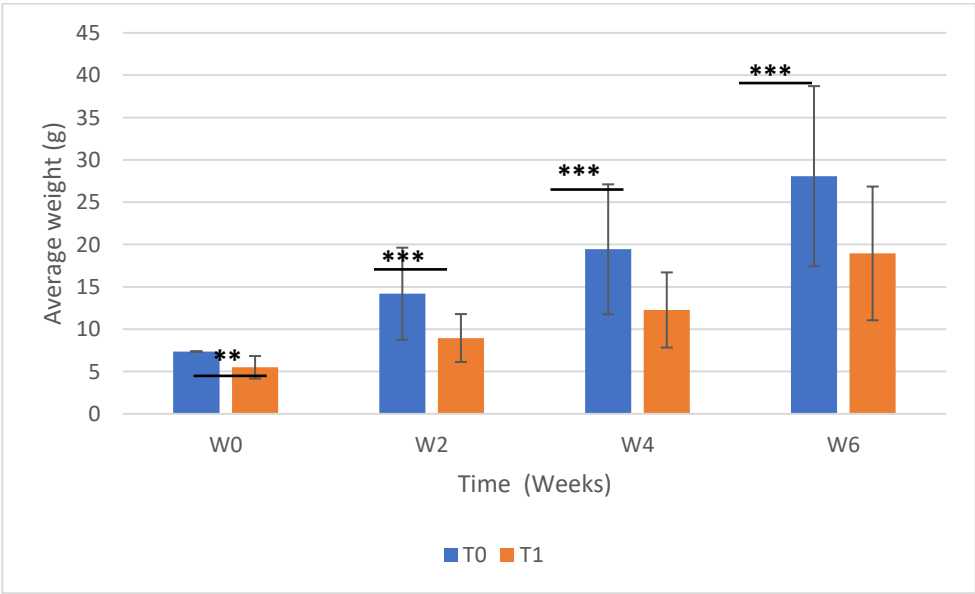

Figure 2. Evolution of fingerlings weight as a function of time.
*p > 0.05; **p < 0.05; ***p < 0.01

Zootechnical performance "Tab. 8", specific growth rate and calculated weight gain, showed a non-significant difference ($p < 0.05$) between two tested feeds. Namely for T_1 diet calculated specific growth and a weight gain were 5.58% and 0.27 g/d/ind respectively, whereas for T_0 diet, slightly higher values were observed (6.74% and 0.46 g/d/ind, respectively). For T_0 feed conversion index was 2.50, with a cost of 3.97 USD per feed unit, whereas for T_1 feed 2.7 with a cost of 1.01 USD per unit. The ANOVA test showed that there were no significant differences ($p> 0.05$) between T_0 and T_1 feed conversion rates.

Table 8

Zootechnical performance of formulated and reference feeds in Red Tilapia

Zootechnical parameter	**T_1**	**T_0**
Number of fish at the beginning of the experiment	30*	30*
Number of fish at the end of the experiment	30*	30*
Initial average weight (g)	5.50±1.33**	7.36±2.90**
Final average weight (g)	18.95±7.90***	28.07±10.64***
Initial average length (cm)	7.56±1.94*	6.66±0.73*
Final average length (cm)	11.43±1.34***	9.64±1.27***
Specific growth rate (%)	5.58*	6.74*
Weight gain (g/d/ind)	0.27*	0.46*
Relative weight gain (%)	2.25*	2.82*
Survival rate (%)	100*	100*
Food conversion index	2.7*	2.5*
Biomass produced (g)	370.58	621.42
Quantity of food ingested (g)	1002.75	1555.86
Cost per feed unit (USD)	1.01	3.97

*: for $p > 0.05$; **: for $p < 0.05$; ***: for $p < 0.01$;

The Feed Conversion Index (CI) is an indicator commonly used in aquaculture farming, it gives an idea of the feed efficiency of a feed or feeding strategy. Slightly higher CI was observed for feed T_1 (2.7). In T_0 calculated CI was 2.5. For both tested feeds their CIs were similar to those obtained by Abdel-Warith et al (2001) which ranged from 1.25 to 2.80 for fingerlings fed with feeds based on avian by-products. Feed conversion rates obtained by Fagbenro et al (1999) ranged from 1.52 to 2.27 depending on whether the diet was based on soybean meal or arugula meal with percentage of incorporation of 19.5, 39 or 58.5%. The feed conversion rates obtained by Giri et al (2000) in their experiment of substituting

fishmeal with chicken viscera meal or vegetable meals (soybean, peanut and mustard) were 3.0 and 2.9, respectively. Sandamali et al (2016) conducted a 12-week feeding trial to evaluate the effects of total fishmeal replacement with different corn co-products on growth performance and feed utilization efficiency in juvenile Nile tilapia *Oreochromis niloticus*. Yacouba (2008) confirmed the benefits of using agricultural by-products as components of tilapia *Oreochromis niloticus* feed in the pre-pregnancy phase. Tested feed was prepared from agricultural by-products (oilcakes of soy and cotton, corn and rice bran) and demonstrated significantly better zootechnical performance in comparison to commercial feed. The results of our research on the efficiency of the feed prepared with fishmeal and tomato meal were quite comparable to those of other researchers. The tomato industry generates a significant volume of waste, estimated between 10 and 30% according to Benakmoum et al (2008). The majority of the works carried out with this by-product, are focused on the antioxidant and pro-vitamin benefits of lycopene, β-carotene and phenolic compounds in this biowaste (Kaur et al., 2008). We have sought to valorize tomato by-products in order to reduce pollution and thus avoid the export of dry matter, while producing a cheaper local fish feed, rich in proteins, vitamins and minerals.

The weight-length relationship in red tilapia alvines is shown in Figure 3a (T_1) and Figure 3b (T_0). In the case of T_0 fish weighed heavier and grew in size with a condition factor of K= 0.005, compared to the T_1 diet (K= 0.10) (fig.3b). In addition, the slopes in the height-weight dependence were lower than the theoretical value of 3 for both treatments (b = 2.84 for T_1 and 2.32 for T_0) (Fig. 3-A, and 3- B), reflecting negative allometric growth (weight grows relatively slower than length in both regimens).

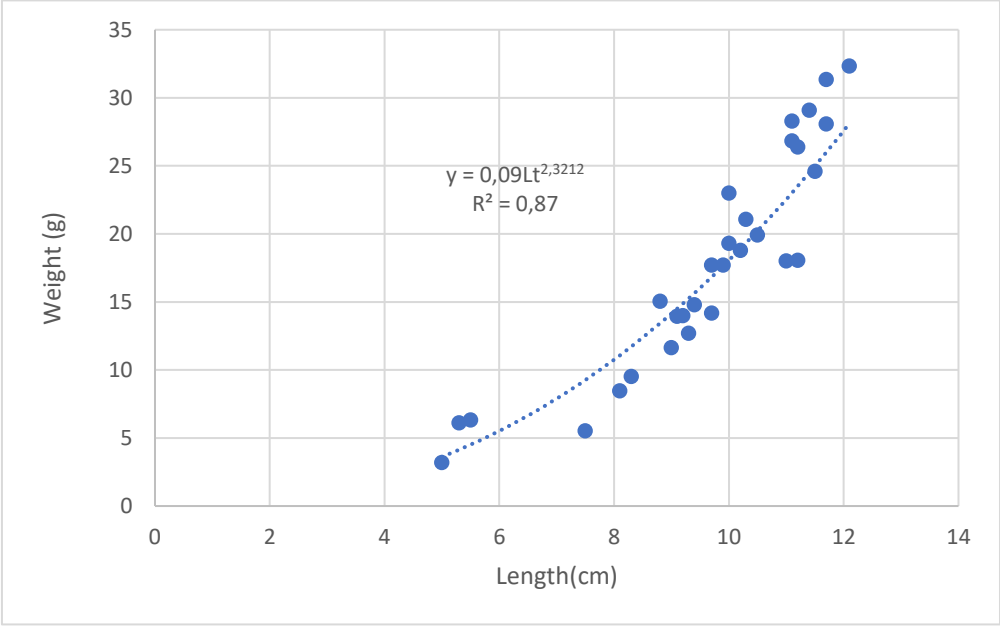

Figure 3a. Weight-length relationship in Red Tilapia alvines fed with T_1

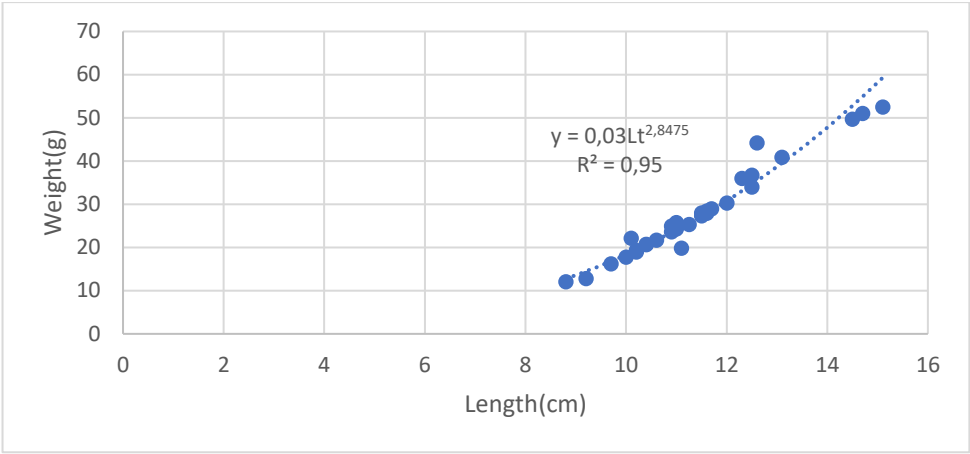

Figure 3b. Weight-length relationship in Red Tilapia alvines fed with T_0

CONCLUSION

In this work a new feed for *Tilapia sp* was formulated and manufactured by incorporating locally-available biowaste, more specifically tomato and sardine waste, offering more economical alternatives to commercially available tilapia feeds. Chemical composition and zootechnical performance of the developed feed was compared with commercially available feed, that was used as a reference. Produced feed, in addition to it`s competitive price which was ~3 times lower in comparison to commercial feed, and simple technology, exhibited appreciable sensory properties (powder appearance, brown color, smell and taste of fish). Chemical analyses reveled that incorporation of sardines biowaste assured high protein (40.76%) and fat (8.82%) contents. Incorporation of tomato biowaste, on the other hand, assured high cellulose content and high energy value.

Formulated feed produced excellent specific growth rate (5.59) of Red tilapia that was close to values reported by several authors for feeds incorporating more than 25% of unconventional protein sources. In addition, the feed conversion index for the formulated feed (2.7) was slightly above the index for the reference, commercial feed (2.5).

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