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Investigation of Pineapple Remnants Used in Fish Aqua Feeds

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ABSTRACT

The present research addressed the pineapple (*Ananas comosus*) residue (peel, crown, and bran) and its impact on the apparent digestibility, productive performance, carcass yield, liver status, and immune system of *Oreochromis niloticus* fry (initial weight: 2.16 ± 0.04 g). In a 14- week trial, fish were fed on 1% and 2% of each pineapple additive, starting from June 16 till September 23, 2020. Results showed a promising enhancement of fish growth performance and feed utilization. The tilapia, fed the pineapple crown, bran, and peel powder at level 2%, showed improvements in fish flesh, liver health, and apparent protein digestibility, followed by those fed the level 1% ($p<0.05$, compared to the control group). The data of the current study indicate the possibility of reusing the pineapple fruit residue as additives to the tilapia feed to sustain fish production with good quality. More studies on fruit residues are required for the sake of fish production and health quality enhancement. This would, in turn, reduce the aquaculture feed cost and solve the environmental problem of accumulating residues.

INTRODUCTION

The last decades have witnessed an outstanding global extension in the Nile tilapia rearing throughout one hundred countries in Asia and Africa **(Gu *et al.*, 2017)**. The global tilapia yield has increased (0.5 to 5.7 Mmt) from 1990 till 2016; with an improving annual rate of 13.5% **(FAO, 2017)**. The *Oreochromis niloticus* is one of the dominant species of world aquaculture especially in Egypt **(Furuya *et al.*, 2008)**. At the intensive culture, The cost of a balanced feed is high cost because animal products and sub-products are being used to form fish meal. Thus, many efforts have been exerted to use alternative plant sources to replace the conventional ingredients, in an attempt to reduce feed costs and optimize the productive yield of the fish **(Lanna *et al.*, 2004)**.

The use of economic diets and inexpensive natural feed sources are considered the main targets of fish nutritionists. A large quantity of by-products is daily generated by the food processing industry such as juices and fresh-cut fruits. Whole processed fruits can generate wastes comprising about 35–85 percentage from these fruits, including peels, seeds and pulp **(Ayala-Zavala *et al.*, 2011).** Pineapple (*Ananas comosus*), belonging to the Bromeliaceae family, is one of the main fruit subjected to fresh-cut minimal processing marketed, almost in all the world. Pineapple is rich in minerals and vitamins, and is a good source of organic acids and phenolics **(Ramirez & Delahaye, 2011; Sabino *et al.*, 2015; de Ancos *et al*., 2017)**. About 50 percent of the fresh fruits produce residues, especially peels and crowns that are not used **(Nor *et al*., 2015)**. Pineapple peel represents about 60% of the total by-product and presents a huge amount of insoluble fibers, phenolic compounds and antioxidants **(Li *et al.*, 2014; Selani *et al*., 2014; Morais *et al*., 2015).** Pineapple waste, including its core, crown, and peel are mostly trash and acts as environmental pollutants. Recently, research efforts have focused on finding better uses for pineapple residues that are rich in phenolics contents, and are antioxidant agents and important bioactive compounds **(Li *et al.*, 2014; Rashad *et al.*, 2015; Roda *et al.*, 2017; Gil & Maupoey, 2018)**. The importance of pineapple by-products may be attributed to the bioactive metabolites, such as calcium, vitamins (A, B, C), pigments, sugars, proteolytic enzyme (bromelain), and organic and amino acids. **(Françasantos *et al.*, 2009; Ketnawa *et al.*, 2012)**.

Li *et al*. (2014) noticed that the polyphenolic metabolites in pineapple peel decreased the oxidative stress-related disease. These agents could be integrated from valuable by-products or food ingredients **(Mediani *et al.*, 2012)**. Hopefully that by 2030, all the bio-processing show the better plans for reusing and recycling pineapple residue, more advanced than those of 2014 **(EEA, 2016)**. Pineapple crown contains bromelain, a proteolytic enzyme which can act as an antioxidant being considered as the important chemical agent that can donate one or more electrons to the free radicals for protecting from the degenerative diseases (**Heo *et al.*, 2006).** Antioxidant agents decrease oxidative stress by preventing the oxidative chain reaction, which inhibits oxidative damage **(Lee *et al.*, 2018)**. The main composition of bromelain is protein; pineapple crown produces 0.27% of dried crude bromelain with total protein content of 44.50%, which is higher than the total protein of fruit. Dried powder of crown has a high mineral content **(USDA, 2019; Grant, 2019**).The total protein content is used to measure the antioxidant activity ratio of crude bromelain **(Nelson & Cox, 2008)**. The pineapple peel contains vitamin B, C, thiamin, pantothenic acid, bromelain and fiber **(de Moraes Crizel *et al.*, 2016)**.

Different pineapple remnants have considerable fiber values; pineapple crown content of total crude fiber is higher than that found in the peel. The pineapple crown also contains insoluble fibers, such as cellulose, hemicellulose and lignin **(Selani *et al.*, 2014; Braga *et al.*, 2015; Miranda *et al.*, 2019)**. According to the nutritional components of pineapple residue, bran composed of pulp pressed and shell is considered an ingredient of energetic source. **Fialho *et al.* (2009)** stated that the ingredients considered as energetic source, which contain 20% of crude protein and fewer than 18% of crude fiber. Several studies have recorded the importance for using pineapple waste for animal feed **(Buliah *et al*., 2019; Kyawt *et al.*, 2020; Zziwa *et al.*, 2021**).

The current work aimed to study the effect of using dried pineapple residues as an additive for the tilapia feed to enhance fish production and health quality and would sequentioally reduce aquaculture feed cost and put an end for the environmental problem of accumulating residues.

MATERIALS AND METHODS

1. Feed additive preparation

Pineapple remnants (crown, peel and bran) were obtained from local fruit shops. The samples were oven-dried at 50ºC; every 6 hours the samples were weighed till they reached the constant weight. Peel sample took almost 48 hrs, while crowns or bran needed 60 hrs; then they were grinded in a mill into powder. The sample was separated using sieves for particle size smaller than 1.25 µm. The powder was packed and maintained at room temperature (25ºC).

2. Experimental design and diet formulation

The experiment was conducted for 14 weeks, using pineapple residues as an additive for the Nile tilapia diets, with an initial body weight of 2.16 ± 0.04 g. A 21- glass aquaria, with capacity of 80 L each were used; 20 fish were stocked in each aquarium at triplicates of seven dietary treatments as follows: P1 (1% pineapple peel), P2 (2% pineapple peel), C1 (1% pineapple crown), C2 (2% pineapple crown), B1 (1% pineapple bran), B2 (2% pineapple bran), and the control (0% additives). The tested diet formula and the chemical composition are shown in Table (1 & 2).

Before starting the trial, fish samples were held for a week under optimal conditions for acclimatization. Fish were fed twice a day at 9:00 am and 14:00 pm. Fish weight was measured biweekly to adjust daily feeding rate (3%). Furthermore, water was partially changed on daily basis.

3. Assessment of ascorbic acid in pineapple byproducts

Powder samples (1.0 g of each) were extracted in 4% oxalic acid, and the solution was brought to 100 ml (V1 ml) and centrifuged at 4032 x*g* for 10 min. Then, 5 ml of the supernatant was mixed with10 ml of 4% oxalic acid; then the solution was titrated against the dye (V2 ml).

Ascorbic acid (mg/100g) = 0.5mg/V1 ml ×V2/5 ml ×100 ml/sample weight (1g) ×100 **(Nwanna *et al*., 2011)**.

4. Measuring total phenolic compounds (TPC) in pineapple residues

The total phenolic compounds content was measured using a Folin–Ciocalteu colorimetric method **(AOAC, 2005)**.

Table 1. Diets formula composition(Dry matter basis)

Ingredient (%)	Control	Experimental diets					
		Pineapple peel		Pineapple crown		Pineapple bran	
		P_1	P_2	C_1	C_2	B_1	B_2
Fish meal	15	15	15	15	15	15	15
Soybean meal	35	35	35	35	35	35	35
Corn gluten	5	5	5	5	5	5	5
Yellow corn	19.5	19.5	19.5	19.5	19.5	19.5	19.5
Wheat bran	15	15	15	15	15	15	15
Rice polishing	8	8	8	8	8	8	8
Sunflower oil	1	1	1	1	1	1	1
Premix*	1	1	1	1	1	1	1
Cr_2O_3	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	99	98	99	98	99	98
Pineapple residues	0	1	2	1	2	1	2
Total	100	100	100	100	100	100	100

*One kg premix contained:

Vitamins:48×10^5I.U (A), 6×10^2mg (B_6), 20 mg (biotin), 8×10^5I.U. (D_3), 144 mg (E), 400 mg (B_1), 1600 mg (B_2), 4×10^3mg (pantothenic acid), 4 mg (B_{12}), 4×10^2mg (niacin), 2×10^5 mg (choline chloride), and 400 mg (folic acid).

Minerals: 12×10^3mg iron, 16×10^3mg manganese, 12×10^2mg copper, 120 mg iodine, 80 mg cobalt, 40 mg selenium, and 16×10^3mg zinc.

5. Measurements of experimental fish

Fish were weighed biweekly and at the end of the experiment to calculate daily weight gain (DWG), mean weight gain (MWG), and specific growth rate (SGR). In addition, feed intake (FI) and protein intake of feed were recorded to determine protein efficiency and feed conversion ratios (PER & FCR, respectively), as well as protein productive value (PPV) according to the equations of **Cho and Kaushik (1985)** as follows:

DWG (g/fish/day) = [BWG (g) / experimental period (days)]

MWG (g/fish)= final weight (g) – initial weight (g)

SGR (%g/day) = (Ln final weight – Ln initial weight) / experimental period (day)*100

FCR = feed intake (g) / body weight gain (g)

PER = gain in weight (g) / protein intake in feed (g)

PPV(%) =100 [protein gain in fish (g) / protein intake in feed (g)]

Survival rate (%) = 100(initial number of fish stocked-mortality) / initial number of fish stocked.

Condition factor (CF) was calculated using the following formula:

CF= (W/L^3) × l00, where W: weight of fish body (g); L: length of fish (cm).

Apparent protein digestibility (APD) was measured by using **Furukawa and Tasukahara (1966)** method. After the end of the trial, the final weight was recorded, then the digestion trial was started where the uneaten diet and feces were collected once daily by siphoning for 15 days. Feed or feces was carefully collected before the first meal, then 30 min after feeding, uneaten feed were collected, and after two hours, feces were collected separately then filtered and dried at 60ºC and stored for the determination of the chemical composition.

Table 2. Chemical composition of experimental diets (Dry matter basis)

Chemical composition	Experimental diets						
	Control	P_1	P_2	C_1	C_2	B_1	B_2
Crude protein %	31.10	30.85	30.61	30.86	30.63	30.86	30.62
Crude fat %	4.33	4.32	4.33	4.32	4.30	4.32	4.33
Crude fiber %	5.26	5.70	6.14	5.69	6.11	5.71	6.15
Ash %	7.06	7.05	7.04	7.05	7.05	7.06	7.05
NFE* %	52.25	52.08	51.88	52.08	51.91	52.05	51.85
Moisture %	10.34	10.43	10.45	10.48	10.50	10.4	10.42
Dry matter %	89.66	89.57	89.55	89.52	89.50	89.60	89.58
GE (MJ/kg)**	19.20	19.18	19.17	19.18	19.16	19.18	19.17
P/E ratio***	1.62	1.61	1.60	1.61	1.60	1.61	1.60

*NFE: Nitrogen free extract.

**Gross energy: GE (MJ/kg) = (CP×5.65+EE×9.65+CF×4.2+NFE×4.2) ×4.182/100

***P/E = Protein /energy ratio

6. Laboratory analysis

6.1. Proximate chemical analysis

Samples of additives and whole fish body (dry weight basis) were analyzed for crude protein (CP), ether extract (EE), crude fiber (CF) and ash, in addition the minerals in the pineapple residues powder were measured using the **AOAC(2012)** reference methods.

6.2. Biochemical and physiological analysis

6.2.1. Liver metabolites

At the end of the trial, liver samples of 15 fish were taken randomly from each pond. Then, liver tissues were homogenized with 5 ml distilled water to determine the following parameters: Liver glycogen was determined (g/100 g fresh tissue) by the formula, using the modified method of **Handle (1965**).

Hepatic glycogen = (absorbance sample/absorbance standard) ×conc. of standard × (V. of dil. factor/ Wt. of tissues).

Total liver lipids was extracted with a mixture of (2 chloroform:1 methanol) described in the study of **Bligh and Dyre (1959)**, then 0.5 ml of sulfuric acid was added to the dried lipid extract, and the hepatic lipid content was measured by using the method of **Zollner and Kirsch (1962)**.

Hepatic lipid = (Absorbance sample/Absorbance standard) x Conc. of standard × (V. of dil. factor/ Wt. of tissues)**.**

Total liver protein was extracted by homogenization in trichloroacetic acid, and was then centrifuged (1008 x*g*), and the hepatic protein content was determined as described by **Gornall *et al.* (1949)** using the following formula:

Hepatic protein= (absorbance sample/absorbance standard) × Conc. of standard ×(V. of dil. factor/ Wt. of tissues).

6.2.2. Amino and fatty acids

Fatty acid and amino acid compositions of pineapple were assessed by using the methods recommended in the studies of **Tidwell *et al*. (1993)** and **Li and Watkin (1998)**.

6.2.3. Blood biochemical parameters

At the end of the trial, blood samples were collected with a heparinized syringe from the caudal vein of fish. Samples were subjected to the determination of plasma protein (PTP) according to the method of **Armstrong and Carr (1964).** Blood was centrifuged at 1008 xg for 15 min. Serum cholesterol was determined according to the method of **Stein (1986)**. The immunoglobulin's (IgM and IgD) were measured by the method of **Feinstein *et al.* (1985)**.

7. Data analysis

The data were subjected to the two-way analysis of variance (ANOVA) to explore effects of additive and level, using general linear models (GLM) procedure; the software used was SPSS (Version 16.0) **(SPSS, 1997)**. Duncan's multiple range tests **(Duncan, 1955)** was used to compare between means of the control and those of the treated groups.
The model of analysis was as follows:

$$Y_{ijk} = \mu + T_i + L_j + E_{ijk}$$

μ = the overall mean,
Ti = the effect of treatment,
L_j = the effect of level
and, E_{ijk} = the random error.

RESULTS AND DISCUSSION

1. Pineapple residue constituents

1.1. Chemical composition

Table (3) reveals that values of chemical composition of pineapple residue were as follows: protein (6.44-7.53%), lipids (2.96-3.14%), Ash (6.35-6.71%), fiber (47.51-49.74%), and dry matter content (81.98-83.58%). Ranges of proximate composition of pineapple peels were 5–9% for crude protein, lipids (2–3%), fiber (1–6%), ash (4–6%), and (50–80%) for carbohydrates, where pineapple bran analysis values were 84.9%, 3.6%, 2.35%, 9.14%, 1.7%, and 83.03% for moisture content, crude protein, crude fat, crude fiber, ash and carbohydrate, respectively **(Romelle *et al.*, 2016; Kodagoda & Marapana, 2017; Morais *et al.*, 2017)**. The crown values were 0.7%, 7.37%, 3.5%, and 62.5% for the total protein, ash, crude fat, and crude fiber, respectively **(Pardo *et al.*,**

2014). The pineapple residue used in this study shows high amount of crude fiber, besides containing carbohydrates and protein. Thus, the values of pineapple residue constituents confirmed that they can be used as good additives for the tilapia feed**.**

1.2. Antioxidants

Carotenoids, vitamin C, and phenolic compounds may contribute to the antioxidant activity of fruits and vegetables, being more effective in removing free radicals. It is worthy to mentioning that pineapple is an important source of those components **(Hossain & Rahman, 2011)**.

Table 3. The proximate analysis of pineapple byproduct powders of dry matter basis

Component	**Pineapple residues**		
	Peels	**Crown**	**Bran**
Protein (%)	6.44	7.53	6.92
Fat (%)	3.14	2.96	3.12
Fiber (%)	49.23	47.51	49.74
Ash (%)	6.71	6.35	6.78
Carbohydrates (%)	34.48	35.65	33.44
Moisture (%)	16.85	18.02	16.47
DM (%)	83.15	81.98	83.58
Phenoliccontent (mg)	11.82	11.71	12.55
Ascorbic acid (mg)	24.22	27.11	29.14

The current findings of the total phenolic compounds (Table 3) demonstrated that crown extracts exhibited the highest phenolic value (2.85 mg/g), followed by peel extracts (2.33 mg/g). The present results varied compared to those of the few previous studies **(Ferreira *et al.*, 2016; Campos *et al*., 2020**).

Results, with respect to vitamin (C), were as follows: 24.22 mg/100g for peel, 27.11 mg/100g for crown, while for bran, it was 29.14 mg/100g (Table 3); almost similar results are recorded in the study of **Achinewhu and Hart (1994),** regarding *Ananas comosus* L. residue.

1.3. Fiber composites

Table (4) shows the fraction of fiber content in the three parts of pineapple waste, and the results cleared that the major amount of cellulose was detected in pineapple bran, while crown contains the highest level of hemicellulose and lignin. Fiber helps to maintain a healthy digestive system, but an excessive intake of it can lead to a restriction of some trace elements **(Siddhuraju *et al.*, 1996)**. The effect of fiber on fish is still little studied and may be related to its composition in cellulose, hemicellulose, lignin, and silica **(Meurer *et al.*, 2003)**. Studies on the pineapple crown recorded a significant content of insoluble fibers, such as hemicellulose (25.4–35.49%), cellulose (12.93-34.6%) and lignin (5.14 –26.40%) **(Braga *et al*., 2015; Miranda *et al*., 2019)**.

Table 4. Fiber fractions of pineapple residue

Parameter (% dry weight basis)	Peel	Crown	Bran
Cellulose	17.54	22.73	30.8
Hemi cellulose	9.5	28.5	11.7
Lignin	13.0	18.27	17.5
Pectin	4.10	2.07	0.89

1.4. Minerals

The present study revealed that the concentrations of potassium, copper, sodium, magnesium, and manganese were higher in crown than in other residues (Table 5). The current results assessed that pineapple crown is a rich source of these essential minerals. It is cleared that calcium, potassium, and phosphorus were the most abundant minerals in all forms of pineapple residues compared to other mineral elements**.** Pineapple wastes contain some important minerals and also huge amount of vitamin C. This advantage can be used as thriftiness supplemental minerals and vitamins (premix) in the diet formulation, which could reduce the cost of feed production.

Table 5. Mineral concentrations of pineapple byproduct (mg/100 g DM)

Element(mg/100g)	Peel	Crown	Bran
Zinc	2.76	1.62	1.43
Calcium	181.25	159.67	183.73
Iron	21.73	12.92	10.61
Manganese	7.60	11.96	8.63
Potassium	92.25	98.62	89.71
Copper	0.30	1.34	0.27
Phosphorus	25.04	27.34	31.17
Sodium	9.55	12.15	11.21
Magnesium	10.72	11.22	10.35

1.5. Amino Acids

Analysis of essential amino acids of pineapple crown, peel and bran (Table 6; Fig1) is considered the important investigates in the current study. The results revealed that pineapple waste powder is a rich source of mainly essential amino and fatty acids, which are highly important for fish health.

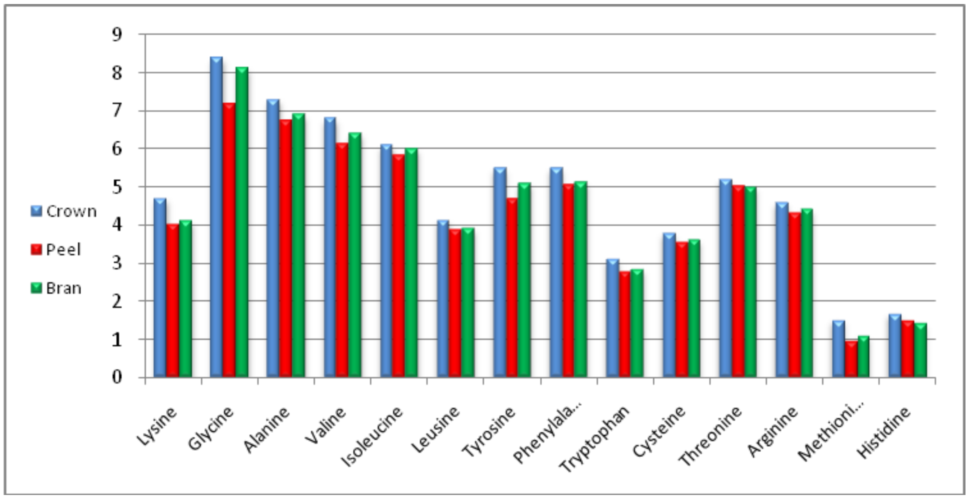

Fig. 1. Amino acids in pineapple residues

Table 6. Amino acid composition in the pineapple remnants

Amino acid (%)	**Crown**		**Peel**		**Bran**	
	Total	**Bromlian**	**Total**	**Bromlian**	**Total**	**Bromlian**
Lysine	4.71	2.21	4.03	2.11	4.11	2.10
Glycine	8.40	4.21	7.18	3.36	8.13	3.87
Alanine	7.3	3.70	6.75	3.28	6.91	3.67
Valine	6.8	3.31	6.13	3.00	6.42	3.15
Isoleucine	6.1	2.94	5.85	2.46	6.01	2.89
Leusine	4.13	1.93	3.90	1.72	3.92	1.43
Tyrosine	5.5	2.38	4.71	2.23	5.1	2.61
Phenylalanine	5.5	2.50	5.05	2.12	5.14	2.41
Tryptophan	3.1	1.42	2.79	1.11	2.85	1.34
Cysteine	3.8	1.71	3.54	1.20	3.61	1.56
Threonine	5.2	2.72	5.02	1.99	5.01	2.32
Arginine	4.6	2.75	4.31	2.01	4.42	2.02
Methionine	1.51	0.68	0.95	0.44	1.09	0.54
Histidine	1.67	0.78	1.51	0.68	1.42	0.67
Aspartic acid	8.42	4.11	6.70	3.26	6.93	3.29
Proline	4.32	1.75	3.85	1.78	4.31	2.31

Bromelain acts as an active agent, which is a major protease in pineapple. Amino acid sequencing and domain analysis of crown, peel, and bran bromelain (Table 6 and Fig. 2) showed many differences and similarities between members of the cysteine protein family. Bromelain contains 44% protein, consistingconsists a considerable amount of essential amino acids composition, such as tryptophan, glysine, alanine, isoleusine and methionine.

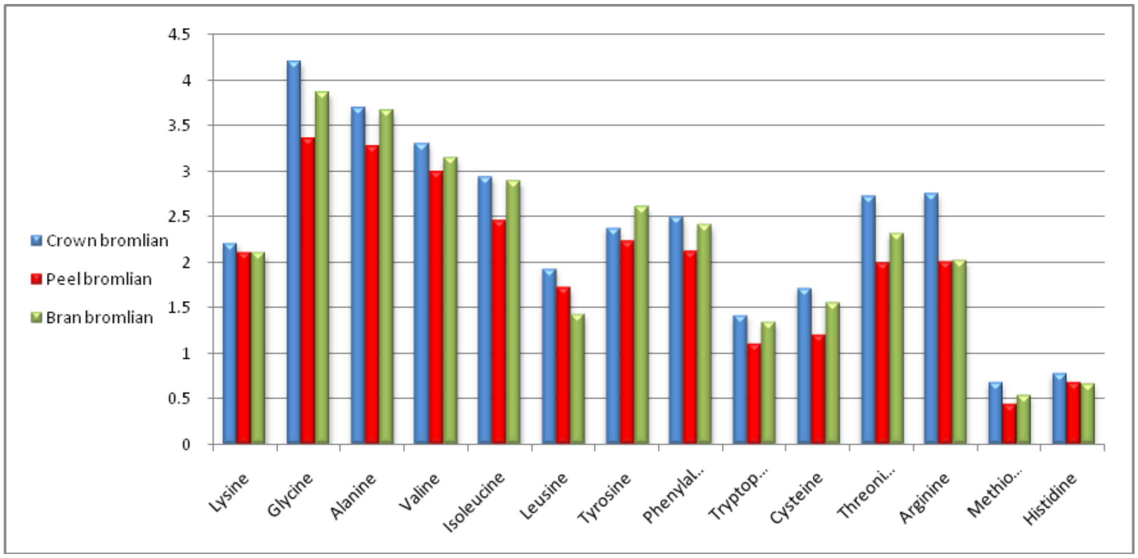

Fig. 2. Constituents of bromlain amino acids in pineapple wastes

1.6. Fatty acids

Results presented in Fig. (3) show that pineapple wastes contained saturated fatty acid (palmitic acid, 7.93±0.9), monounsaturated (oleic acid, 3.89±0.45) and polyunsaturated fatty acids (linoleic acid, 2.03±0.21 and linolenic acid, 1.62±0.10). Ntably, the ratio between the unsaturated and saturated fatty acids was 0.95.

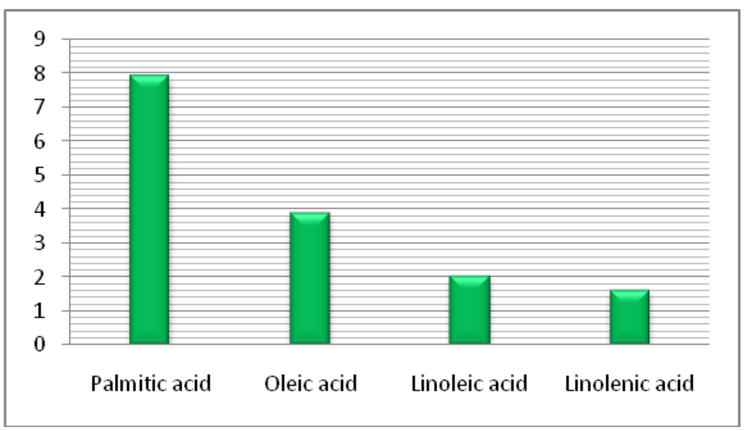

Fig. 3. Fatty acids profile of pineapple wastes

2. Growth performance of experimental fish

2.1. Growth and utilization parameters

The current study showed that the use of natural pineapple additives improved all fish growth parameters, for the three additives (peel, crown, or bran) at the two levels (Table 7 and Fig. 4). The mean weight gain values were (17.51, 19.02, 18.95, 21.01, 18.06, and 20.64) for fish fed P1, P2, C1, C2, B1, and B2, respectively. The best growth performance was detected for those fed on crown at level 2, compared to the control. A statistically significant difference (P < 0.05) was recorded in MWG, ADG, and SGR of treated fish fed on pineapple additive, and an improved fish growth was observed compared to those fed the control diet.

The present findings coincided fish treated by 2% pineapple crown supplementation and recorded the maximum fish growth that caused thehighest PER accompanied with the lowest FCR value (P < 0.05). These results may be attributed to a fast metabolism in fish that are fed pineapple waste supplemented diet, which would in turn result in better FCR (Table 7 and Fig.5).

The protease enzyme breaks down proteins to release short peptides into the diet. It is the key factor for improving protein digestibility, enhancing more absorption and growth factors **(Torrissen & Male, 2000)**. Thus, the present results of the fish that ate pineapple remnants showed better growth than those fed the control diet **(Yuangsoi *et al*., 2018)**.

Table 7. Growth performance of fish fed (14 weeks) on three forms of pineapple residue

Parameter	**Experimental diets**						
	Control	**Pineapple peel**		**Pineapple crown**		**Pineapple bran**	
		P$_1$	**P$_2$**	**C$_1$**	**C$_2$**	**B$_1$**	**B$_2$**
Initial weight	2.17 ± 0.44	2.15 ± 0.37	2.16 ± 0.29	2.17 ± 0.31	2.15 ± 0.11	2.15± 0.13	2.14±0.21
Initial length	4.97±0.13	5.05±0.15	5.02±0.12	4.98±0.11	4.94±0.17	5.06±0.14	5.02±0.13
Final weight	18.19^{e} ± 2.14	19.66^{dB}±1.83	21.18^{bcA}±1.81	21.12^{cB}±1.55	23.16^{aA}±1.61	20.21^{bcB}±1.46	22.78^{bA}±1.75
Final length	10.15^{f}±0.24	10.38^{cB}±0.16	10.39^{cA}±0.21	10.36^{dB}±0.14	10.55^{bA}±0.23	10.30^{eB}±0.15	10.66^{aA}±0.17
MWG	16.02^{e}±1.52	17.51^{dB}±1.6	19.02^{cA}±1.73	18.95^{dB}±1.50	21.01^{aA}±1.50	18.06^{cB}±1.71	20.64^{bA}±1.62
DWG	0.16^{d}±0.00	0.18^{cB}±0.02	0.19^{bA}±0.03	0.19^{bB}±0.03	0.21^{aA}±0.02	0.18^{cB}±0.01	0.21^{aA}±0.02
SGR	2.17^{d}±0.10	2.26^{cB}±0.14	2.33^{bA}±0.09	2.32^{bB}±0.13	2.43^{aA}±0.12	2.29^{bcB}±0.08	2.41^{aA}±0.10
FI	31.83^{e}±3.25	33.82^{dB}±1.9	33.89^{cdA}±2.61	33.79^{dB}±2.92	34.97^{bA}±3.16	34.15^{cB}±3.0	35.99^{aA}±3.2
FCR	1.99^{e} ±0.07	1.93^{dB}±0.04	1.78^{cA}±0.04	1.78^{cB}±0.06	1.66^{aA}±0.03	1.89^{dB}±0.02	1.74^{bA}±0.03
PER	1.80^{f}±0.03	1.87^{eB}±0.03	2.05^{cA}±0.05	2.03^{cB}±0.01	2.19^{aA}±0.01	1.91^{dB}±0.03	2.09^{bcA}±0.03
PPV	25.96^{g}±0.90	28.64^{fB}±1.00	34.16^{cA}±1.60	33.39^{cB}±1.70	39.03^{aA}±1.75	31.38^{eB}±2.01	35.82^{bA}±1.81
APD (%)	76.01^{g}±3.35	78.92^{fB}±2.62	83.14^{cA}±2.85	82.65^{dB}±3.05	85.17^{aA}±3.16	80.11^{eB}±3.06	85.01^{bA}±3.30
CF	1.74^{e}	1.76^{dB}	1.89^{bA}	1.90^{bB}	1.97^{aA}	1.85^{cB}	1.88^{bA}
Survival (%)	96	97	97	98	98	98	99

Mean values in the same raw with different letters (capital and small), are significantly different (P < 0.05).

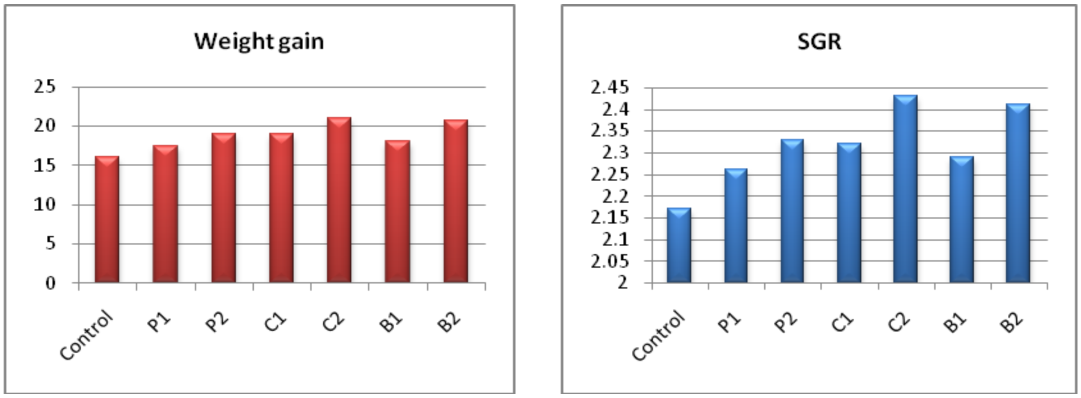

Fig. 4. Growth performance

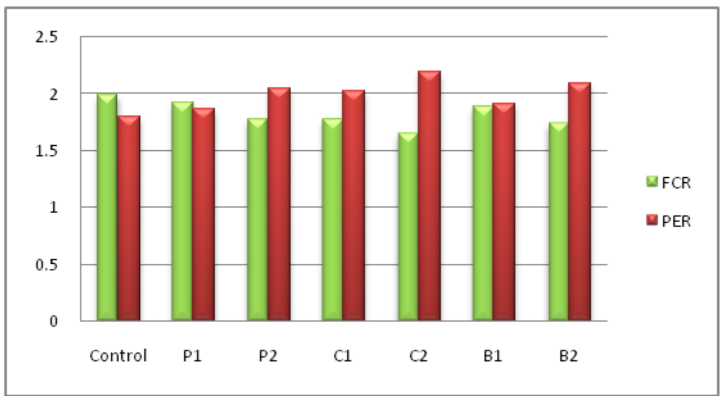

Fig. 5. Feed utilization ratios

Table (7) and (Fig. 6) show a growing trend, an increasing ($p<0.05$) of APD and PPV values for all fish treated groups with the three additives ($p<0.05$), compared to the control diet. Pineapple residue contains considerable amounts of dietary fibers (47.51%-49.74%), which improved the digestibility, promoting growth performance and feed utilization of the test fish. The levels of pineapple waste used in the current experiment enhanced the protein digestibility of fish; the results of digestibility represented were satisfactory for fish as vegetal source. These results cleared that pineapple acts as a source of proteolytic enzyme such as bromelain that breaks the peptide bond of proteins and peptides **(França-santos *et al*., 2009)**.

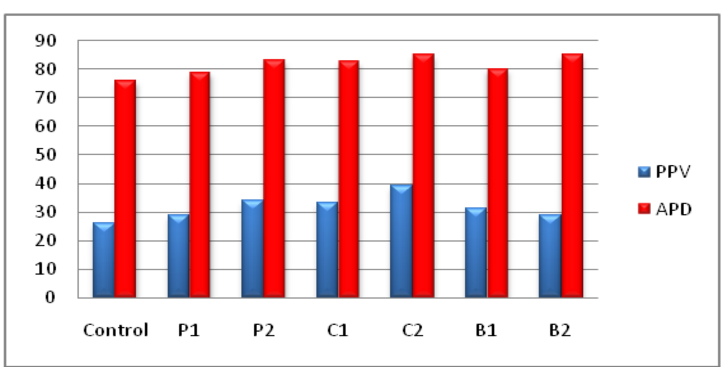

Fig. 6. Protein digestibility utilization

Protease enzymes play an important role in digestion of proteins in the digestive system and lead to a better protein digestion **(Drew *et al.*, 2005; Farhangi & Carter 2007; Shi *et al.*, 2016)**. **Shi *et al.* (2016)** stated that supplementing exogenous protease could improve the growth, apparent digestibility of dry matter and crude protein, and enhance the retention of protein and lipid for carp. Additionally, fiber helps to maintain the health of gastrointestinal tract **(Siddhuraju *et al.*, 1996)**.

2.2. Condition indicators

Regarding fish body condition and survival rates (Table 7 and Fig. 7), the present findings verified that they were optimized for fish fed the examined diets compared to the control group **(Raky *et al.*, 2021a)**.

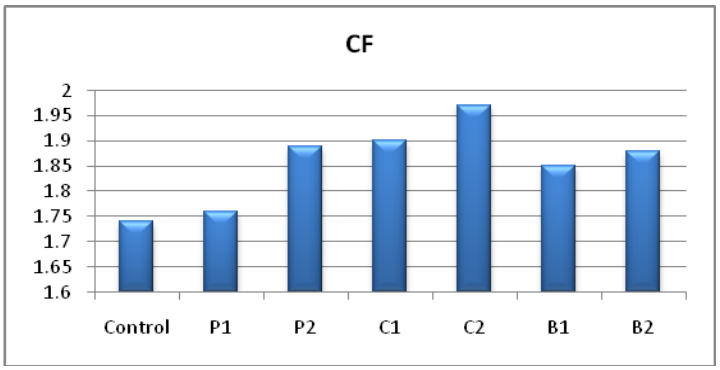

Fig. 7. Fish condition factor

3. Carcass composition.

It was deduced that, the pineapple residues contained good amount of protein, assuming that they may be essential in the functional food formulations. Even though fruits and vegetables are a poor source of lipids, some fat is essential for bioactivity and 20-35% of calories should come from fat as per the dietary reference intakes published by **USFDA (2019)**.

Carcass composition, in Table (8), reveales an increasing fish protein content at the two levels of the three additives, while an inverse trend is recorded for the fat content. These results may be due to the presence of amino acid content in pineapple wastes. **Raky *et al.* (2021a)** recorded the highest protein accompanied with the lowest fat content when the tilapia was fed orange peel. The protein quality of feed has a strong effect on the protein composition of body muscles **(Osek *et al.*, 2011).** Furthermore, **Melesse *et al*. (2011)** mentioned that, the quality of protein and amino acid content in food enhanced muscle protein content.

Table 8. Whole body composition (DM basis)

Carcass parameter	Experimental diets						
	Control	**P_1**	**P_2**	**C_1**	**C_2**	**B_1**	**B_2**
DM %	24.84^{f} ±0.93	25.42^{eB} ±1.03	26.55^{cA} ±1.02	26.21^{dB} ±1.06	27.86^{aA} ±1.04	26.23^{cdB} ±0.97	27.11^{bA} ±1.11
CP (%)	57.91^{d} ±1.34	60.13^{cB} ±1.38	62.84^{abA} ±1.37	62.75^{bB} ±1.42	63.92^{aA} ±1.45	62.55^{bB} ±1.29	63.20^{abA} ±1.51
Fat (%)	20.02^{a} ±0.64	18.84^{bA} ±0.58	17.03^{dB} ±0.53	17.22^{dA} ±0.49	16.30^{fB} ±0.81	17.67^{cA} ±0.71	16.76^{eB} ±0.72
Ash (%)	17.84^{a} ±0.37	17.72^{bA} ±0.94	17.33^{dB} ±0.69	17.45^{cB} ±0.47	17.76^{abA} ±0.62	17.46^{cB} ±0.83	17.74^{abA} ±0.85
NFE (%)	4.23^{a} ±0.05	3.31^{bA} ±0.01	2.80^{cB}± 0.01	2.58^{dA}± 0.03	2.02^{fB}± 0.01	2.32^{eA}± 0.04	2.30^{eB}± 0.02
Moisture %	75.16^{a} ±1.10	74.58^{bA} ±1.04	73.45^{dB} ±1.09	73.79^{cA} ±1.06	72.14^{fB} ±1.03	73.77^{cA} ±1.07	72.89^{eB} ±1.05

Mean values in the same raw with different letters (capital and small), are significantly different ($P < 0.05$).

Dry matter and ash content were also improved for fish fed C2 and C1 in the results of the presenttrials; the highest mineral content in the pineapple wastes powder may increase the mineral in fish muscles. **Nwanna *et al.* (2011)** stated that the high mineral content of diet increased the mineralization of fish body.

4. Biochemical results

4.1. Liver analyses

Results obtained (Table 9) showed that fish treated with pineapple peel, crown, and bran at the two levels recorded an improvement in the results of liver protein and glycogen levels and may act as liver health prophylactic. **Raky *et al.* (2021b)** recorded an increasing trend for liver protein and glycogen, while hepatic lipids showed an opposite trend.

Table 9. Liver metabolites (g/100 tissue)

Liver composition	Control	Experimental diets					
		Peel		**Crown**		**Bran**	
		P_1	**P_2**	**C_1**	**C_2**	**B_1**	**B_2**
Hepatic protein	18.32^{g} ±1.11	19.21^{fB} ±0.88	21.27^{cA} ±1.21	20.13^{dB} ±1.18	21.95^{aA} ±1.09	19.66^{eB} ±1.54	21.46^{bA} ±1.01
Hepatic lipids	10.35^{a} ±0.54	9.74^{bA} ±0.09	9.08^{cB} ±0.06	9.14^{cA} ±0.08	8.04^{eB} ±0.04	9.43^{cA} ±0.08	8.91^{dB} ±0.06
Hepatic glycogen	1.90^{d} ±0.05	2.01^{cB} ±0.10	2.09^{bA} ±0.01	2.10^{bB} ±0.02	2.18^{aA} ±0.03	2.03^{cB} ±0.06	2.18^{aA} ±0.06

Mean values in the same raw with different letters (capital and small), are significantly different ($P < 0.05$).

4.2. Blood analyses

Table (10) clarifies that fish fed on the experimental diets showed a decrease in cholesterol levels compared to those fed the control diet. More attention has been paid to monounsaturated fatty acids (MUFAs) over the past few decades due to their beneficial effects on cardiovascular disease **(Mann *et al.*, 2004)**. A diet rich in MUFAs may tend to reduce low density lipoprotein cholesterol **(Singh *et al*., 1997)**.

Immunoglobulin (IgM & IgD) and plasma total protein (PTP) revealed better results for the test fish groups fed the level 2% of pineapple remnants' additives than those fed pineapple residues at level 1% (Table 10 and Fig.8). Generally, pineapple residue caused enhancement of immunity with regard to the treated fish fed the two levels of experimental diets when compared to the control diet (p<0.05).

Table 10. Blood biochemical concentrations of the treated fish fed pineapple residue

Blood parameter	Experimental diets						
	Control	Peel		Crown		Bran	
		P_1	P_2	C_1	C_2	B_1	B_2
Plasma total protein (g/dl)	3.29^{e} ±0.05	3.57^{dB} ±0.05	4.06^{abA} ±0.03	3.85^{cB} ±0.04	4.26^{aA} ±0.02	3.73^{cdB} ±0.05	4.01^{bA} ±0.04
Albumin (g/dl)	1.65^{d} ±0.01	1.83^{cB} ±0.04	1.97^{abA} ±0.02	1.80^{cB} ±0.03	2.00^{aA} ±0.01	1.82^{cB} ±0.04	1.90^{bA} ±0.02
Globulin (g/dl)	1.64^{e} ±0.02	1.74^{dB} ±0.01	2.09^{bA} ±0.03	2.05^{bB} ±004	2.26^{aA} ±0.05	1.91^{cB} ±0.03	2.11^{bA} ±0.01
Cholesterol (mg/dl)	129.33^{a} ±1.05	126.23^{bA} ±1.17	117.25^{eB} ±1.09	119.32^{dA} ±1.08	114.25^{gB} ±1.24	121.82^{cA} ±1.14	115.24^{fB} ±1.09
IgM	9.95^{a} ±0.44	9.54^{bA} ±0.31	8.74^{dB} ±0.19	8.82^{dA} ±0.11	8.41^{eB} ±0.38	9.31^{cA} ±0.52	8.44^{eB} ±017
IgD	136.55^{f} ±1.12	137.55^{eB} ±1.23	140.01^{cA} ±1.51	138.05^{dB} ±1.34	144.93^{aA} ±1.26	138.1^{dB} ±1.42	142.71^{bA} ±1.35

Mean values in the same raw with different letters (capital and small), are significantly different (P < 0.05).

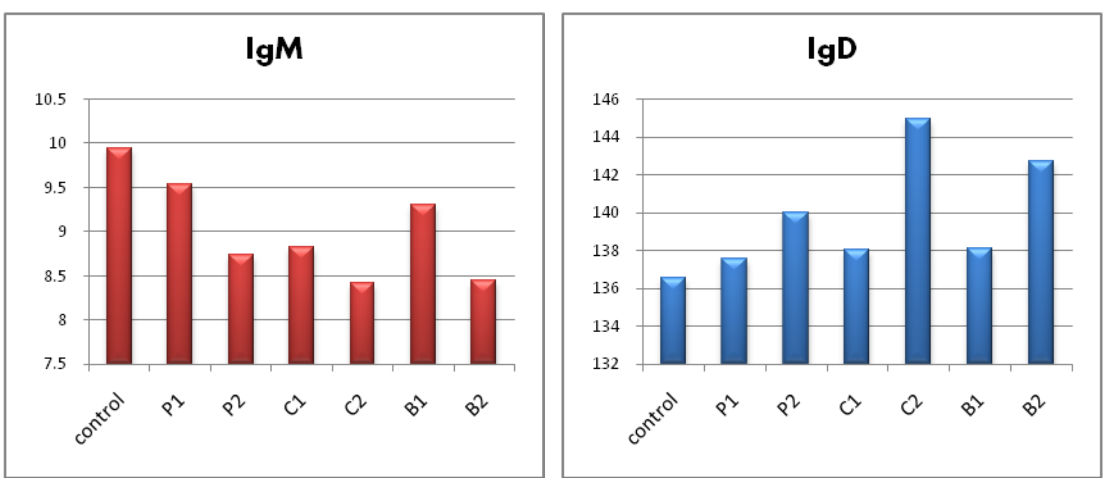

Fig. 8. Immunity indicators

The fish fed on pineapple crown recorded the optimum results of immune activity. To clarify the current findings, it makes sense to observe a start of an increase in specific immune response (IgM) only when the infection becomes harmful, and then it returns to the optimum levels when the infection is reduced **(Williams & Hoole, 1992; Hoole *et al*., 2003)**. These results may be related to the presence of abundant amounts of the phenolics, minerals, vitamins (A, B, and C), and bromelain in the pineapple residue **(França-santos *et al.,* 2009)**. Vitamin C and crude bromelain have an antioxidant activity which enhanced immunity while the IgM decreased. The protein content in bromelain ranged from 36% to 45% **(Aehle, 2007)**. The total protein content is used to determine the ratio of antioxidant activity of crude bromelain **(Saptarini *et al.*, 2019)**.

Pineapple waste, in the form of crown, peel, stem, and core are rich sources of bioactive components. Moreover, it contains insoluble fibers, pectins, sugars, protein, vitamins, minerals, phenolic compounds and bromelain **(Silvestre *et al.*, 2012; Diaz-Vela *et al*., 2013; Said-Al Ahl *et al*., 2017; Hikal *et al.*, 2020, 2021**). Serum proteins are responsible for the fish innate immune response, and a high level of it provides strong response **(Wiegertjes *et al*., 1996; Sahu *et al*., 2007)**. The raise in plasma proteins could be resulted from the higher digestion of dietary protein **(Lundstedt *et al.*, 2002)**. **Rostika *et al.* (2018)** obtained better results of growth and immune response indicators (total protein and total immunoglobulin) in fish fed additives (papain and bromelain crude enzyme extract) compared to those fed the conventional feed. Almost similar data were determined in the study of **Raky *et al.* (2021a)** when they used different forms of orange peel additive to the Nile tilapia diets. The afore- mentioned authors mentioned that torange peel enhanced growth performance and immune response system of fish.

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