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Fermented By-Products from Fruits as a Protein Source in the Basa Fish (*Pangasius bocourti*) Diets

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ABSTRACT

Four isonitrogenous and isocaloric experimental diets were developed to substitute soybean with fermented fruits wastes (FFWs), specifically fermented grape pomace (FGP), fermented apple peels (FAP), and a mixture of fermented grape pomace and apple peels (FGAPm) at a level of 25%. Four experimental groups, each comprising twenty-five basa fish (*Pangasius bocourti*, 7.5±0.5g*)*, were maintained for twelve weeks with three repetitions per group. The growth performance, feed utilization, apparent digestibility coefficients (ADC), biochemical, enzymatic, and hematological characteristics of the fish were documented and assessed. Fish fed with 25% FGP exhibited considerably superior growth, survivability, and feed utilization in comparison with other experimented groups. The energy ADC of the FFWs diet exceeded that of the control. According to linear regression, basa fish (*Pangasius bocourti*) received 25% fishmeal, as a soybean protein substitute exhibited the maximum growth performance. FGAPm induced hyperglycemia, elevated plasma triglycerides, hepatic glycogen and albumin levels, enhanced alkaline protease and amylase activities, and reduced plasmatic proteins, cholesterol, and hepatopancreas AST levels. The dietary FGAPm caused a reduction of blood indices, alongside an elevation in erythrocytes counts. It was determined that fermented grape waste (FGP) can effectively substitute soybean protein by up to 25% regarding growth and feed efficiency.

INTRODUCTION

Fish farmers face significant economic challenges due to losses from diseases and elevated feed ingredient costs **(Lim *et al*., 2021)**. Consequently, the development of feeds from recirculated raw materials like fruits wastes is crucial for lowering production expenses and improving aquaculture productions **(FAO, 2020)**.

Aquaculture sector in Egypt is the largest between African countries and nowadays is considered as the principal source of fish supply by about 78.8% of total

production (1.56 million tonnes). It reached 1.8 million tonnes in 2018, representing 85.7% of total production, indicating an increase of six hundred (600) million tonnes or a 50% gain since 2015 **(Wally, 2016)**. The swift growth of aquaculture has created numerous job possibilities for qualified workers and farm technicians. Furthermore, nascent enterprises and financial services that facilitate aquaculture are creating employment opportunities **(FAO, 2020)**.

The pangasius stands out as one of the most successful species in aquaculture due to its high market demand, ease of production, and adaptability to local circumstances **(Rahman & Ali, 2012)**. Its implementation in aquaculture in Egypt's coastal districts, characterized by underutilized water resources, has the potential to significantly enhance protein intake for low-income families creating new employment and revenue opportunities.

The aquaculture feed sector has grown simultaneously and taken on a more prominent role. According to their protein content and balanced fatty acids composition, fishmeal has been extensively utilized as the principal protein source in the aquafeed sector **(El-Sayed, 2020)**. To offer economical fish feed, various plant or grain by-products were utilized as substitutes for fish meal, which is derived from wild-caught fish and is costly **(Daniel, 2018)**.

Aquaculture represents a sustainable method for global food diversification, offering nutritious food options and profitable opportunities within the industry. Utilizing alternative ingredients, such as plant by-products or waste, is advisable due to their high nutritional value and low cost, as evidenced by documentation from various countries. Plant by-products obtained from reputable food sources are favored over animal by-products due to their lower risk of bacterial, fungal, and parasitic infections that could negatively impact human health **(Dawood *et al.*, 2022)**.

About 75% of grape outputs is allocated to the wine industry, while the other components, including skins, stalks and seeds account for 25% of total grapes' weight nonemployee. Seedless pomace constitutes around 48–62% of total grape pomace, serving as fibers and phenolics source, whilst seeds account for roughly 38–52%, mostly providing oils rich in unsaturated fatty acids **(Beres *et al.*, 2017)**.

Grape by-products may act as dietary supplements (nutraceuticals or food components) that provide health goods **(Chauhan *et al.*, 2013; Frank *et al.*, 2020)**. Grape pomace extracts are rich in antioxidant properties and fermentation substrates due to the high concentration of bioactive materials that demonstrate notable antioxidant activities, including polyphenols (flavanols, procyanidins, anthocyanins), phenolic acids, and fibres **(Caponio *et al.*, 2023)**.

Dawood *et al.* (2022) discussed the utilization of fruits' by-products processing, specifically highlighting grape seeds extract, seeds oil, microencapsulated products, and pomace meal. The derivatives of grape can be used in various forms, including micro capsulated products, grape pomace flour, grape seeds extract, grape seeds oil. Processing

of extraction technologies, including enzyme supplementation and pre-treatment process, are frequently employed to enhance the availability of grape by-products due to its high tannin content, which serves as antioxidants but at the same time has an antinutrients functions **(Alfaia *et al.*, 2022)**. Nutrient-dense fruit byproducts have an economical energy source for fish, potentially resulting in protein-sparing effects that promote growth **(Pérez-Pacheco *et al.*, 2022)**.

The apple is a natural source of antioxidants and, in addition to providing a large amount of them, serves as an excellent substrate for producing microorganisms via solid-state fermentation. These microorganisms can break down cellulosic structures and release the phenolic compounds contained within, making them available to consumers of the ferment **(Ajila *et al.*, 2011; Dhillon *et al.*, 2012)**. By using this material as an ingredient in animal diets, the producer faces several problems, such as its deficiency of digestible protein, its high moisture content and its low pH. Therefore, the development of microbial protein in this medium is presented as an excellent alternative to increase its protein level and to modify its pH, so it can be used for animal feed as a high-quality food supplement, providing protein at a lower cost than animal meal, and with similar digestibility **(Rodríguez-Muela *et al.*, 2010)**.

Chokeberry, apples, and mango peels wastes have been utilized as heavy metals' bio absorbents in the field of biomaterials industry **(Maulani *et al.*, 2021)**. A comprehensive understanding is essential for achieving a coherent conclusion regarding the application of fruits waste in aquaculture. Comparative analyses examining various fruits wastes, liver and intestinal histology, serum biochemistry, and pathogenic tests yield significant insights into the potential of fruits wastes to improve both growth and health of fishes **(Van Doan *et al.*, 2021)**. Blood serum constituents serve as indicators of fish metabolism, corroborated by liver cell structure **(Fazio *et al.*, 2021)**.

The escalating consumption of fruits, along with the dispose of their wastes items into the environment, results in pollution issues and indicates the deprivation of vital nutrients biomass **(Osorio *et al.*, 2021)**. By-products from fruits processing, including rinds, pulps, skins, and seeds are an important source of compounds such as antioxidants, antimicrobials, fundamentals vitamins and phytochemicals (flavonoids, carotenoids) that exhibit nutritional attributes and several biological activities **(Qiang *et al.*, 2019)**.

Therefore, this study examined the impact of fruits wastes, fermented apple peels, and grape pomace combined with urea and molasses as a protein source in a contemporary diet on the growth and feed efficiency of basa fish **(***Pangasius bocourti***)**.

MATERIALS AND METHODS

Preparation of tanks

Twelve tanks, each with a capacity of 120 litres, were utilized. Aerated freshwater, after a duration of one day, serves as the medium for fish culture to enhance the dissolved oxygen in the culture water. Daily water exchange with one third of tank volume was

done. Quality parameters of water (temperature, pH, and ammonia (NH_3) were periodically observed to the end of experiment. Water temperature was monitored daily using a multi-parameter device. Nonetheless, ammonia (NH_3) and pH were biweekly measured by using colorimetric analysis (Table 1).

Table 1. Quality of the culture water utilized in the experiment for basa fish **(***Pangasius bocourti***)**

Parameter	Control	FGP (25%)	FAP (25%)	FGAPm (25%)
Temp **(**C°**)**.	27.43 ± 0.03	27.27 ± 0.12	27.37 ± 0.07	27.16 ± 0.08
DO_2 **(**ppm**)**	7.82 ± 0.05	7.00 ± 0.08	6.80 ± 0.06	6.98 ± 0.04
PH	7.64 ± 0.00^{c}	8.16 ± 0.00^{a}	8.17 ± 0.00^{a}	8.12 ± 0.01^{b}
NH_3 **(**ppm**)**	0.104 ± 0.00153^{a}	0.085 ± 0.00^{b}	0.065 ± 0.00^{c}	0.042 ± 0.00^{d}

Fish rearing

All healthy basa fish (*Pangasius bocourti*) were acquired from the Fish Research Center, Faculty of Agriculture, Suez Canal University, Ismailia–Egypt. Fish were acclimatized in 120L fiberglass tanks for 10 days before the start of treatments and were fed on a commercial feed powder two times daily (09:30 am and 14:30 pm). Fish with 7.5±0.5 g was used in the current trials each tank had 25 fish. Fish biomass was provided with feed to the point of satiation twice daily.

Fermented grape and apple wastes and feed preparation

Grape and apple wastes were obtained from juice factories in the 10th of Ramadan City, Egypt. The fruit wastes were washed thoroughly, dried at 50°C for 48 hours, ground, and then fermented using the required ingredients. The fermented grape pomace and apple peels (FGP+FAP) mixture consisted of 1kg of chopped grape and apple waste, 2.5kg of molasses, 3% urea, and 1 liter of distilled water. The components were combined and incubated weekly in a closed tank for a total of 28 days. Following fermentation, the mixture was dried in an oven at 60°C for 72 hours and was then blended into a homogeneous state.

The fermented fruit wastes (FFWs) were used to replace 25% of soybean protein in experimental diets. Four isogenic diets were formulated: a control diet and three diets with 25% replacement by FGP, FAP, and FGAPm (designated as FGP25%, FAP25%, and FGAPm25%, respectively) (Table 2). Other ingredients were adjusted to ensure nutritional values were comparable across all treatments.

Proximate composition (crude protein, ash, moisture, fat, and fiber) was analyzed following the guidelines of the Association of Official Analytical Chemists **(AOAC, 2019)**. Proximate analysis was carried out on feed materials, including fish meal, fermented grape and apple waste flour, and commercial feed (Table 3). Prior to

substitution, the protein content of the commercial feed was consistent with the protein content determined in the fermented products and the grape and apple waste flour combined with fish meal.

Commercial basa fish (*Pangasius bocourti*) feed was pulverized and sifted to achieve a soft consistency. The feed ingredients were then mixed with the fermented blend of fish meal and grape–apple waste flour. Pellets were produced using a pellet press, dried for 24 hours, labeled, and stored in plastic bags under moisture-free conditions.

Table 2. Compositional structure of fruits wastes utilized in the formulation of the tested diets

Treatments Parameter	Grape pomace		Apple peels	
	Before fermented	After fermented	Before fermented	After fermented
Protein	9.46±0.5	20.7±0.5	6.0 ± 0.38	18.33±0.7
Lipid	4.48±0.65	1.93±0.06	0.98 ±0.76	3.89 ± 0.10
Ash	3.53±0.55	16.33±0.05	3.72 ±0.37	6.41 ± 0.01
Fiber	33.79±0.9	31.35 ±0.10	36.66 ±3.08	24.60±0.10
Carbohydrates	45.89± 1.53	32.54 ± 0.31	53.57 ±3.16	49.65±0.33
Energy (kcal/kg diet)	2839.34±0.21	2686.08±0.16	2627.98 ±0.11	3438.90±0.10

Table 3. Feed formulation and compositional structure of the tested diets

Item	CON	FGP25%	FAP25%	FGAPm25%
	Feed formula (%)			
Fish meal 65%	10	10	10	10
Soybean meal 44%	53.41	44.11	44.11	44.11
Yellow corn meal	26.59	30.01	30.01	30.01
Fermented apple peels	-	-	5.88	2.94
Fermented grape peels	-	5.88	-	2.94
Fish oil	3.00	3.00	3.00	3.00
Soya oil	3.00	3.00	3.00	3.00
Min. and Vit. premix[1]	3.00	3.00	3.00	3.00
Cr_2O_3	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00
Proximate composition (%)				
Dry matter	89.87	90.37	89.44	89.87
Crude protein	35.18	35.64	34.70	34.84
Crude lipid	8.46	8.29	8.29	8.29
Fiber	5.99	6.13	6.12	5.83
Ash	12.08	10.81	9.14	9.10
Nitrogen free extract[2]	38.29	39.13	41.75	41.94
Gross energy (kcal kg^{-1})[3]	4277.38	4318.92	4343.64	4277.13

1- Vitamin and mineral premix (kg-1 diet): A, 5,000 IU; D3, 1,000 IU; E, 5,000 mg; K, 2,000; B1, 2,500 mg; B2, 1,000 mg; B6, 1,000, mg; B12, 10 mg; inositol, 1000 mg; pantothenic acid, 3,000 mg; niacin acid, 3,000 mg; C, 10,000 mg; folic acid, 300 mg; biotin, 10 mg; calcium phosphate, 80; calcium lactate, 100; ferrous sulfate, 1. 24; potassium chloride, 0.23; potassium iodine, 0.23; copper sulfate, 1.2; manganese oxide, 1.2; cobalt carbonate, 0.2; zinc oxide, 1.6; magnesium chloride, 2.16; sodium selenite, 0.10.
2- NFE (nitrogen free extract) = 100 – (% moisture + % protein + % fat + % ash + % fiber).
3- Gross energy value was calculated based on combustion values of 5.5 protein, 9.1 fat, and 4.1 kcal g-1 carbohydrates **NRC (2011).**

Data acquisition

This study primarily examined growth performance, feed utilization, haematological indicators, and economic evaluation of basa fish) *Pangasius bocourti*). Each 15 days, fish were weighed all over 84-days of trial period.

Growth parameters (Dighiesh *et al.*, 2024)

Weight gain **(**g**)** WG = *final body weight – initial body weight;*

Average daily gain **(**ADG, g fish-1 day-1**)** = *weight gain (g)/ experimental time (days);*

Specific growth rate **(**SGR, % BW day-1**)** = *100 x (Ln final body weight – Ln initial body weight)/ experimental time (days);*

Feed utilization (Dighiesh *et al.*, 2024)

Feed conversion ratio **(**FCR**)** = *total feed intake/ weight gain;*

Feed conversion efficiency **(**FCE %**)** = *100 x (final body weight – initial body weight)/ dry feed consumed (g);*

Protein efficiency ratio **(**PER**)** = *wet weight gain (g)/ protein intake (g) ;*

Apparent digestibility (Altan & Korkut, 2011)

ADC of dry matter **(**%**)** = *100 – [(100 × Cr2O3 of diet/ Cr2O3 of feces)]X nutrient in diet/nutrient in feaces*

Survivability (Dighiesh *et al.*, 2024)

Survivability **(**%**)** = *100 × (final number of fish/ initial number of fish)*.

Digestibility trial

Animals and experimental framework

Fish were provided with feed twice daily at 09:30 and 14:30 until satiation was achieved. Thirty minutes post the final feeding, all tanks were cleaned, and then water was replaced. Fecal collectors extracted approximately 300 mL into Falcon conical tubes, which were individually affixed to the bottoms of tanks and kept in polystyrene containers with ice to reduce fecal decomposition. Samples were collected in vials, left overnight, and retrieved the following morning at 6:30 a.m. The samples were then oven-dried for 48 hours. The dried material was centrifuged at 2800 × g for 10 minutes and transferred into labeled plastic bottles. These bottles were subsequently preserved at −80°C until the end of the experiment.

Apparent digestibility coefficient calculation

The ADCs calculations for dry matter, crude protein, crude fat, energy, phosphorus, and amino acid availability in the diets were conducted using the formulae established by **Cho and Kaushik (1990)**. ADCs of dry matter (%) was determined by multiplying 100 by the difference between dietary Cr_2O_3 consumption and faecal Cr_2O_3, then dividing by faecal Cr_2O_3. ADC of nutrient or energy (%) = 100 × (I - Cr_2O_3 in diet / Cr_2O_3 in faeces) × (% nutrient in faeces / nutrient in diet).

Collection of samples

At 84 days, 5 fish/ tank (n=25) were randomly selected for blood, liver, and whole digestive tract sampling for haematology plasma and histological analysis. Before blood collection, the fish underwent a 24-hour fasting period.

Haematology and plasma analysis

After anaesthetizing the fish by clove oil, blood was extracted from the caudal vertebral vein and was collected into tubes containing sodium heparinate as an anticoagulant (20U L-1) for the assessment of haemoglobin (Hb), red blood cells (RBCs), and white blood cells (WBCs). Fresh blood specimens were inserted into glass capillary tubes and centrifuged for 10 minutes using a microhematocrit centrifuge to determine packed cell volume (PCV). A micropipette was used to transfer 2mL of haemoglobin (Hb) sample, which was then combined with 5mL of Drabkin's solution and was let to stand for 5 minutes. Colorimetric tests were performed to assess the synthesis of cyanomethaemoglobin using the procedure published by **Van Kampen and Zijlstra (1961)**. White blood cell counts were determined using a Neubauer haemocytometer, as described by **Kaplow (1955)**. The erythrocytic indices in the blood were determined using the method reported by **Handy *et al.* (1999)**. The hematocrit **(**Hct**)** was measured by centrifuging the samples at 12,000 x g for five minutes (Microspin, model Spin 1000, Jaboticabal, Brazil) following **Goldenfarb *et al.* (1971)** protocol. Red blood cell count was tested using a Neubauer chamber, resulting in 106/μL. The concentration of haemoglobin **(**Hb**)** was measured using Drabkin's reagent and a spectrophotometer at 540 nm **(Drabkin, 1948)**. Haematimetric indices were determined using these equations: The formula for mean corpuscular volume **(**MCV**)** is Hct × 10 / Ery **(**×10^6 μL**)** and measured in femtolitres. Mean corpuscular hemoglobin **(**MCH**)** was calculated using the formula Hb × 10 / Ery and expressed in picograms **(**pg**)**. To determine the mean corpuscular haemoglobin concentration **(**MCHC**)**, the formula Hb × 100 / Hct, expressed in g dL^{-1} was applied. Blood glucose levels were assessed via a digital glucometer **(**Accu-Chek Performa/Roche®, São Paulo, Brazil**)**. 1.5ml of blood was extracted from the heart via 5000 UI heparinised syringes. Two blood aliquots were collected; the first aliquot **(**~0.50 ml**)** was used for blood parameter analysis, while the second aliquot **(**~1.00 ml**)** was

utilized to obtain plasma for biochemical assessments. Two aliquots were dispensed into 2.50ml tubes. The second sample of blood was centrifuged at 4°C at 3000× g for 10 minutes to isolate the plasma, which was thereafter kept at −80°C. Whole liver and intestinal samples were obtained and kept at−80°C before examination. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured calorimetrically at a wavelength of 540nm, using the approach established by **Reitman and Frankel (1957)**. Plasma triglycerides, total proteins, albumin, and cholesterol were quantified utilizing commercial kits **(**Labtest®; Vista Alegre, Brazil**)** and a semiautomatic biochemical analyzer **(**Doles®, model D-250, Goiânia, Brazil**)**.

Histopathological test

At the end of the feeding trial, five fish were randomly selected from each tank for histopathological examination. Intestine and liver tissue samples from each fish were preserved in 10% buffered formalin solution for at least 24 hours. Samples then underwent dehydration through a graded ethanol series (70, 80, 90, 95, and 100%), followed by overnight clearing with methyl benzoate and treatment with xylene before embedding in paraffin wax. Tissue sections of 6µm thickness were prepared using an automated microtome, stained with hematoxylin and eosin (H&E), and photographed under a Leica microscope in accordance with prior study **(Eissa *et al.*, 2024)**.

Economic assessment

The five experimental diets were economically evaluated, considering both fish and feed costs. The following ingredient prices, expressed in Egyptian pounds (LE) per kilogram, were used to calculate diet expenses: fishmeal (70.00 LE), soybean meal (18.50 LE), maize meal (14.00 LE), oil (60.00 LE), and vitamin–mineral premix (300.00 LE). Fermentation costs were estimated based on expenses for collection, washing, drying, grinding, and screening.

The feed cost required to produce one kilogram of fish biomass was assessed through a basic economic analysis using prevailing local retail prices of dietary ingredients during the study period **(Eid & Mohamed, 2008)**. Calculations were performed as follows:

- **Cost per kilogram of diet (LE):** Cost ÷ kilograms of diet.
- **Feed conversion ratio (FCR):** Feed intake per fish ÷ final weight gain per fish.
- **Cost of feed per kilogram of fresh fish (LE):** Step 1 × Step 2.
- **Relative feed cost per kilogram of fish (%):** (Step 3 ÷ highest value in Step 3) × 100.
- **Feed cost per kilogram of gain (LE):** Feed input ÷ weight gain.
- **Relative feed cost per kilogram of gain (%):** (Step 5 ÷ highest value in Step 5) × 100.

Ethical approval

This study was approved by the Ethical Committee of the Faculty of Agriculture, Suez Canal University (Reference Code: SCU-Agr-REC 30/2025).

Statistical analysis

Data are presented as means ± standard deviation (SD). Statistical analyses were conducted using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was employed to assess treatment effects. When significant differences were detected ($P< 0.05$), group means were compared using Duncan's multiple range test **(Duncan, 1955)**.

RESULTS

Growth performance

Regarding growth performance and feed utilization of basa fish (*Pangasius bocourti*), fish fed the FGP diet showed a statistically significant increase in weight ($P< 0.05$) compared to the control group (Table 4). Weight increase in the control and FAP groups was considerably reduced ($P< 0.05$) in comparison with the FGAPm group. The ideal feed conversion ratio was seen in fish administered FGP, FAP, and FGAPm, exhibiting a significant difference from the control group. The outcomes regarding growth, feed utilization, and survivability of basa fish (*Pangasius bocourti*) over a 84-day experimental period are presented in Table (40. Fish subjected to various diets exhibited a superior growth pattern with the FGP 25% diet compared to all other diets. This was followed in descending order by the FAP25%, FGAPm25%, and control diets, which demonstrated the least growth. The inclusion in diets at a rate of 25% resulted in decreased energy, adversely impacting growth and feed efficiency. The nutrient digestibility results demonstrated the highest levels of PER and ADC in fish fed a diet containing 25% FGP, followed by those fed 25% FAP, 25% FGAPm, and the control diet group. Diets incorporating fruits wastes significantly influenced growth performance in a linear manner ($P< 0.05$). Significant negative effects ($P< 0.05$) were noted for the control diet in comparison with the fruits wastes diets regarding final weight, WG, and SGR. Nonetheless, FGP25% is the most optimal. Consequently, linear regression analysis indicates that the basa fish (*Pangasius bocourti*) receiving 25% FAP exhibited a reduced growth performance. Furthermore, fish receiving a diet comprising 25% FGP exhibited significantly higher WG and SGR compared to those fed 25% FAP, FGAPm, and the control group. The apparent digestibility coefficients of CP, crude energy, and dry matter exceeded 90%. Regarding crude energy ADC of FFW, it was significantly greater than that of the RD ($P< 0.05$) (Table 4). The survivability in the FGP, FAP, and FGAPm groups was significantly higher ($P< 0.05$) than that of the control group. Survivability during the feeding trial was significantly induced ($P> 0.05$) by the replacement of FFW.

Table 4. Growth performance, feed utilization, and survivability of basa fish (*Pangasius bocourti*) fed for 84 days (mean ± SD)

Parameter	**Fruit Wastes**			
	Control (CN)	**(FGP)**	**(FAP)**	**(FGAPm)**
Initial body weight **(g)**	7.5 ± 0.61	7.5 ± 0.31	7.5 ± 0.42	7.5 ± 0.61
Final body weight **(g)**	49.69 ± 0.16^{c}	52.57 ± 0.44^{a}	50.85 ± 0.11^{b}	51.1 ± 0.12^{a}
Body weight gain **(g)**	42.19 ± 0.16^{c}	45.07 ± 0.06^{a}	43.34 ± 0.18^{b}	43.50 ± 0.11^{b}
SGR **(**% d^{-1}**)**	2.25±0.01^{b}	2.31 ± 0.04^{a}	2.27 ± 0.08^{b}	2.28 ± 0.04^{b}
FCE **(**%**)**	47.70 ± .01^{c}	67.16±.01^{a}	50.51±0.67^{b}	46.27±0.01^{c}
FCR	2.1 ± .01^{a}	1.51±1.41^{d}	1.72 ± 0.05^{b}	1.64 ± 0.11^{c}
PER	1.21±0.03^{c}	1.29±0.04^{a}	1.24±0.01^{b}	1.24±0.01^{b}
ADG **(**g day^{-1}**)**	0.50±0.02^{d}	0.54±0.05^{a}	0.52±0.01^{c}	0.52±0.05^{b}
Survivability **(**%**)**	96.04^{b}	100^{a}	100^{a}	100^{a}

Means in the same row followed by the same letter did not differ statistically **(***P*> 0.05**)**.

The body composition of basa fish (*Pangasius bocourti*) indicated that the control group exhibited a significant high fat level (*P*< 0.05) compared to those subjected to treatments with fruits wastes. The fish exhibited a significantly higher protein content (*P*< 0.05) in the FGP group compared to the other groups, while the ash content was notably lower (*P*< 0.05) than the control after 84 days (Table 5).

Table 5. Body composition **(**% wet weight**)** of basa fish **(***Pangasius bocourti**)*** fed for 84 days (mean ± SD)

Parameter	Fruit Wastes			
	Control **(**CN**)**	**(**FGP**)**	**(**FAP**)**	**(**FGAPm**)**
Moisture	71.25 ± 0.46	72.6 ± 0.12	71.31 ± 0.50	72.01 ± 0.12
Crude protein	56.1 ± 0.09^{d}	66.5 ± 0.15^{a}	58.3 ± 0.04^{c}	61.3 ± 0.29^{b}
Crude lipid	32.3 ± 0.12^{a}	25.5 ± 0.11^{d}	29.9 ± 0.10^{c}	31.02 ± 0.01^{b}
Ash	10.6 ± 0.20^{a}	7.7 ± 0.09^{c}	9..4 ± 0.06^{b}	6.42 ± 0.07^{d}

Means in the same row followed by the different letters differ statistically **(***P*< 0.05**)**.

Digestibility

The diets demonstrated effective digestion and yielded high ADC values for CP, crude energy, and dry matter, indicating their suitability for inclusion in commercial diets for basa **(***Pangsius bocourti***)** culture, as evidenced by the study. The substitution of the examined fruit waste at the designated ratio resulted in elevated ADC values of protein across all samples. Notably, the diet incorporating 25% fermented grape pomace **(**FGP**)** exhibited optimal performance, maintaining the digestibility of crude protein and dry matter while enhancing the digestibility of raw energy in the diet (Table 6).

Table 6. Apparent digestibility coefficient in the control and waste fruit trial in basa fish (*Pangasius bocourti*) fed for 84 days (mean ± SD)

Variable	Control	FGP	FAP	FGAPm
		25%	25%	25%
Crude protein (%)	96.90 ± 0.20 [b]	97.90 ± 0.50 [a]	94.70 ± 0.50 [c]	92.91 ± 0.50 [d]
Crude energy (kcal kg^{-1})	93.85 ± 0.43[b]	98.63 ± 1.29[a]	95.43 ± 1.29[b]	94.43 ± 1.29[b]
Dry matter (%)	91.80 ± 0.89[c]	94.30 ± 1.68 [a]	93.70 ± 1.68 [b]	92.80 ± 1.68 [b]

Means in the same row followed by the different letters differ statistically ($P < 0.05$).

Hematological and biochemical serum indices

The inclusion of various fruits wastes has demonstrated significant effects on the biochemical indicators in the blood serum of basa fish (*Pangasius bocourti*). Fish that were fed FGP and FAP exhibited significantly lower ($P < 0.05$) AST concentrations relative to the control and FGAPm groups (Table 6). Triglyceride levels in the blood were lower ($P < 0.05$) in treated fish that received fruit wastes supplements compared to the reference diet. Fruits clusters treated with waste exhibited significantly higher levels of PCV, Hb, and RBC ($P < 0.05$) in comparison with the control. The fish received a diet comprising 25% FGP, its blood glucose and hepatopancreas glycogen levels are significantly elevated compared to those who were fed the FAP and FGAPm diets which resulted in lower plasma cholesterol levels than the control ($P < 0.05$). Linear regression test indicated a statistically significant decrease ($P < 0.05$) in Hct, MCV, and MCH in basa fish (*Pangasius bocourti*) due to dietary FGP squared regression analysis ($P < 0.05$). Results indicated that MCHC levels were elevated in fish that received a diet comprising 25% FGP (Table 7).

Table 7. Effects of fruits wastes on serum biochemical indices and enzymes of *Pangasius bocourti* fed for 84 days (mean ± SD)

Parameter	Fruit Wastes			
	Control	(FGP)	(FAP)	(FGAPm)
		Plasma analysis		
Glucose (mg/dl)	166± 0.11d	272.91± 0.08a	150.04 ± 0.12c	251.12 ± 0.19b
Triglycerides (mg/dl)	552.0 ± 0.12b	539.01 ± 0.05b	536.0 ± 0.04a	522.03 ± 0.09a
Total Protein (g/dl)	2.80 ± 0.30b	3.10 ± 0.25b	3.10 ± 0.12a	3.0 ± 0.11a
Albumin (g dL-1)	0.81 ± 0.10	0.93 ± 0.05	0.87 ± 0.05	0.80 ± 0.09
Cholesterol (mg/dl)	269.02 ± 0.05b	242.02 ± 0.05b	235.04 ± 0.04a	155.30 ± 0.09a
		Hepatic variables		
Glycogen(μmoles)	13.49 ± 0.77c	18.15 ± 0.88a	17.49 ± 0.99b	13.45 ± 0.89c
AST (U/ L)	307.00 ± 0.14b	300.00 ± 0.13c	302.00 ± 0.18c	311.00 ± 0.11a
ALT (U/ L)	21.00 ± 0.05c	22.00 ± 0.10c	23.00 ± 0.08b	24.00 ± 0.06a
		Hematology Indices		
PCV (g dL—1)	24.12 ± 0.04c	26.60 ± 0.14a	25.09 ± 0.10b	25.55 ± 0.09b
Hb (g/dl)	9.00 ± 0.15c	11.6 ± 0.08a	10.83 ± 0.06b	10.65 ± 0.15b
RBC (× 1012 L—1)	2.1 ± 0.14b	3.12 ± 0.04a	2.90 ± 0.21b	2.8 ± 0.11b
WBC (× 108 L—1)	14.37 ± 0.05b	15.04 ± 0.10c	15.86 ± 0.04a	15.74 ± 0.08a

Hematocrit **(**%**)**	30.00±0.01	33.00±0.01	31.00±0.21	31.00±0.01
Hct **(**%**)**	28.17 ± 0.75	28.13 ± 0.54	27.15 ± 0.68	26.98 ± 0.58
M.C.V**(**FL**)**	146.01±0.10	105.01±0.10	106.04±0.10	110.01±0.10
M.C.H**(**pg**)**	48.10±0.2	32.19±0.2	37.24±0.2	37.86±0.2
M.C.H.C**(**g/dL**)**	31.01±05	36.12±05	34.84±05	34.19±0.05
Neutrophils **(**x103/ul**)**	3.8±0.04	0.3±0.04	0.3±0.04	0.3±0.04
Neutrophils **(**x103/ul**)**	9.40±0.05	6.7±0.05	8.30±0.05	7.8±0.05
Lymphocytes **(**x103/ul**)**	7.50±0.06	5.6±0.02	7.8±0.08	6.2±0.08
Monocytes **(**x103/ul**)**	1.31±0.05	1.3±0.05	0.3±0.05	0.6±0.05
Eosophils **(**x103/ul**)**	3.8±0.00	0.1±0.04	0.2±0.03	0.1±0.03
	Digestible enzymes **(**Ul mg-1**)**			
Amylase **(**U/ L**)**	280.00 ± 0.11a	284.00 ± 0.11b	281.00 ± 0.11c	283.00 ± 0.11c
Alk Protease **(**Ul mg-1**)**	5.08 ± 0.41	6.70 ± 0.23	5.71 ± 0.18	5.55 ± 0.37

Means in the same row followed by the different letters differ statistically **(***P* < 0.05**)**.

Histopathological analysis of both basa fish (*Pangasius bocourti*) liver and intestine

The livers of fish that were administered FGP, FAP, and FGAPm had normal morphology and structure and staining properties of hepatocytes. This indicates that the fish fed on these diets sustained a healthy condition. Nevertheless, other samples demonstrated irregular venous congestion in the livers of fish fed FAP and control diets (Fig. 1), but no congestion was observed in those receiving alternative diets. The intestines from FGP, FAP, and FGAPm exhibited no inflammatory effects in the villi; however, the villi length in fish from FGP was significantly greater (Fig. 2A). The intestines of fish in the control group exhibited no signs of inflammation (Fig. 2D). The villi in FAP, FGAPm, and control groups were significantly shorter ($P< 0.05$) compared to those in FGP, and exhibited a reduced density along with varying degrees of inflammation (Fig. 2B, C, D).

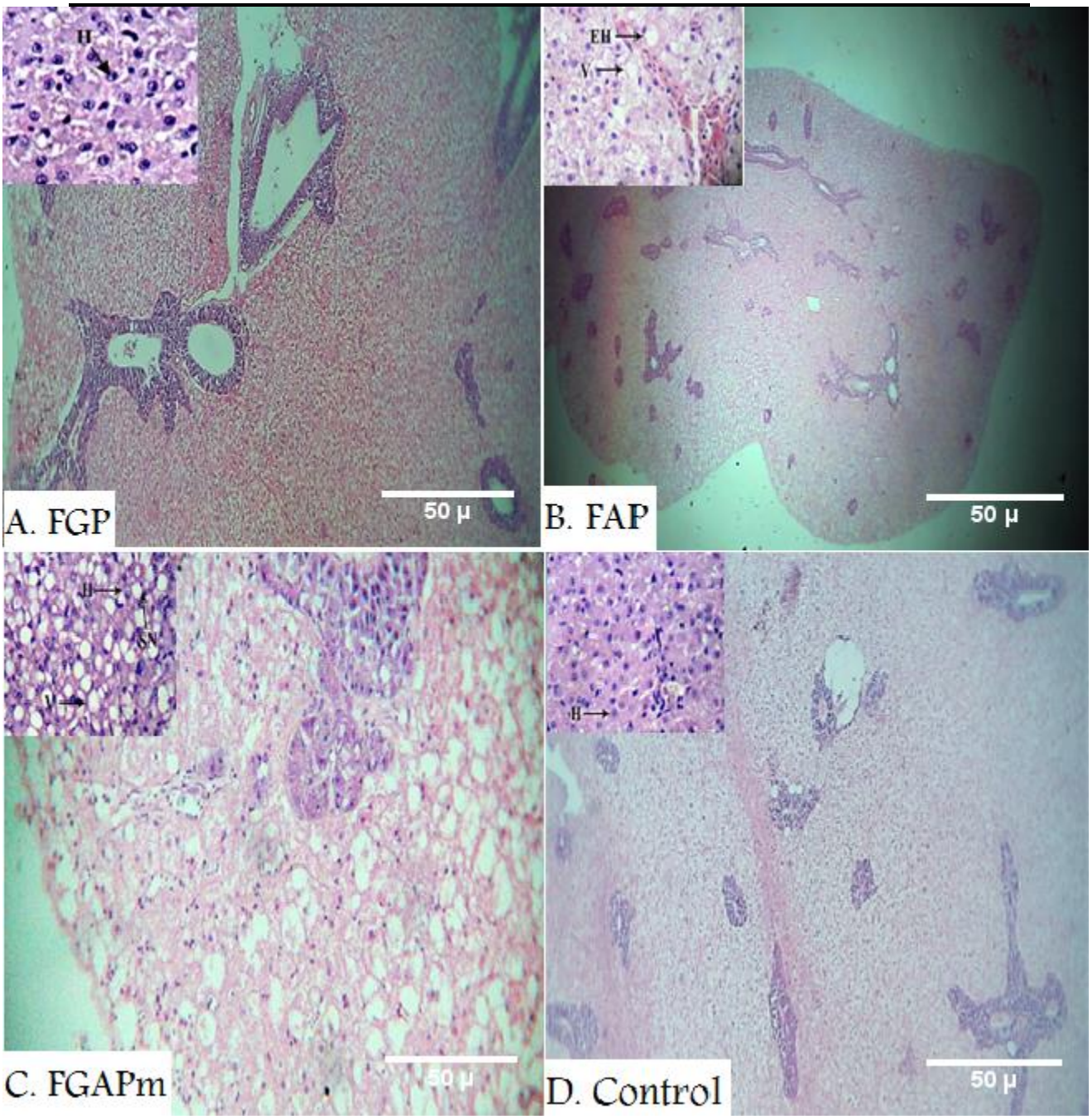

Fig. 1. Liver histopathology of Basa Fish **(***Pangasius bocourti*) fed on different fruits wastes. Control, Grape Pomace (FGP), Apple peels (FAP), Mix of Grape pomace and Apple peels (FGAPm). H, hepatocyte; V, vacuolization; EH, enlarged hepatocyte; SN, shrunken nucleus. 50µm (H&E)

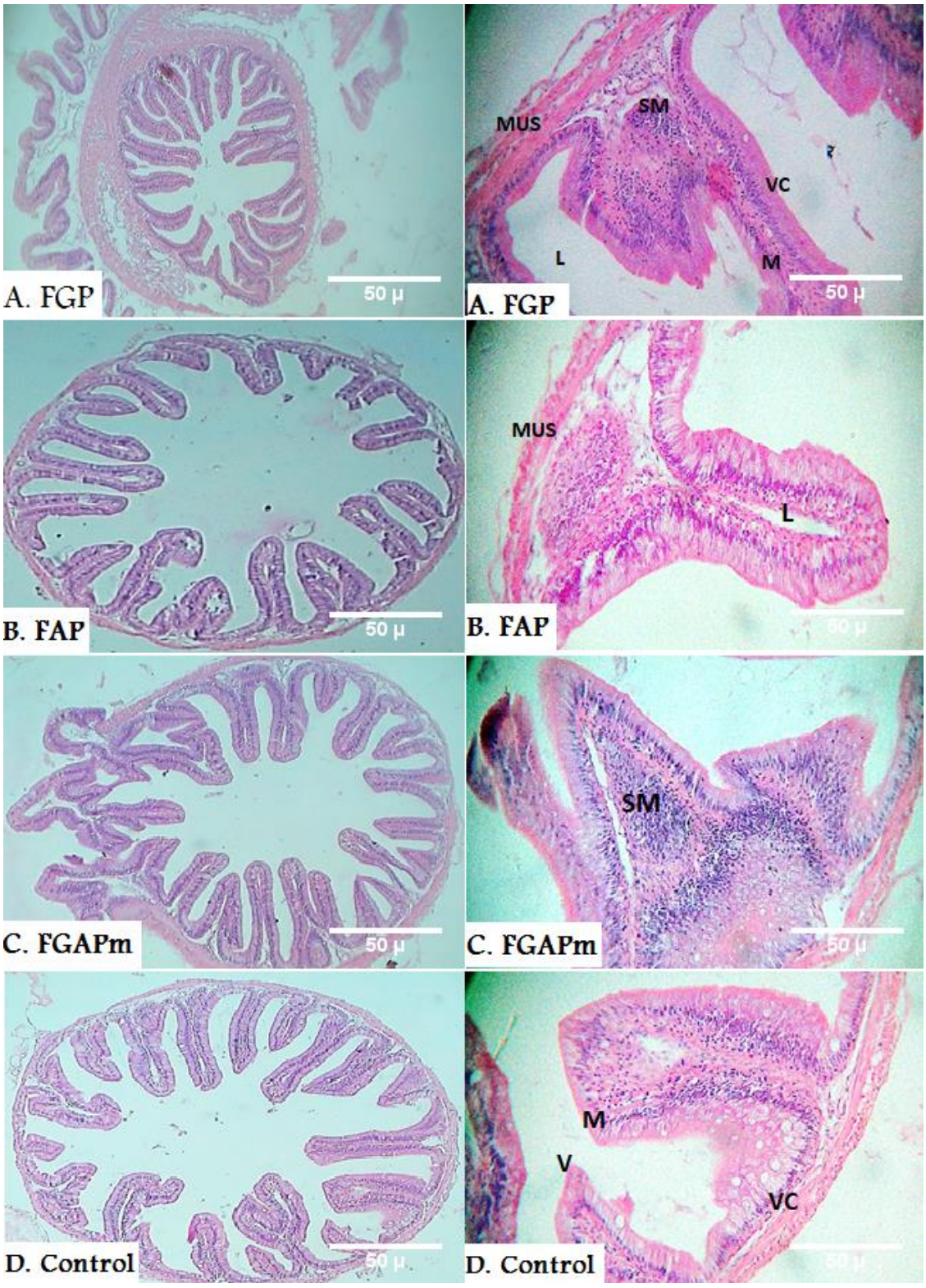

Fig. 2. Intestinal histopathology of Basa fish (*Pangasius bocourti*) fed on fruits wastes. Control, Grape Pomace (FGP), Apple peels (FAP), Mix of Grape pomace and Apple peels (FGAPm)., Muscularis 'Mus'; Lumen 'L'; Sub-Mucosa 'sM'; Villi 'V'; Mucosa 'M'; with suer nuclei 'VC'; 50µm (H&E)

Assessment of economic factors

Table (8) presents the economic evaluation of the experimental relationships utilized in the study. The implementation of FGP, FAP, and FGAPm led to a reduction in the feed cost associated with the production of one kilogram of fish. The data presented in Table (8) indicate that the costs for one kilogram of diet using fermented wastes in the experimental diets were 27, 26, 24, and 23 LE for the control diet, FGP, FAP, and FGAPm, respectively. The costs associated with a one-kilogram increase in weight were 55.59, 39.26, 41.28, and 37.72 LE. The findings demonstrate that the inclusion of fermented wastes in basa fish diets decreased the cost per kilogram of the diet by 29.38, 25.74, and 32.15% for FGP, FAP, and FGAPm, respectively, in comparison with the control (100% of the price). The cost reduction or increase per kilogram of the treatment comprising 25% FGP and other fermented components aligned with growth performance and feed utilization metrics.

Table 8. Economic evaluation of experimental diets used in the study

Item	Experimental diets			
	Cont.	FGP	FAP	FGAPm
Cost/kg	27	26	24	23
Feed intake	1.1	0.94	1.12	1.13
Feed intake cost (LE/kg)	29.70	24.44	26.88	25.99
Relative to feed cost (%)	100	82	91	88
FCR	2.1	1.51	1.72	1.64
Feed cost/kg gain	56.7	39.26	41.28	37.72
Relative to control	100	69.24	72.80	66.53
Decrease in feed costs (L.E/kg weight gain %)	0.00	30.76	27.2	33.47

DISCUSSION

Currently, there is a global surge in commercial fruit cultivation, which may lead to substantial fruit output and waste in the future **(Nor & Ding, 2020)**. A significant portion of fruit and vegetable wastes can be used as components in aquaculture diets **(Sulaiman *et al.*, 2022)**. In addition to serving as growth enhancers, certain waste materials can improve the welfare and health of many aquaculture species **(Qiang *et al.*, 2019)**. Furthermore, the disposal of fruit waste in rivers or landfills may exacerbate organic pollution and pose environmental risks **(Galintin *et al.*, 2021)**.

The nutritional composition of fruit waste varies, particularly in protein, fat, and ash contents **(Van Doan *et al.*, 2021)**. Different fruit by-products possess unique beneficial characteristics that can be efficiently incorporated into fish feed formulations **(Pérez-Pacheco *et al.*, 2022)**. Water quality is a critical determinant of aquaculture performance, as poor water quality increases stress in aquatic organisms, reducing survival and growth **(Lim *et al.*, 2021)**. In the present study, all water quality parameters remained within the optimal range for *Pangasius*, consistent with the findings of **Rodrigues *et al.* (2023)**.

This study revealed that fermented grape pomace (FGP) had significantly higher protein levels, whereas apple peel (FAP) showed an increased lipid content. The incorporation of fruit residues into the diet markedly influenced the growth and weight gain of basa fish (*Pangasius bocourti*). Fish fed FGP exhibited significantly greater growth ($P< 0.05$) compared to those fed FAP, FGAPm, or the control diet. The reduced growth in the FAP and FGAPm groups may be attributed to the presence of phenolic compounds and other bioactive substances that hinder the digestion of proteins and soluble carbohydrates, reducing digestibility and nutrient absorption **(Van Doan *et al.*, 2021)**.

The optimal feed conversion ratio (FCR) in basa fish was observed in the FGP group, likely due to differences in nutritional composition and palatability. Certain fruit by-products, containing ferulic and gallic acids, enhance flavor and promote growth in fish and mammals **(Cai *et al.*, 2020)**. For instance, **Enyidi and Nduh-Nduh (2016)** reported that the African catfish (*Clarias gariepinus*) juveniles fed neem and lettuce seeds exhibited growth comparable to those fed *Artemia*. Similarly, **Afreen and Ucak (2020)** found that *Oreochromis niloticus* given 15% sweet potato peel waste showed improved growth and feed efficiency. Papaya leaf meal has also been shown to enhance nutrient utilization, feed conversion, and weight gain in the tiger shrimp (*Penaeus monodon*) **(Sánchez-Muros *et al.*, 2020)**.

In the present study, FGP-fed fish had the highest protein efficiency ratio (PER) and apparent digestibility coefficient (ADC), followed by FAP, FGAPm, and the control. These results align with those of **Yossa *et al.* (2022)** and **Intharathat *et al.* (2024)**, who reported that tilapia fed a 25% fruit by-product diet showed the highest apparent digestibility for protein and energy compared with 50–75% inclusions and the control. A low-energy diet may induce amino acid catabolism, depriving fish of sufficient energy for metabolism and resulting in reduced growth and protein efficiency **(Molina, 2016)**. Similarly, **Peña *et al.* (2020)** observed an improved digestibility in the rainbow trout fed 18% grape pomace for 90 days. These findings are further supported by **Mehrinakhi *et al.* (2021)** and **Quagliardi *et al.* (2024)**.

Fermentation was shown to increase the protein content of fruit waste, largely due to microbial breakdown of complex carbohydrates and the production of microbial protein. In this study, molasses–urea fermentation increased protein levels by up to 12% compared to pre-fermentation values, consistent with **Fatmawati *et al.* (2018)**. Moisture content of raw fruit waste ranged from 74.88 to 86.60g/ 100g, making it essential to accurately assess digestible and non-digestible carbohydrates to avoid compositional errors **(Garcia-Amezquita *et al.*, 2018)**.

Nutritional values of grape pomace (GP) have been reported as high in crude fiber (31.35–33.79%), protein (9.46–20.7%), ash (3.53–16.33%), carbohydrate (32.54–45.89%), and crude lipid (1.93–4.48%) **(Rosas *et al.*, 2022)**. Basa fish are known for high ADC values and efficient nutrient digestion **(Guimarães *et al.*, 2014)**. Herbivorous and

omnivorous fish also utilize dietary carbohydrates more efficiently than carnivorous species, owing to structural and physiological differences in their digestive systems **(Do Carmo Gominho-Rosa *et al.*, 2015)**. Additionally, apple peel powder (0.1–0.2%) has been shown to reduce cholesterol, triglycerides, ALT, and AST in genetically modified organisms **(Qiang *et al.*, 2019)**.

Blood glucose regulation is another critical factor. Rising plasma glucose levels increase the diffusion gradient into red blood cells, but fish erythrocytes are relatively impermeable to glucose, preventing hyperglycemia and delayed glycoprotein synthesis **(Polakof *et al.*, 2012; Walker *et al.*, 2020)**.

An emerging area of aquaculture research is the use of fermented grape peel (FGP) as a sustainable feed ingredient. This not only reduces feed costs but also promotes circular use of agricultural by-products. To date, little evidence exists on the effects of fermented fruit waste on the liver and intestine of basa fish. Previous studies indicated that liver histology serves as an effective indicator of fish nutritional status **(Shiu *et al.*, 2015; Zakaria *et al.*, 2022)**. In this study, FGP supplementation induced histological, morphological, and functional changes in liver tissues of *Clarias gariepinus* after eight weeks. Negative effects of soybean meal (SBM) on fish liver health have also been widely reported. However, **Sulaiman *et al.* (2022)** found no signs of inflammation, congestion, or vacuolization in the livers of mahseer fed various fruit wastes, suggesting good hepatocyte maintenance.

All treatments in this trial, including the control, showed normal intestinal walls without villi inflammation. Interestingly, fish fed fruit waste exhibited densely packed villi without significant differences in length ($P > 0.05$) compared to the control. **Toutou *et al.* (2019)** noted that larger villi in pomegranate-fed fish enhanced absorptive efficiency, supporting the findings of the 25% FGP treatment in the present study.

Finally, this study also evaluated the economic implications of using fermented grape peel in fish diets. Previous research indicated that incorporating fermented fruit waste can reduce diet costs without compromising performance **(Eid *et al.*, 2024)**.

CONCLUSION

This study demonstrated that fermented grape pomace **(**FGP**)** serves as substitute protein source in the fish diets, constituting 25% of dietary protein, with positive effects on growth performance, feed utilization, and economic evaluation for basa **(***Pangasius bocourti***)** fingerlings under the experimental conditions applied.

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