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Incorporation of Silk Warm By-Products Feeds as Un-Traditional Source of Protein in Diets of the Nile Tilapia (*Oreochromis niloticus*) Fingerlings

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ABSTRACT

This study aimed to investigate incorporated silk warm by-product feeds as an unconventional source of protein for the Nile tilapia, *Oreochromis niloticus* fingerlings diets, and its impact on their growth performance and physiological status. In this study, 160 monosex Nile tilapia fingerlings with an average live weight of 2.6 ± 0.04g were used. They were divided into four groups and distributed among 16 aquariums, with 10 fries per aquarium. The aim was to investigate the impacts of including silkworm by-product feeds (SWBF) as an unconventional source of protein in the Nile tilapia fingerlings' diets on their growth performance, feed utilization, apparent protein digestibility, blood parameters, body composition, energy retention percentages (ER%), protein productive value percentages (PPV%), and intestinal villi absorptive capacity. Four levels (0, 10, 20, and 30%) of SWBF were incorporated into the experimental diets to partially or completely replace a portion of the soybean meal. The feeding trial was extended for 84 days, and fish were hand-fed at a rate of 3%. The results indicated that *Oreochromis niloticus* fingerlings fed on a diet containing 10% silk-warm by-products showed improvements in growth, and survival ratio, with a reduction in the number of dead fish and mortality rate. Moreover, dry matter and crude protein intakes increased. Both feed conversion & protein efficiency ratio and apparent protein digestibility were significantly improved. Blood contents which include albumin, globulin, albumin: globulin ratio, red blood cell count (RBC's), ASAT, ALAT, and urea were significantly ($P< 0.05$) affected, and values of ASAT, ALAT, and urea were decreased. The body composition of dry matter and villi length of the duodenum, ileum, and rectum significantly ($P< 0.05$) increased. From the results obtained in our study, it is clear that 10% silkworm by-products can be successfully used as an unconventional source for rearing the Nile tilapia fingerlings, significantly affecting their growth and survival. Additionally, the energy retention and protein productive value improved, with no adverse effects on the apparent protein digestibility, blood constituents, body composition, energy retention percentages, protein productive value (PPV), and intestinal villi absorptive capacity of the Nile tilapia fingerlings.

INTRODUCTION

Abdel-Mobdy *et al.* (2021) noted that in developing countries, fish play an important role in resolving the sustainability of people's food and livelihoods. Moreover, **Gomma (2005)** highlighted that fish are regarded as one of the most essential and high-quality sources of protein. **El-Dakar *et al.* (2021, 2023)** emphasized that successful cultured fish production necessitates cost reduction.

Mangbanua and Ragaza (2022) recorded that tilapia culture is one of the largest sectors of global aquaculture and several tilapia strains having better growth performance. **Kaleem and Sabi (2021)** and **El-Dakar *et al.* (2022)** reported that Egypt has the largest aquaculture production in Africa, with a total production reaching 1.8 million tons annually. Fishmeal, soybean meal, and corn gluten, among others, are generally classified as highly valuable sources of dietary protein for many fish species **(Turchini *et al.*, 2019)**.

Therefore, the research team investigated the incorporation of silk worm by-products feeds as an unconventional source of protein in the Nile tilapia fingerlings' diets. Researchers examined its effects on various parameters including growth performance, feed utilization, apparent protein digestibility, blood constituents, body composition, energy retention percentages, protein productive value, and intestinal villi absorptive capacity.

MATERIALS AND METHODS

Our study was carried out at the Fish Experimental Station belonging to the Animal Production Department, Faculty of Agriculture, Al-Azhar University, Cairo, the Plant Protection Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt in co-operation with the Animal Production Department, National Research Center, 33 El-Bohouth Street, P.O. Box: 12622, Dokki, Cairo, Egypt.

Experimental unit

Mono sex of the Nile tilapia *Oreochromis niloticus* fingerlings with average initial body weight of 2.6± 0.041g were obtained from a hatchery (GAFRD) that was found at a distance of 21km from Alexandria Governorate. After acclimatization, fish were randomly classified into the experimental aquariums. A total number of 160 fry was distributed in 16 aquariums (1.5 x 1.00 x 1.00m) and randomly divided into four equal experimental groups, each of which contained 40 fry distributed in 4 equal replicate, and each aquarium contained 10 fry. The aquariums were supplied all day with air blowers. Water temperature was maintained at 28- 25°C. The part of water aquarium was daily exchanged by 10% of fresh water.

Water quality

The water temperature and dissolved oxygen were daily measured using oxygen meter (Lutron model Do- 5509, Taiwan), and the pH values were recorded by a digital pH meter (Hanna model PHEP, USA). Total ammonia concentration was measured using comparison apparatus using HACH kits (Hach Co., Loveland, Colorado, USA).

The percentages of unionized ammonia (NH_3) were calculated from multiplying the total ammonia value by the appropriate factor according to the following equation:
Ammonia concentration (mg/ L as NH_3) = A/ 100 x 1.2 x total ammonia.
Where, A is a coefficient related to water pH and temperature.

Experimental diets

Four levels of silk warm by-products feeds (SWBF) were incorporated into the diets at 0, 10, 20 and 30%, replacing soybean meal, which constituted 30% in component of the first group (D_1), designated as the control diet. These levels were incorporate in the experimental diets as an alternative source of protein that replaced partial or complete replacement of soybean meal that was used in formulating the control diet (D_1), as exhibited in Table (2).

The experimental diets were continued for 84 days. These diets were formulated to be iso-caloric and iso-nitrogenous and were provided in the form of pelleted floating rations with a diameter between 2- 3mm. The fish were hand-fed at rate of 3%, with the feed distributed three times a day (9, 12, and 3pm) throughout the experimental period (84 days) which extended from March to May, 2022. The chemical composition of feed ingredients are presented in Table (1).

Table 1. Chemical composition of feed ingredients

Item	Ingredient					
	SWBF	Soybean Meal	Corn gluten	Fish meal	Wheat bran	Yellow corn
Dry matter (DM)	92.95	94.5	92.85	93.58	91.50	92.07
Chemical composition on dry matter basis:						
Organic matter (OM)	92.41	93.72	98.14	78.00	94.78	98.28
Crude protein (CP)	13.18	44.16	68.00	65.00	14.08	9.15
Crude fiber (CF)	8.95	4.15	1.87	00.00	10.09	2.50
Ether extract (EE)	6.06	1.08	2.83	8.22	3.05	2.85
Nitrogen free extract (NFE)	64.22	44.33	25.44	4.78	67.56	83.78
Ash	7.59	6.28	1.86	22.00	5.22	1.72
Gross energy kcal/ kg DM	4351	4608	5241	4644	4305	4365
Metabolizable energy Kcal/ kg DM	332.83	362.23	417.85	376.18	324.00	356.79
Protein energy ratio (mg CP/ Kcal ME)	39.60	121.91	162.74	172.79	43.46	25.65

SWBF: Silk warm by-products feeds
Gross energy (kcal/ kg DM) was calculated according to **Blaxter (1968)** and **MacRae and Lobley (2003)**. Where, each g of CP = 5.65Kcal, g of EE = 9.40kcal and g of CF and NFE = 4.15Kcal.
Metabolizable energy (ME) was calculated using values of 4.50, 8.15 and 3.49Kcal for protein, fat and carbohydrate, respectively. Protein energy ratio (mg CP/ Kcal ME) was alculated according to the method of **NRC (2011)**.

Growth performance parameters

Fish growth performance parameters included weight gain, average body weight gain, daily gain and survival rate were determined by the following equations:
Body weight gain (BWG) = (W_1) – (W_0).
Where, W_0: means initial weight and W_1: means final weight.
Survival rate (SR %) = Number of fish at final / Number of fish at start x100.

Calculation of feed conversion ratio (FCR)

The feed conversion ratio (FCR) was expressed as the proportion of total dry matter intake (TDMI) in grams to total live body weight gain (TBWG) in grams, calculated using the following equation:

FCR = total dry matter intake, (TDMI), g / total body weight gain (TBWG), g.

Calculation of crude protein efficiency ratio (CPER)

(PER) = Total body weight gain (TBWG) in grams/ total crude protein intake (TCPI) in grams.

The composition and chemical analysis of the experimental diets are shown in Table (2).

Table 2. Composition and chemical analysis of the experimental diets

Ingredient	Experimental diets containing			
	0 % SWBF	10 % SWBF	20 % SWBF	30 % SWBF
	D_1	D_2	D_3	D_4
Composition of the experimental diets				
Fish meal	8	8	16	20
Soybean meal	30	20	10	0
Silk warm by-products feeds (SWBF)	0	10	20	30
Corn gluten	15	21	20	22
Yellow corn	20	22	15	10
Wheat bran	26	18	18	17
Vitamin & mineral mixture*	1	1	1	1
Chemical analysis (%)				
Dry matter (DM)	92.95	92.90	92.84	92.77
Chemical composition on dry matter basis				
Organic matter (OM)	93.40	93.53	91.78	90.75
Crude protein (CP)	34.14	34.17	34.96	35.22
Crude fiber (CF)	4.65	4.49	4.78	5.07
Ether extract (EE)	2.76	3.26	4.19	4.89
Nitrogen free extract (NFE)	51.85	51.61	47.85	45.57
Ash	6.60	6.47	8.22	9.25
Gross energy kcal/ kg DM	4533	4565	4553	4551
Metabolizable energy kcal/ kg DM	357.08	360.45	358.47	357.38
Protein energy ratio (mg CP/ Kcal ME)	95.61	94.80	97.53	98.55

Each kg of vitamin & mineral mixture premix contained Vitamin A: 4.8 millionIU, D3: 0.8 millionIU, E: 4g, K: 0.8g, B1: 0.4g, Riboflavin: 1.6g, B6: 0.6g, B12: 4mg, Pantothenic acid: 4g, Nicotinic acid: 8g, Folic acid: 0.4g, Biotin: 20mg, Mn: 22g, Zn: 22g, Fe: 12g, Cu: 4g, I: 0.4g, Selenium: 0.4g, and Co: 4.8mg.

Gross energy (kcal/ kg DM) was calculated according to method of **Blaxter (1968)**, **MacRae and Lobley (2003)**. Where, each g of CP = 5.65Kcal, g of EE = 9.40kcal and g of CF and NFE = 4.15Kcal.

Metabolizable energy (ME) was calculated using values of 4.50, 8.15 and 3.49Kcal for protein, fat and carbohydrate, respectively. Protein energy ratio (mg CP/ Kcal ME) was calculated according to guidelines of **NRC (2011)**.

Feed efficiency

Generally, the following equation was used in the calculation of the feed efficiency

Feed efficiency (FE %) = [weight gain (g) / feed intake (g)]

Protein productive value (PPV %) = [PR_1– PR_0 / PI] 100.
Where, PR_1 = The total fish body protein at the end of the experiment. (On dry matter basis). PR_0 = The total fish body protein at the start of the experiment. (On dry matter basis) PI = Protein intake.

Energy retention percentages (ER %)

The energy retention percentage was calculated according the following equation: Energy retention (ER %) = E-E_0 / E_F X 100
Where, E= The energy in fish carcass (kcal) at the end of the experiment.
E_0= The energy in fish carcass (kcal) at the start of the experiment.
E_F = The energy (kcal) in feed intake.

Digestibility study

Feces and diets were meticulously collected during the final 14 days of the experimental period. Any uneaten feed was carefully siphoned out of each tank approximately 30 minutes after the final feeding. Feces were separately collected from each replicate aquarium by a siphoning method in the morning. It was filtered, oven dried at 60^oC and kept in airtight containers to estimate the chemical analysis and apparent protein digestibility using the method described in the study of **Furukawa and Tuskahara (1966)**.

Blood measurements

At the end of the feeding trial, 32 fish, eight from each treatment, were captured and anesthetized for blood sampling from the caudal vein. From each aquarium, five fish were weighed and prepared to collect blood samples from caudal vein by heparinized syringes. Then, blood samples were centrifuged at 4000rpm for 20 minutes to prepare blood plasma. The plasma was kept frozen at -18°C for subsequent analysis.

Body constituents of tested group fish

In the begining of the feeding trial, 20 fingerlings of the Nile tilapia fish were used to determine their whole body composition. Meanwhile, at the end of the feeding trial, thirty two fish, eight from each treatment, were randomly chosen also to evaluate their whole body composition, and calculate their values of both energy and protein retention.

Analytical procedures

Chemical analysis of feed ingredients, experimental diets, feces samples of body composition of fish were analyzed according to **AOAC (2016)** methods.

Blood samples were evaluated according to the method described by **Weiss and Wardrop (2010)** that included the red blood cell count (RBC's), white blood cell count (WBC's) and hemoglobin (Hb) concentration. Serum total proteins and albumin were determined according to the methods of **Doumas *et al.* (1971)** and **Henary *et al.* (1974)**, respectively. The activities of aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were determined according to the procedure of **Reitman and Frankel (1957)**. Serum urea, uric acid and creatinine were determined according to the methods of **Patton and Crouch (1977)** and **Houot (1985)**, respectively. On the other hand, globulin and albumin: globulin ratio (A: G ratio) were calculated by difference. Moreover, test kits supplied by bioMérieux-France were used.

Calculated data

According to **Blaxter (1968)** and **MacRae and Lobley (2003)**, the gross energy (kcal/ kg DM) of the basal diet and the body composition of the tested group of fish were calculated to determine the energy retention percentages. This was done using the following values: each gram of crude protein (CP) = 5.65kcal, each gram of ether extract (EE) = 9.40kcal, and each gram of crude fiber (CF) and nitrogen-free extract (NFE) = 4.15kcal.

Statistical analysis

The collected data were subjected to statistical analysis as one way analysis of variance according to **SPSS (2020)**. Duncan's multiple range test **(Duncan, 1955)** was used to separate means when the dietary treatment effect was significant according to the following model: $Y_{ij} = \mu + T_i + e_{ij}$

Where, Y_{ij} = observation

μ = overall mean.

T_i = effect of experimental diets for i = 1–4, 1 = G_1: The Nile tilapia fish received diets containing 0.00% SWBF and assigned as control group, G_2: the Nile tilapia fish received diets containing 10% SWBF, G_3: the Nile tilapia fish received diets containing 20% SWBF, and G_4: the Nile tilapia fish received diets containing 30% SWBF.

e_{ij} = The experimental error.

RESULTS AND DISCUSSION

Chemical composition of feed ingredients

The data of Table (1) reveal that silk warm by-products feeds (SWBF) contained moderate quantity of crude protein (13.18%); it seems close to the crude protein percentages that was found in wheat bran (14.08%), and it was more than what was found in yellow corn (9.15%). Therefore, it can be considered that SWBF is a suitable alternative cheap source of crude protein. Furthermore, SWBF was considered a suitable source of energy, providing 4351 kcal/ kg DM. **Srikanth (1986)** reported that the dead silk worm pupae and moths could also be used as fish feed. Meanwhile, **Blair (2008)** noticed that deoiled silk worm pupae have higher protein content, making them suitable as a dietary protein source for fish.

The experimental diets (composition and chemical analysis)

The data in Table (2) elucidate that the diets were formulated to cover fish crude protein percentages ranging from 34.14 to 35.22% among the four experimental diets. Gross energy (GE) ranged between 4533- 4565, and metabolizable energy (ME) ranged between 357.08- 360.45. On the other hand, protein energy ratio ranged from 94.80 to 98.55mg CP/ Kcal ME among the four tested diets.

Water quality

The data obtained in Table (3) generally show that the recorded values of temperature, dissolved oxygen (DO), pH, ammonia (NH_3), and nitrite (NO_2) are considered suitable for fingerlings. The present results of ammonia (NH_3) are in harmony with the results noted by **El-Sayed (2006)** and the results obtained by **Abdelhamid *et al.* (2011a, b)** for pH and DO. Additionally, our results coincide with those of **Stickney (1979)** concerning DO and pH. Moreover, our results agree with those of **Eid *et al.* (2021)** regarding water temperature, pH, and DO. In this respect, **Abdel-Hakem *et al*. (2009)** and **El-Dakar *et al.* (2023)** carried out an experiment to study the impact of feeding regimes on growth performance and recorded that water quality was maintained at quite acceptable levels throughout the experiment.

Table 3. Water quality of the different experimental aquariums of mono sex *Oreochromis niloticus* fingerlings for 84 days

Item	Range	Average ± SD
Water Temperature °C	27.5 - 29.4	28.45 ±1.40
Dissolved oxygen (DO)	5.8 – 6.0	5.90 ±0.21
Hydrogen ion concentration (pH)	7.3 - 7.6	7.45 ±0.28
Ammonia (NH3)	0.22 - 0.24	0.23 ±0.002
Nitrites (NO2)	0.03 - 0.05	0.04 ±.001

Growth performance and survival ratio of different experimental groups

The presented results in Table (4) show that SWBF at different levels (10, 20 and 30%) caused an improvement in their contents. Moreover, values of survival ratio, number of dead fish and mortality rate percentages improved. The best values of FW, TBWG, ADG, survival ratio, number of dead fish and mortality rate percentages were recorded with the group of fish that fed on 10% of SWBF containing diet (D_2). **Boscolo *et al.* (2001)** used 168 tilapia fingerlings (Thai strain)and divided them into five treatments and noted that the average final weight was higher and feed intake and survival rate did not differ among the treatments. **Hardy (2010**) concluded that it is not necessary to include the evaluated feeds, at a level of 5%, to determine feed intake or to promote the Nile tilapia fingerlings growth. **Lavefve *et al.* (2019)** noted that polyphenols and their metabolites, once in the gut, can modulate the microorganism populations. **Rahman *et al.* (1996)** noticed that incorporated silk worm pupae meal improved their growth and reduced its cost of feed. **Keshavappa (1988)** reported that silk worm pupae meal at 30% provided a significant change in growth. **Joshi *et al.* (1980)** found that the replacement of fishmeal with silk worm pupae meal did not produce any adverse effect on productive performance. **Cho (2010)** mentioned that shellfish species can be successfully reared with silk worm pupae meal.

Effect of inclusion SWBF on feed utilization and digestibility

The values of feed utilization of experimental groups that are shown in Table (5) clarify the inclusion of 10% SWBF containing diet (D_2) in the Nile tilapia. The *O. niloticus* achieved a significant increase in the intake matter intake (41.28) compared to the others groups that recorded 38.15, 39.18 and 38.39g for D_1, D_3 and D_4, respectively. Generally, the values of feed conversion ratio (FCR) were improved with the inclusion of SWBF in the diet compared to the control (D_1). The best FCR was recorded when fish fed diet containing 10% SWBF (FCR, 1.156g DM intake/ g gain), meanwhile the values of FCR were 1.320, 1.188 and 1.196g DM intake/ g gain for D_1, D_3 and D_4, respectively. On the other hand, values of crude protein intake (CPI) were significantly ($P< 0.05$) increased with incorporated SWBF in fish diet. In addition, the values of PER were improved with the inclusion of SWBF in diet. The best values of CPI and PER were recorded for the fish group fed on 10% SWBF (D_2) in comparison with the other groups. Moreover, the present results (Table 5) show that values of apparent protein digestibility (APD) were significantly (P< 0.05) increased after introducing the diet with SWBF. The best value of APD was recorded by fish group fed a 10% SWBF in comparison with the other groups. **Hossain *et al.* (1992)** evaluated the protein digestibility in silk worm pupae meal (85.74%), and deoiled silk worm pupae meal (84.95%). Furthermore, in another study, **Hossain *et al.* (1997)** noticed a difference between the apparent protein digestibility (83.04%), true protein digestibility (86.87%) and the apparent lipid digestibility (91.21%) in silk worm pupae meal compared to fish meal diet.

Table 4. Effect of incorporation SWBF on growth performance and survival ratio of mono sex *Oreochromis niloticus* fingerlings reared in the different experimental aquariums for 84 days

Parameter	**Experimental groups containing**					
	0 % SWBF	**10 % SWBF**	**20 % SWBF**	**30 % SWBF**		**Sign.**
	D_1	**D_2**	**D_3**	**D_4**	**SEM**	**P<0.05**
Initial weight, g (IW)	2.70	2.50	2.60	2.60	0.041	NS
Final weight, g (FW)	31.60^{c}	38.30^{a}	35.58^{ab}	34.70^{b}	0.746	*
Total body weight gain, g (TBWG)	28.90^{c}	35.80^{a}	32.98^{ab}	32.10^{b}	0.759	*
Daily gain, g (DG)	0.34^{c}	0.43^{a}	0.39^{b}	0.38^{b}	0.010	*
Number of fish at the starter	40	40	40	40	-	-
Number of fish at the end	35	38	37	36	-	-
Survival ratio	87.50%	95.00%	92.50%	90.00%	-	-
Number of dead fish	5/40	2/40	3/40	4/40	-	-
Mortality rate percentages	12.50%	5.00%	7.50%	10.00%	-	-

a, b and c: Means in the same row having different superscripts differ significantly (P< 0.05).
**: Significant. NS: Not.*
SWBF: Silk warm by-products feeds

Table 5. Effect of incorporation SWBF on feed utilization and digestibility of mono sex *Oreochromis niloticus* fingerlings reared in the different experimental aquariums for 84 days

Parameter	**Experimental groups containing**					
	0 % SWBF	**10 % SWBF**	**20 % SWBF**	**30 % SWBF**		**Sign.**
	D_1	**D_2**	**D_3**	**D_4**	**SEM**	***P*<0.05**
TBWG	28.90^{c}	35.80^{a}	32.98^{ab}	32.10^{b}	0.759	*
Feed intake (FI), g	38.15^{b}	41.28^{a}	39.18^{ab}	38.39^{b}	0.476	*
Feed conversion ratio (FCR)	1.320^{b}	1.156^{a}	1.188^{a}	1.196^{a}	0.017	*
Crude protein %	34.14	34.17	34.96	35.22	-	-
Crude protein intake (CPI), g	13.02^{d}	14.11^{a}	13.70^{b}	13.52^{c}	0.101	*
Protein efficiency ratio (PER)	2.22^{c}	2.54^{a}	2.41^{b}	2.37^{b}	0.031	*
Apparent protein digestibility (APD)	76.12^{d}	76.56^{a}	76.39^{b}	76.28^{c}	0.042	*

a, b, c and d: Means in the same row having different superscripts differ significantly (P< 0.05).
SWBF: Silk warm by-products feeds

Effect of inclusion SWBF on blood parameters

The results of blood parameters (Table 6) showed values of total protein, hemoglobin (Hb) concentration, white blood cell count (WBC's), uric acid and createnine. However, other parameters including albumin, globulin, albumin: globulin ratio, red blood cell count (RBC's), ASAT, ALAT, and urea were significantly (P< 0.05) affected by the incorporation of SWBF in fish diets. The values of ASAT, ALAT and urea were decreased for fish fed diets containing different levels of SWBF (D_2, D_3, and D_4) in comparison with the control one (D_1). **Khalaf-Allah *et al.* (2015)** noticed that ASAT, ALAT, and GGT enzyme activities showed in significant decrease.

Body composition of different experimental groups

As shown in Table (7), treatments had no effect on ether extract content of body composition among the four tested experimental groups, meanwhile the other values were affected when SWBF was incorporated in the diets. The values of gross energy kcal/ kg were not significantly affected (P< 0.05) among the different groups, and these values varied from 5800 to 5809kcal/ kg. The highest value of CP content (66.21%) was found in the group of fish fed on 20% of SWBF containing diet (D_3). The present results match those of **El-Dakar *et al*. (2023)**, who also observed differences in lipid and gross energy content in fish body composition. Additionally, the previous authors noted that no significant differences among the treatments (P> 0.05) in both low and high stocking density. On the other hand, **Abo-State *et al.* (2021)** showed no statistical differences recorded in the whole body moisture, ether extracts, and ash content.

Table 6. Physiological parameters of mono sex *Oreochromis niloticus* fingerlings reared in the different experimental aquariums for 84 days

Item	Experimental diets containing				SEM	Sign. P<0.05
	0 % SWBF D_1	10 % SWBF D_2	20 % SWBF D_3	30 % SWBF D_4		
Total protein (g/ dl)	4.22	4.21	4.20	4.19	0.006	NS
Albumin (g/ dl)	2.18^{ab}	2.15^{b}	2.20^{a}	2.16^{b}	0.007	*
Globulin (g/ dl)	2.04^{ab}	2.06^{a}	2.00^{c}	2.03^{b}	0.007	*
Albumin: globulin ratio	1.07^{b}	1.04^{c}	1.10^{a}	1.06^{bc}	0.007	*
Hb concentration (g/dl)[1]	11.50^{6}	11.60	11.58	11.57	0.008	NS
RBC's cell count (x106/µl) [2]	1.55^{b}	1.57^{ab}	1.59^{a}	1.60^{a}	0.007	*
WBC's cell count (x106/µl) [3]	4.76	4.74	4.75	4.73	0.007	NS
<u>*Liver function*</u>						
AST (U/l)[4]	21.86^{a}	21.80^{ab}	21.78^{b}	21.82^{ab}	0.012	*
ALT (U/l)[5]	9.45^{ab}	9.40^{b}	9.43^{ab}	9.46^{a}	0.009	*
<u>*Kidneys function*</u>						
Urea mg/dl	4.28^{a}	4.26^{ab}	4.24^{ab}	4.22^{ab}	0.009	*
Uric acid mg/dl	1.83	1.81	1.80	1.82	0.006	NS
Createnine mg/dl	0.336	0.334	0.332	0.335	0.007	NS

a, b and c: Means in the same row having different superscripts differ significantly (P< 0.05).
SWBF: Silk warm by-products feeds.
[1]Hb: Hemoglobin concentration.

Table 7. Body composition of mono sex *Oreochromis niloticus* fingerlings reared in the different experimental aquariums for 84 days

Item	Experimental diets containing				SEM	Sign. *P*<0.05
	0 % SWBF	10 % SWBF	20 % SWBF	30 % SWBF		
	D_1	D_2	D_3	D_4		
Moisture	74.13^b	74.21^{ab}	74.29^a	74.18^b	0.022	*
Dry matter (DM)	25.87^a	25.79^{ab}	25.71^b	25.82^a	0.022	*
Chemical analysis on DM basis						
Organic matter (OM)	88.15^b	88.08^c	88.21^a	88.19^{ab}	0.017	*
Crude protein (CP)	66.17^{ab}	66.12^b	66.21^a	66.15^{ab}	0.014	*
Ether extract (EE)	21.98	21.96	22.00	22.04	0.019	NS
Ash	11.85^b	11.92^a	11.79^c	11.81^{bc}	0.017	*
Gross energy kcal/ kg	5805	5800	5809	5809	1.389	NS
Gross energy cal/ g DM	5.805	5.800	5.809	5.809	0.002	NS

a, b and c: Means in the same row having different superscripts differ significantly (P< 0.05).
SWBF: Silk warm by-products feeds.

Effect of inclusion SWBF on energy retention and protein productive value

The presented data of Table (8) show an improvement in the value of energy retention percentages (ER%), with the incorporation of SWBF at different levels. The corresponding values of ER% were 110.19, 107.39 and 106.73 in D_2, D_3 and D_4, respectively, compared to the control ($D_{1)}$ that recorded 97.02%. Moreover, the values of protein productive value improved the PPV% in comparison with the control. The corresponding values of PPV% were 167.75, 159.42 and 157.03% for the three experimental groups G_2, G_3 and G_4, respectively, in comparison with the control group that received diet which did not contain SWBF and recorded a PPV value of 146.85%. Superior values of energy and protein productive value retention percentages were recorded by the group of fish that received a 10% of SWBF containing diet (D_2). Our results concur with those of **El-Nadi *et al*. (2022)**, who found improvements in values of ER % upon adding jojoba oil at zero, 0.05, and 0.10%. Moreover, the values of PPV% were also improved.

Morphometric assessment of intestinal villi absorptive capacity

The data on the intestinal villi absorptive capacity presented in Table (9) indicate that the values of mucosal length and villi length of the duodenum, ileum, and rectum increased with SWBF inclusion in the fish diets. **Krogdahl *et al*. (2000**) and **Hartviksen (2015)** noted that, the use of raw materials of non- marine origin may modulate the intestinal morphology and possibly compromise the barrier function. Therefore, it is important to monitor the effect of the alternative raw materials on the mucosal morphology.

Table 8. Effect of incorporation SWBF on energy retention and protein productive value of mono sex *Oreochromis niloticus* fingerlings reared in the different experimental aquariums for 84 days

Item	Experimental diets containing				SEM	Sign. $P< 0.05$
	0 % SWBF	10 % SWBF	20 % SWBF	30 % SWBF		
	D_1	D_2	D_3	D_4		
Initial weight (IW), g	2.70	2.50	2.60	2.60	0.041	NS
Final weight (FW), g	31.60^{c}	38.30^{a}	35.58^{ab}	34.70^{b}	0.746	*
Percentages of energy in body fish	5.805	5.800	5.809	5.809	0.002	NS
Energy at the end in body fish (E)	183.44^{d}	222.14^{a}	206.68^{b}	201.57^{b}	4.167	*
Energy at the start in body fish (E_0)	15.67^{a}	14.50^{c}	15.10^{b}	15.10^{b}	0.126	*
Energy retained in body fish ($E-E_0$)	167.77^{d}	207.64^{a}	191.58^{b}	186.47^{c}	4.290	*
Energy of the feed (Cal / g feed)	4.533	4.565	4.553	4.551	-	-
Quantity of feed intake	38.15^{b}	41.28^{a}	39.18^{ab}	38.39^{b}	0.476	*
Total energy feed (EF)	172.93^{d}	188.44^{a}	178.39^{b}	174.71^{c}	1.808	*
Energy retention (ER) %	97.02^{d}	110.19^{a}	107.39^{b}	106.73^{c}	1.499	*
Percentages of crude protein in body fish	66.17^{ab}	66.12^{b}	66.21^{a}	66.15^{ab}	0.014	*
Total protein at the end in body fish (PR_1)	20.91^{d}	25.32^{a}	23.56^{b}	22.95^{c}	0.476	*
Total protein at the start in body fish (PR_2)	1.79^{a}	1.65^{b}	1.72^{ab}	$1.72a^{b}$	0.019	*
Protein Energy retained in body fish (PR_3) = ($PR_1 - PR_2$)	19.12^{d}	23.67^{a}	21.84^{b}	21.23^{c}	0.491	*
Crude protein in feed (CP%)	34.14	34.17	34.96	35.22	-	-
Total Protein intake (PI), g	13.02^{d}	14.11^{a}	13.70^{b}	13.52^{c}	0.119	*
Protein productive value (PPV) %	146.85^{d}	167.75^{a}	159.42^{b}	157.03^{c}	2.265	*

a, b, c and d: Means in the same row having different superscripts differ significantly ($P< 0.05$).
SWBF: Silk warm by-products feeds.

Table 9. Morphometric assessment of intestinal villi absorptive capacity of mono sex *Oreochromis niloticus* fingerlings reared in the different experimental aquariums for 84 days

Item		Experimental diets containing				SEM	Sign. $P< 0.05$
		0 % SWBF	10 % SWBF	20 % SWBF	30 % SWBF		
		D_1	D_2	D_3	D_4		
Duodenum	Mucosal length	408^{d}	495^{c}	550^{b}	588^{a}	20.65	*
	Villi length (um)	325^{d}	425^{c}	462^{b}	492^{a}	19.05	*
Ileum	Mucosal length	466^{d}	618^{c}	654^{b}	691^{a}	25.83	*
	Villi length (um)	383^{d}	475^{c}	570^{b}	607^{a}	26.36	*
Rectum	Mucosal length	301^{d}	323^{c}	411^{b}	517^{a}	25.73	*
	Villi length (um)	203^{d}	209^{c}	339^{b}	365^{a}	22.25	*

a, b, c and d: Means in the same row having different superscripts differ significantly ($P<0.05$).
SWBF: Silk Warm by-Products Feeds.

CONCLUSION

Based on the current findings, it is clear that incorporating 10% silk warm by-products can be successful as an unconventional source for rearing *Oreochromis niloticus* fingerlings. This incorporation does not have any adverse effects on their performance, apparent protein digestibility, blood constituents, body composition, energy retention percentages, protein productive value (PPV), and intestinal villi absorptive capacity.

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