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Evaluation of Pell-Gel-Dry® as a Feed Binder and Growth Promoter Agent for the Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758)

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ABSTRACT

Aqua-feed binders are one of the most important agents in commercial fish feeds. They help to bind all the ingredients together, which leads to better digestion and absorption of nutrients by fish. The present study was conducted over 90 days (as an *in vivo* experiment) to evaluate the dietary addition of Pell-Gel-Dry® as a growth promoter for the Nile tilapia, *Oreochromis niloticus*. In two separate experiments, fish were fed two different diets (floating and sinking); each diet was bounded by the graded levels (0, 200, 300, and 400g/ ton of Pell-Gel-Dry®). Thus, a total of 2000 *O. niloticus* fingerlings were randomly distributed into four treatments in each diet and fed daily at a rate of 4% of their live body weight. Before the beginning of the *in vivo* experiment, the *in vitro* experiment was conducted to determine the water stability percentage nb for the above-tested diets. The obtained results showed that fish fed the floating and sinking diets bounded with the graded levels of Pell-Gel-Dry® revealed the highest growth performance, feed utilization, chemical composition of the fish body and muscles, economic efficiency parameters, and water stability than those fed the control diet, especially at levels of 400 and 300g/ ton of Pell-Gel-Dry®, respectively. Thus, it could be concluded that the useful use of Pell-Gel-Dry® at these levels demonstrated a promising growth promoter and feed binder agent for *O. niloticus* with predictable sustainability and economic properties for fish farmers.

INTRODUCTION

Aquaculture requires an optimization of nutrition to efficiently raise fish for food production. Resources for a successful livestock industry mainly include the provision of ample feed for the animals. Feed procurement accounts for about 70% of the production costs **(Calicioglu *et al*., 2019)**. Due to human population growth, global demand for animal protein has significantly increased, meaning that the food supply must increase by 25- 70% in 2050 **(Hunter *et al*., 2017)**. Aquaculture is a critical industry for meeting increased protein demand and maintaining future global food security. Furthermore, aquaculture is expected to grow its share of global fish production from 46% currently to 53% in 2030 **(FAO, 2020)**. However, there are many issues related to fish nutrition that need to be considered to achieve balance in food production and sustainability. The sustainability of the aquaculture industry is an environmental, economic, and social concern **(Hixson, 2014)**. On the other hand, in the past few years, the negative effects of climate change on profitability and aquaculture operations are expected to be impacted in terms of productivity and agricultural procedures **(Islam *et al*., 2022)**.

Nutrient requirements are recommended for feed formulations. Formulating cost-effective feeds that meet the essential amino acid requirements of fish and shrimp can represent a challenge. Aqua feeds are different from other livestock feeds due to the wide variability of their composition in terms of digestible protein, lipids, carbohydrates, and digestible energy contents **(Encarnação *et al*., 2004; Bureau & Encarnação, 2006)**. The impact of diet composition on essential amino acids (EAAs) utilization and requirements of fish and shrimp has been the focus of a limited number of studies, and thus remains poorly understood and controversial **(Encarnação *et al*., 2006)**. Significant differences in opinion exist as to how EAAs requirement data should be expressed, and EAAs levels deemed adequate in feed formulations should be calculated **(Bureau & Encarnação, 2006)**. This situation limits the ability to review and interpret information on EAAs requirements of fish and make recommendations that are widely applicable to practical conditions, for example, the wide variability in protein and digestible energy levels to which commercial feeds for a given species are formulated **(Encarnação *et al*., 2004, 2006; Bureau & Encarnação, 2006)**.

Binders are substances used in diets to improve their palatability, enhance their durability, preserve their physical form during storage, and preserve water stability **(Paolucci *et al*., 2012)**. Binders are incorporated into pelleted aqua feeds to improve stability in water, increase pellet firmness, and reduce the amount of fines produced during processing and handling **(Zaabwe *et al*., 2020)**. Numerous natural binders have been employed to produce firm pellets to increase their stability in water and reduce nutrient loss **(Orire & Emine, 2019)**. Any binder chosen for fish feed pellets should have water stability for a minimum period of 3 hours. The binder, apart from enhancing palatability, must also serve as a source of carbohydrates in the feed. A binder should be inexpensive and easily available **(Yadav *et al*., 2019)**. These binding agents are particularly important in shrimp and tilapia feeds due to the feeding habits of those organisms, which typically result in the feed remaining in water for a relatively long period before consumption. Among the most widely used binders are sodium and calcium bentonites, lignosulfonates, hemicellulose, carboxymethylcellulose, alginate, and guar gum **(Hardy & Barrows, 2003)**. Cereal grains contain starch that, when gelatinized, gives a durable, water-stable pellet. Hence, formulations pelleted by extrusion processing do not require pellet binders; gelatinized starch provides sufficient binding capacity **(Glencross *et al*., 2012)**. Certain feed ingredients, such as whey, wheat gluten, pregelatinized starches, and molasses, are considered nutritional binders in that they improve pellet stability and also contribute to nutritional value **(Ma *et al*., 2021)**. Other binders, including those composed of clays or cellulosic materials, are considered to be inert and have limited or no nutritional value. Such binding agents tend to increase the density of agglomerated particles produced by compression pelleting **(Olugbade *et al*., 2019)**. Thus, the present study was conducted to evaluate the effect of dietary graded levels of Pell-Gel-Dry® as a novel commercial feed binder on growth performance, feed utilization, chemical composition, and economic efficiency of the Nile tilapia, *Oreochromis niloticus* fingerlings for 90 days as an *in vivo* experiment, along with the *in vitro* assessment of its effects on the stability percentage of floating and sinking diets in the aqueous media.

MATERIALS AND METHODS

The current study was conducted from 11th August to 11th November 2022 for 90 days. This study was conducted in two stages. The first stage was an *in vitro* experiment, and the second stage was an *in vivo* experiment for 90 days.

1. *In vitro* experiment

The *in vitro* experiment was conducted in the Fish Research Laboratory, Animal Production Department, Faculty of Agriculture, Mansoura University, Al-Mansoura, Egypt. The purpose of this experiment was to evaluate the water stability percentage of different types of diets (floating and sinking) bounded by graded levels (0.0, 200, 300, and 400g/ ton) of Pell-Gel-Dry® as a novel feed binder agent. The samples were placed in glass cups filled with 500 cubic centimeters of tap water (three replicates for each treatment), and their stability (non-disintegration) was observed for periods of 20 and 30 minutes. The stability percentage was calculated from the following equation:

The stability (%) = (number of feed pellets dismantled / total number of feed pellets used) × 100

2. *In vivo* experiment

The present work was conducted in a private fish farm in Kafr El-Sheikh Governorate, Egypt, during the summer season of 2022 to evaluate the effect of Pell-Gel-Dry® as a novel growth promoter for *O. niloticus* for 90 days.

2.1. Experimental diets

A basal diet was formulated from commercial ingredients including soybean meal, fish meal, yellow corn, wheat bran red, rice bran, protein meal, oil, and some additives such as salt and vitamins and minerals premix. The composition of feed ingredients and chemical analysis of the experimental diets are presented in Table (1). The contents of the amino acids in the experimental diets are shown in Table (2). The tested product (Pell-Gel-Dry®) was produced by the ATCO Company in the Middle East and Africa (as a cooperation between Egypt and Italy). Pell-Gel-Dry® is the first feed additive of the ATCO supplemented with AAs. The composition of Pell-Gel-Dry® is granular gelatin and each 1.0kg contains some EAAs and nonessential AAs, as shown in Table (3). The fine dietary ingredients were divided into two equal parts. Then, Pell-Gel-Dry® was added to each dietary ingredient at different levels (0.0, 200, 300, and 400g/ ton) and harmoniously mixed with the fine ingredients. The first part of the experimental diet was manufactured using the extruder technique in the El-Doaa fish feed factory, Industrial Zone, Mutobas City, Kafr El-Sheikh Governorate, Egypt, to get the floating diet. While, another amount of ingredients was pelleted as a sinking experimental diet in the same factory.

Table 1. Ingredients and chemical composition of the experimental diets

Ingredient	Floating diet (kg/ton diet)				Sinking diet (kg/ton diet)			
	0	200	300	400	0	200	300	400
Soybean meal	420	420	420	420	420	420	420	420
Fish meal	15	15	15	15	15	15	15	15
Yellow corn	120	120	120	120	120	120	120	120
Wheat bran red	175	175	175	175	175	175	175	175
Rice bran	163	163	163	163	163	163	163	163
Protein meal	86	86	86	86	86	86	86	86
Soy oil	4	4	4	4	4	4	4	4
Salt	4	4	4	4	4	4	4	4
Limestone	6.2	6	5.9	5.8	6.2	6	5.9	5.8
Vits. & Mins.	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vit. C	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sodium butyrate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Mono calcium	4.	4.	4.0	4.0	4.0	4.0	4.0	4.0
Pell-Gel-Dry®	0.0	0.2	0.3	0.4	0.0	0.2	0.3	0.4
Chemical analysis (%)								
Moisture (%)	8.00	8.16	8.17	8.30	9.30	9.16	9.60	9.10
Protein (%)	30.05	30.14	30.16	30.00	30.23	30.20	30.30	30.36
Ether extract (EE, %)	5.30	5.24	5.51	5.29	5.84	5.61	5.8	5.72
Ash (%)	9.10	9.13	9.27	8.95	9.19	9.27	9.26	9.13
Crude fiber (%)	4.38	4.38	4.30	4.49	4.22	4.24	4.48	4.44
NFE[1]	51.17	51.11	50.77	51.40	50.5	50.67	50.00	50.35
Gross energy (MJ/100 g DM)[2]	1817.0	1815.7	1820.9	1819.4	1830.9	1824.1	1822.7	1826.9

[1]Nitrogen-free extract (NFE) = 100 - (protein + EE + ash + fiber)

[2]Gross energy (MJ/ 100g DM) was calculated by multiplication of the factors 17.11, 23.54, and 39.54MJ GE/ kg DM for carbohydrate, protein, and EE, respectively (**NRC, 2011**).

Table 2. Amino acids-typical analysis of the experimental diets

Amino acid (%)	Floating diet				Sinking diet			
	0	200	300	400	0	200	300	400
Aspartic (ASP)	3.05	3.16	2.52	2.99	3.12	3.16	2.63	2.44
Therionine (THR)	1.19	1.21	0.96	1.18	1.23	1.20	0.98	0.93
Serine (SER)	1.55	1.55	1.26	1.58	1.64	1.55	1.25	1.18
Glutanic (GLU)	5.43	5.34	4.48	5.26	5.25	5.23	4.47	4.30
Glycine (GLY)	1.62	1.55	1.32	1.50	1.60	1.67	1.37	1.32
Alanine (ALA)	1.51	1.46	1.27	1.40	1.47	1.52	1.32	1.26
Valine (VAL)	1.56	1.56	1.37	1.54	1.54	1.53	1.28	1.29
Isoleucine (ISO)	1.29	1.32	1.11	1.31	1.27	1.29	1.13	1.13
Leucine (LEU)	2.35	2.29	1.97	2.27	2.29	2.35	2.05	1.93
Tyrosine (TYR)	0.94	1.08	0.31	1.06	1.06	0.57	0.20	0.72
Phenylalanine (PHE)	1.55	1.54	1.30	1.49	1.50	1.51	1.36	1.29
Hisitidine (HIS)	0.74	0.72	0.60	0.71	0.74	0.73	0.61	0.60
Lycine (LYC)	1.70	1.65	1.37	1.61	1.68	1.70	1.42	1.36
Argnine (ARG)	2.09	2.09	1.52	2.07	2.17	2.04	1.59	1.69
Proline (PRO)	1.85	1.79	1.50	1.74	1.74	1.91	1.52	1.52
Cystine (CYS)	0.69	0.72	0.59	0.49	0.64	0.70	0.83	0.53
Methionine (MET)	0.53	0.50	0.45	0.51	0.57	0.50	0.47	0.35

Table 3. Amino acids-typical analysis of Pell-Gel-Dry®

Amino acid	%	Amino acid	%
Aspartic Acid	6.59	Methionine	0.39
Threonine	2.02	Isoleucine	1.70
Serine	3.61	Leucine	1.80
Glutamic	10.99	Tyrosine	0.14
Proline	15.76	Phenylalanine	3.64
Glycine	26.51	Histidine	0.36
Alanine	10.00	Lysine	4.25
Cystine	0.70	Arginine	8.77
Valine	2.76		

2.2. Fish culture system

Fish were randomly distributed to two experiments, each containing eight hapas (a net enclosure measuring 3×6×1m in width, length, and depth, respectively) suspended in an earth pond of 4200m^3. The fish were stocked at a density of 7 fish/ m^3 (with a total of 125 fish/ hapa). *O. niloticus* fingerlings were separately fed with the floating diet (as a first experiment) and the sinking diet (as a second experiment), which were supplemented with different levels (0.0, 200, 300, and 400g/ ton) of Pell-Gel-Dry® of each type of the experimental diet. The fish were fed with the experimental diets containing 30% CP and 3800kcal/ 100g DM as gross energy at a feeding rate of 4% of fish total biomass in each hapa (six days a week). The fish were fed manually with the experimental diets two times a day (8.00 a.m. and 2.00 p.m.). The amounts of each diet were adjusted at approximately 14-day intervals in response to the fish weight gain. At the beginning of the experiment, thirteen fish were randomly taken and kept frozen at -20°C for chemical analysis. The water exchange rate was 15% of the total pond per day.

2.3. Water quality parameters

The water temperature, pH, and dissolved oxygen (DO) were measured using a thermometer, a portable digital pH meter (ADWA Model AD32, Romania), and a waterproof portable DO meter (HANNA, Model HI 9147, Romania), respectively. The total ammonia nitrogen (TAN), nitrite (NO_2), and nitrate (NO_3) were measured by the Hanna HI Model 97715, Romania, and salinity was measured by a portable refractometer (RFT-PSI 0~100‰ salinity 1‰, China), and total dissolved solids (TDS) was measured by the ADWA Model AD32, Romania.

All the above water quality parameters were weekly measured during the experimental period. The average of these water quality parameters during the experimental period was 28.0– 29.6°C for water temperature, 8.0– 8.6 for pH, 6.0– 6.4mg/ L for DO, 0.34– 0.78mg/ L for TAN, 0.24– 0.27mg/ L for NO_2, 0.38– 0.46mg/ L for NO_3, 2.1– 2.58g/ L for salinity, and 140.0– 147.4g/ L for TDS. All water quality parameters measured in the present study are within the acceptable limits for rearing *O. niloticus* fingerlings, according to **Boyd and Tucker (1998)**.

2.4. Growth performance and feed efficiency parameters

The average total weight gain (ATWG), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV), and energy utilization (EU) of the experimental fish were calculated according to the method of **Lovell (2001)**, as shown in the following equations:

- ATWG (g) = Average final weight (g) – Average initial weight (g).
- ADG (g/fish/day) = [ATWG (g) / experimental period (day)].
- SGR (%/day) = [Ln final body weight – Ln initial body weight] × 100 / experimental period (day).
- FCR = Feed Intake (g) / live weight gain (g).
- PER = Live weight gain (g) / protein intake (g).
- PPV (%) = 100 × [Final fish body protein content (g) – Initial fish body protein content (g)] / crude protein intake (g).
- EU (%) = [Retained energy / consumed feed energy] × 100.

2.5. Proximate analysis

The dry matter (DM), crude protein (CP), ether extract (EE), crude fiber, and ash contents of the tested feed ingredients and the whole body of fish at the beginning and end of the experiment, and the fish muscles at the end of the experiment were performed according to the guidelines of **AOAC (2016)**, using a device (NIR, FOSS, DS 2500F, Danmark).

2.6. Indicators of the economic efficiency

At the end of the experiment, the total yield of *O. niloticus* in each treatment was estimated to calculate the total fish production and the total return for each treatment, as well as the total costs, which were calculated according to the following equations:

- Total costs (LE/treatment) = total feed costs + total fingerlings price
- Total outputs (LE/treatment) = fish price × total fish production
- Net return (LE/treatment) = total outputs – total cost
- Economic efficiency per treatment (%) = (net return / total costs) × 100

Where,

The price of feed was 13.70LE/ kg, the price of Pell-Gel-Dry® was 200LE/ kg; and the selling price for *O. niloticus* was 32.00LE/ kg. All these prices are according to the Egyptian market at the time of the experiment in 2022.

3. Statistical analysis

All data were statistically analyzed by one-way ANOVA using SAS™ software for Windows version 9.1.3 **(SAS, 2006)**, and were statistically compared for the significance at $P \leq 0.05$.

RESULTS

1. Stability percentage of the sinking diet

The data in Fig. (1) illustrate the stability percentage of the sinking diet supplemented with different levels of Pell-Gel-Dry® after 20 and 30 minutes in the aqueous medium. After 20 and 30 minutes, the highest stability ($P \leq 0.05$) was recorded in the sinking diet bounded with 400, and 300g/ ton of Pell-Gel-Dry®, respectively. Generally, from the obtained results, it could be noticed that, the stability of the sinking diet was severely affected by increasing the staying time of the diet in the aqueous medium. On the other hand, the floating diet bounded with the graded levels of Pell-Gel-Dry® achieved the highest stability percentage (100%) for all dietary treatments in the aqueous medium, which continued for 5 hours without negative effects on the stability percentage.

2. Growth performance parameters

2.1. Average total weight gain (ATWG) of fish

Data in Fig. (2A) display the ATWG (g) of *O. niloticus* fingerlings fed with the floating diet supplemented with different levels of Pell-Gel-Dry® at the different interval times of the experiment. No significant ($P \geq 0.05$) differences were observed in ATWG of fish among all different levels of Pell-Gel-Dry® at the first 30 days of the experiment. While, after 60 and 90 days of the experiment, the significant ($P \leq 0.05$) differences between the treatments appeared clearly. Moreover, ATWG significantly increased in fish fed with 400g of Pell-Gel-Dry® ton compared to all other levels.

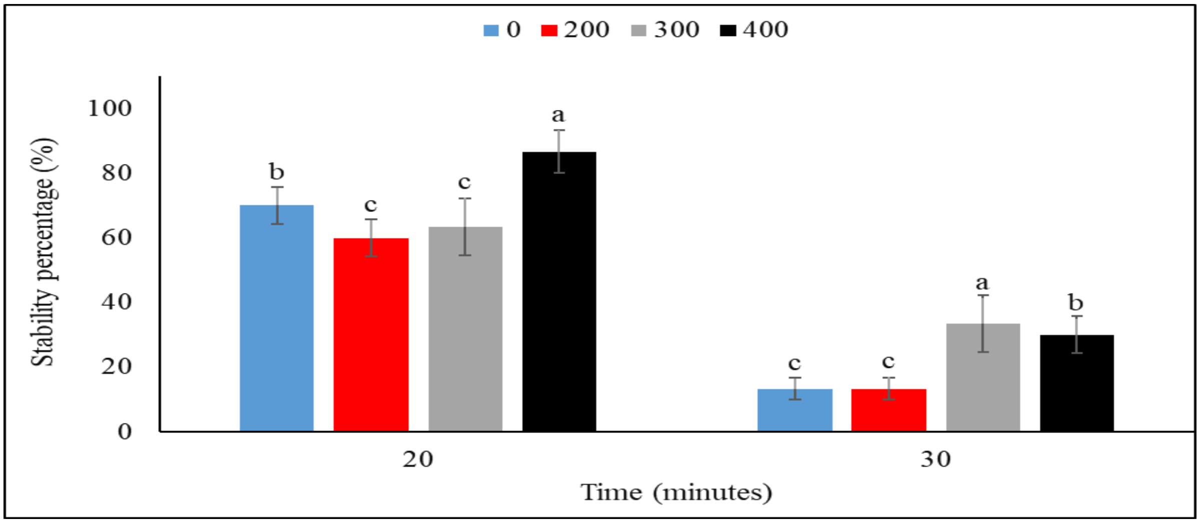

Fig. 1. The stability percentage of the sinking diet bounded with graded levels of Pell-Gel-Dry®

Regarding *O. niloticus* fed with the sinking diet supplemented with different levels (0.0, 200, 300, and 400g/ ton) of Pell-Gel-Dry® at different interval times (30, 60, and 90 days), their ATWG is shown in Fig. (2B). After 30 days, no significant ($P \geq 0.05$) differences were detected in the ATWG of fish among all additional levels of Pell-Gel-Dry® to the sinking diet. Meanwhile, after 60 days, the ATWG of fish fed with a sinking diet supplemented with 300g/ ton of Pell-Gel-Dry® significantly ($P \leq 0.05$) increased among other levels. After 90 days, no clear trend among the addition levels was observed; however, fish fed a sinking diet supplemented with 300g/ ton of Pell-Gel-Dry® showed the highest ($P \leq 0.05$) increase of ATWG than those fed with 400g/ ton, and with non-significant ($P \geq 0.05$) differences with those fed with 200g/ ton or those fed with free Pell-Gel-Dry® supplemented with the sinking diet (as a control group).

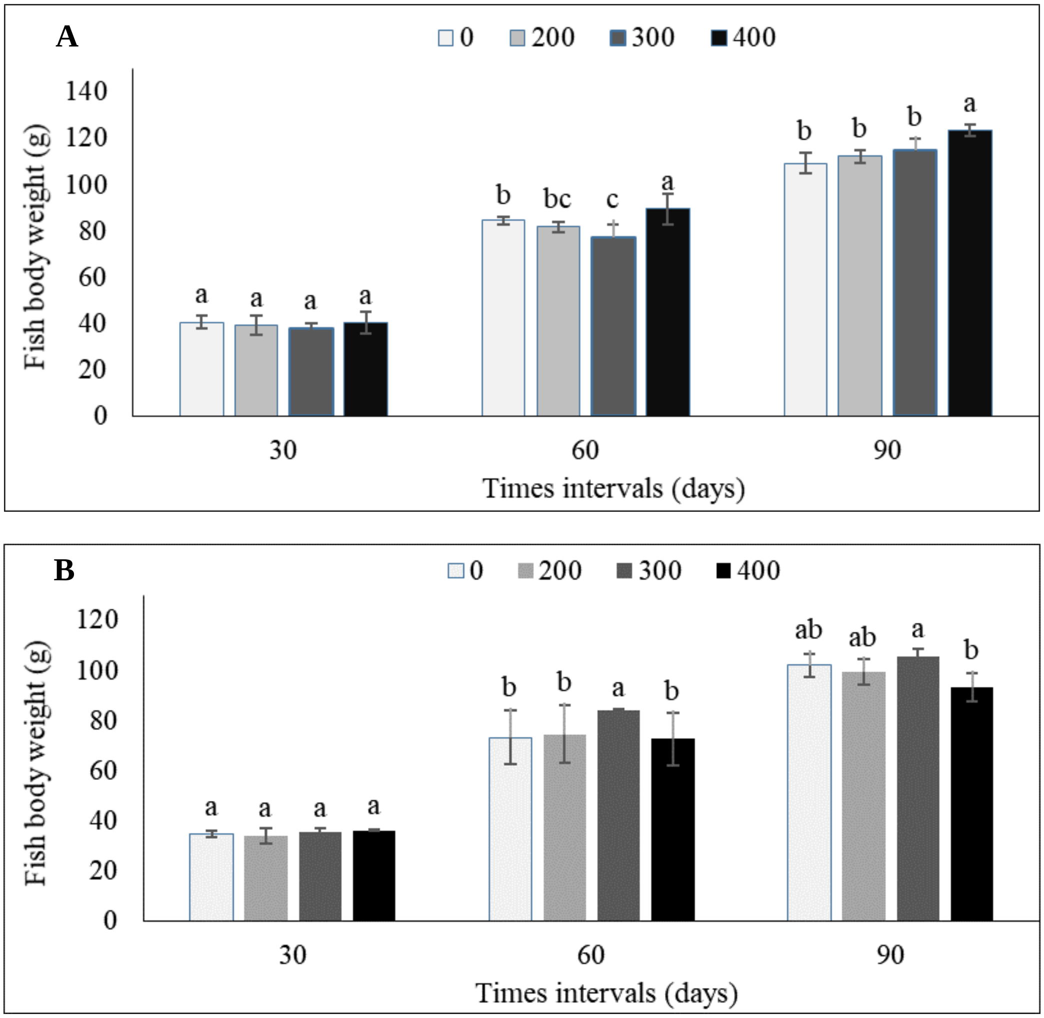

Fig. 2. Fish body weight of the Nile tilapia body fed different levels of Pel-Gel-Dry in **(A)** Floating and **(B)** Sinking feed during intervals times

2.2. Average daily gain and specific growth rate

The results of ADG and SGR of *O. niloticus* fed with a floating diet and a sinking diet supplemented with different levels of Pell-Gel-Dry® at the end of the experiment (90 days) are shown in Fig. (3). The fish fed a floating diet supplemented with 400g Pell-Gel-Dry®/ ton showed significantly ($P \leq 0.05$) increased ADG (Fig. 3A) and SGR (Fig. 3B) compared to those fed a floating diet supplemented with the different levels of Pell-Gel-Dry®. On the other hand, fish fed a sinking diet supplemented with 300g Pell-Gel-Dry®/ton revealed the highest ($P \leq 0.05$) increase of ADG (Fig. 3C) and SGR (Fig. 3D), compared to those fed a sinking diet supplemented with 400g of Pell-Gel-Dry®/ ton and non-significantly ($P \geq 0.05$) differences were observed with fish fed a sinking diet supplemented with 200g of Pell-Gel-Dry®/ ton or those fed a free Pell-Gel-Dry® sinking diet (as a control group).

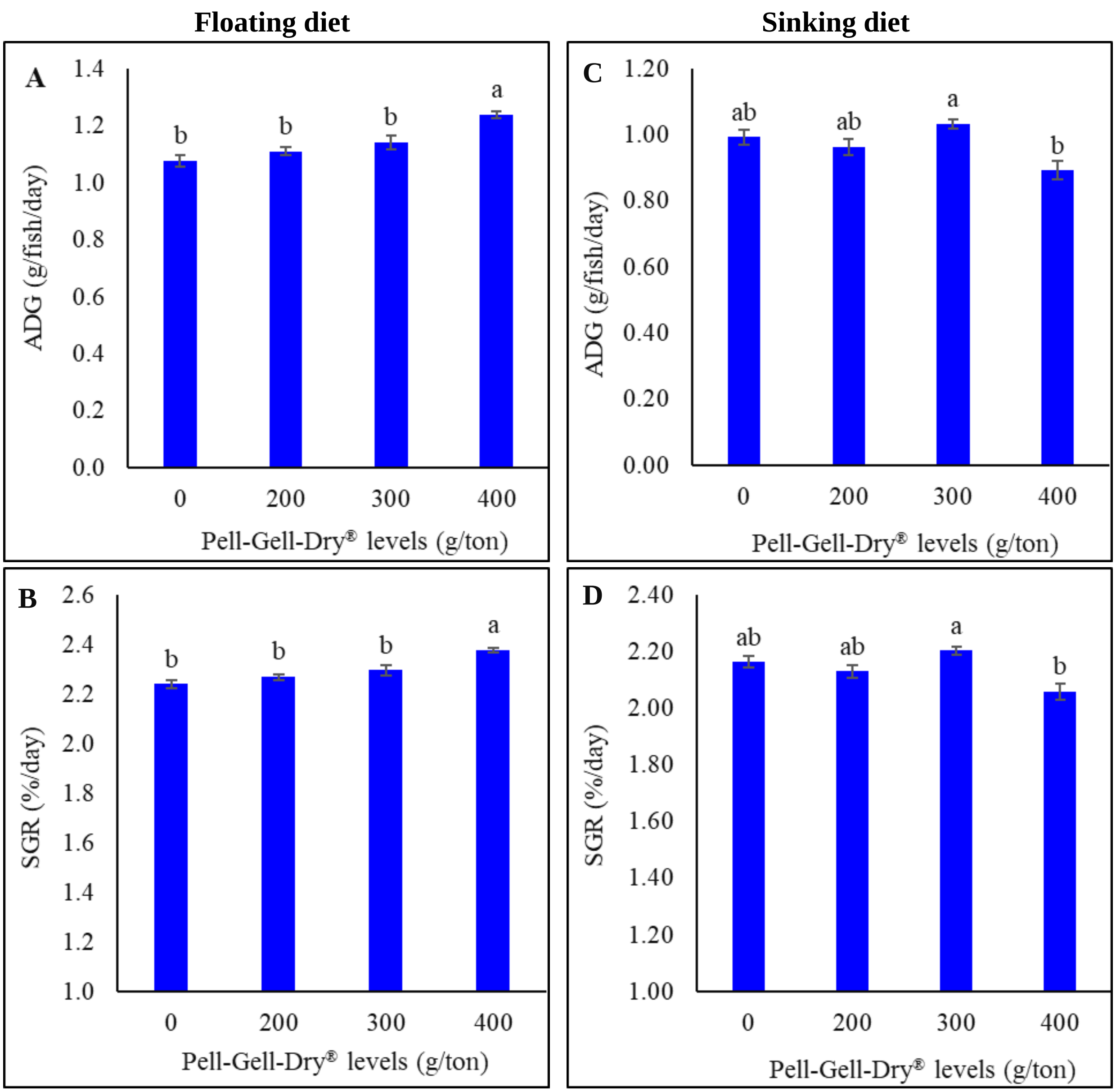

Fig. 3. Average daily gain (ADG: **A** and **C**) and specific growth rate (SGR: **B** and **D**) of *O. niloticus* fed different levels of Pell-Gel-Dry® in floating and sinking diet

3. Feed efficiency

The feed efficiency parameters such as feed intake (FI), FCR, PER, PPV, and EU are illustrated in Table (4). *O. niloticus* fed the floating diet supplemented with Pell-Gel-Dry® at a level of 400g/ ton significantly ($P \leq 0.05$) increased FI, PER, PPV, and EU, as well as significantly improved FCR among all treatments. In the case of a sinking diet, fish fed a non-supplemented Pell-Gel-Dry® sinking diet (as a control group) significantly ($P \leq 0.05$) increased PPV and EU, compared to those fed a sinking diet supplemented with different levels of Pell-Gel-Dry®. However, FI, FCR, and PER were not significantly affected among all treatments (Table 4).

4. The chemical composition of fish body

The result of the chemical compositions of the whole fish body of *O. niloticus* fed floating or sinking diets supplemented with different levels of Pell-Gel-Dry® is shown in Table (5). In the case of the floating diet, DM, EC, CP, EE, and ash were significantly ($P \leq 0.05$) increased in the whole body of fish fed a Pell-Gel-Dry® supplemented diet at levels of 400, 300, and 200g/ ton, respectively, compared to those fed the control diet. Regarding the sinking diet, a significant ($P \leq 0.05$) increase in body CP, EE, EC, and ash contents were observed in fish fed a diet supplemented with 200, 400, and 300g of Pell-Gel-Dry®/ ton, respectively, compared to those fed the control diet. However, DM was significantly increased in the whole body of fish fed the control diet among all groups (Table 5).

Table 4. Effect of different levels of Pell-Gel-Dry® supplemented with floating and sinking diets on feed efficiency parameters of *O. niloticus*

Parameter	Pell-Gel-Dry® level (g/ ton)				*P-value*
	0	200	300	400	
Floating diet					
FI (g)	140.6±3.45^{ab}	132.8±3.85^{ab}	128.1±1.17^{b}	143.7±4.03^{a}	0.0368
FCR	1.50±0.01^{a}	1.37±0.02^{b}	1.29±0.04^{b}	1.33±0.03^{b}	0.0023
PER	2.21±0.01^{b}	2.41±0.04^{ab}	2.56±0.08^{a}	2.47±0.05^{a}	0.0058
PPV (%)	34.87±0.22^{bc}	31.31±0.93c	38.21±1.09^{ab}	38.91±0.76^{a}	0.0006
EU (%)	24.52±0.15^{bc}	23.33±0.17^{c}	26.32±0.74^{b}	28.96±0.57^{a}	0.0002
Sinking diet					
FI (g)	136.3±6.21	131.8±5.95	143.2±0.98	131.0±4.94	0.3507
FCR	1.44±0.03	1.43±0.03	1.45±0.03	1.54±0.01	0.0879
PER	2.32±0.05	2.32±0.05	2.28±0.05	2.18±0.02	0.1561
PPV (%)	38.06±0.95^{a}	34.66±0.75^{b}	31.98±0.68^{b}	33.46±0.28^{b}	0.0017
EU (%)	24.41±0.60^{a}	21.66±0.46^{bc}	20.39±0.43^{c}	23.25±0.20^{ab}	0.0011

Mean in the same row having different small letters are significantly different ($P \leq 0.05$). FI: Feed intake, FCR: Feed conversion ratio, PER: Protein efficiency ratio, PPV: Protein productive value, and EU: Energy utilization.

5. The chemical composition of fish muscles

The data of the chemical composition of muscles of *O. niloticus* fed floating and sinking diets supplemented with different levels of Pell-Gel-Dry® are shown in Table (6). The DM, CP, and EC were significantly ($P \leq 0.05$) increased in the muscles of fish fed a floating diet supplemented with 400, 200, and 300g of Pell-Gel-Dry®/ ton, respectively, compared to those fed the control diet. EE and ash contents of muscles significantly increased in fish fed the control diet among all groups of fish fed a floating diet supplemented with different levels of Pell-Gel-Dry®. With respect to the sinking diet, DM was significantly ($P \leq 0.05$) increased in fish-fed diets supplemented with 300, while EE and EC increased in 400g of Pell-Gel-Dry®/ ton compared to those fed other levels. The ash content of the muscles was significantly increased in the muscles of fish fed a sinking control diet followed by those fed a supplemented diet with 300 and 200g of Pell-

Gel-Dry®/ ton, and the lowest value was recorded in fish fed sinking diet supplemented with 400g/ ton. However, CP in fish muscles was insignificant ($P \geq 0.05$) among all dietary treatments.

Table 5. Effect of different levels of Pell-Gel-Dry® supplemented with floating and sinking diets on the chemical composition of the whole fish body of *O. niloticus*

Pell-Gel-Dry® level (g/ton)	DM (%)	On dry matter basis (%)			
		Protein	EE	Ash	EC
Floating diet					
0	26.66±0.01[b]	56.99±0.04[b]	26.52±0.04[c]	16.49±0.02[b]	2396±0.88[c]
200	22.59±0.11[d]	52.63±0.03[d]	29.25±0.10[a]	18.11±0.08[a]	2401±3.46[c]
300	24.43±0.02[c]	59.14±0.03[a]	25.99±0.03[d]	14.86±0.02[d]	2426±0.58[b]
400	27.38±0.16[a]	55.51±0.18[c]	28.60±0.11[b]	15.88±0.12[c]	2443±3.00[a]
P-value	<.0001	<.0001	<.0001	<.0001	<.0001
Sinking diet					
0	26.16±0.02[a]	59.78±0.03[b]	22.73±0.04[b]	17.49±0.02[a]	2312.0±1.00[c]
200	23.28±0.02[c]	61.86±0.01[a]	22.03±0.11[c]	16.10±0.11[b]	2333.3±4.48[b]
300	22.76±0.01[d]	59.87±0.03[b]	22.43±0.08[b]	17.70±0.10[a]	2302.0±3.51[c]
400	25.67±0.04[b]	57.27±0.05[c]	26.21±0.06[a]	16.52±0.11[b]	2390.0±3.46[a]
P-value	<.0001	<.0001	<.0001	<.0001	<.0001

Mean in the same column having different small letters are significantly different ($P \leq 0.05$). DM: Dry matter, EE: Ether extract, and EC: Energy content.

Table 6. Effect of different levels of Pell-Gel-Dry® supplemented with floating and sinking diets on the chemical composition of *O. niloticus* muscles

Pell-Gel-Dry® level (g/ton)	DM (%)	On a dry matter basis (%)			
		Protein	EE	Ash	EC
Floating diet					
0	16.38±0.15[b]	86.35±0.83[c]	5.20±0.32[a]	8.45±0.51[a]	2247±7.02[b]
200	17.69±0.15[a]	91.45±0.38[a]	2.57±0.15[c]	5.99±0.27[b]	2263±5.17[ab]
300	18.01±0.06[a]	89.13±0.14[b]	4.07±0.08[b]	6.80±0.06[b]	2268±0.88[a]
400	18.04±0.09[a]	91.10±0.14[ab]	1.66±0.10[d]	7.24±0.08[ab]	2219±1.76[c]
P-value	<.0001	0.0002	<.0001	0.0023	0.0002
Sinking diet					
0	15.42±0.01[b]	85.63±0.03	3.38±0.03[b]	10.98±0.00[a]	2158.3±0.33[b]
200	15.36±0.26[b]	87.72±0.04	2.37±0.02[c]	9.90±0.02[a]	2167.6±0.33[b]
300	17.70±0.03[a]	87.15±0.10	2.90±0.03[bc]	9.93±0.07[a]	2175.3±1.33[b]
400	17.27±0.25[a]	86.98±1.05	5.20±0.39[a]	7.81±0.66[b]	2262.0±9.54[a]
P-value	<.0001	0.1099	<.0001	0.0010	<.0001

Mean in the same column having different small letters are significantly different ($P \leq 0.05$). DM: Dry matter, EE: Ether extract, EC: Energy content.

6. Economic efficiency indicators

The data in Table (7) exhibit the economic efficiency indicators of *O. niloticus* fed the floating or sinking diets supplemented with the different levels of Pell-Gel-Dry® (as a novel feed binder). The fish fed the floating diet or sinking diet bounded by 400 or 300g/ ton, respectively, achieved the highest ($P \leq 0.05$) economic efficiency parameters and the lowest costs compared to those fed the control diet (0.0 Pell-Gel-Dry®/ ton).

Table 7. Effect of different levels of Pell-Gel-Dry® on the economic efficiency indicators of *O. niloticus*

Pell-Gel-Dry® level (g/ton)	Output (LE)	Input (LE)	Net return (LE)	Economic efficiency (%)
Floating feed				
0	374.8±7.16^{b}	240.8±5.89^{a}	134.0±1.25^{d}	55.70±0.85^{c}
200	386.7±4.68^{b}	228.1±6.61^{b}	158.6±1.94^{c}	67.13±1.36^{b}
300	390.3±3.96^{b}	220.3±2.02^{b}	175.1±1.10^{b}	75.68±2.44^{a}
400	431.0±3.81^{a}	241.9±3.49^{a}	185.5±1.58^{a}	76.73±1.75^{a}
P-value	0.0003	0.0378	<.0001	<.0001
Sinking feed				
0	346.1±7.53^{ab}	221.3±4.85	124.8±6.49^{b}	53.49±1.62^{bc}
200	335.4±8.20^{ab}	214.5±4.68	127.5±0.62^{b}	58.10±0.70^{ab}
300	360.0±5.31^{a}	224.8±1.53	141.1±3.43^{a}	62.81±1.80^{a}
400	319.1±4.76^{b}	212.6±3.91	106.2±0.88^{c}	49.94±0.50^{c}
P-value	0.0135	0.1842	0.0012	0.0005

Mean in the same column having different small letters are significantly different ($P \leq 0.05$).

DISCUSSION

Water stability is an important physical characteristic of fish feed; it is influenced by the composition of food formulation **(Onada & Ogunola, 2020)**, where the values of the water stability for the diets showed the quality of pellets to stay in water for long periods in water **(Secci *et al.*, 2020)**. In the present study, the water stability of the sinking diet bounded by Pell-Gel-Dry® obtained after 20min (70.00- 86.70%) is nearly the same as the water stability of yam starch-bound diets recorded by **Orire *et al.* (2010)**. The current findings are also similar to those obtained by **Momoh *et al.* (2016)** and **Zaabwe *et al.* (2020)**. However, **Oke *et al.* (2021)** reported that the highest values of water stability ranged from 85.20 to 88.90% for the *O. niloticus*-fed diet bounded by *C. olitorius* leaf than those obtained in the present study. The differences in the water stability of diets showed that the inclusion of maize or wheat alongside the binders is of great importance. Several factors can increase the level of stability of fish feed in water, such as the type of tested binders since each binder has different properties from one to another **(Ruscoe *et al.*, 2005; Haetami *et al.*, 2017)**. On the other hand, the current findings revealed that the floating diet has 100% water stability, and the floating pellets are still stable in the aqueous medium for 6 hours. This highest water stability is seriously related to the highest obtained growth performance (Figs. 1, 2) and feed efficiency (Table 4) of *O. niloticus* fed the floating diet compared to those fed the sinking diet. In addition, the increase in feed hardness and water stability was only attributed to the gelatin or CMC supplementation in the gibel carp, *C. gibelio* diet **(Gao *et al.*, 2020)**. More recently, **Maharsih *et al.* (2023)** stated that the integration of 0.75% w/ v beeswax in the edible coating significantly affects the water stability of *O. niloticus* feed.

Feed additives are employed as nonnutritive ingredients or as nonnutritive components of ingredients that are included in the formulation to either influence the physical or chemical properties of the diet or affect fish performance or the quality of resulting products **(Borrws & Hardy, 2000)**. In the present study, significant improvements of ATWG, ADG, and SGR of *O. niloticus* fed the floating diet supplemented with Pell-Gel-Dry® at levels of 400 and 300g/ ton, respectively, among other levels. On the other hand, an improvement was found in ATWG, ADG, and SGR of fish fed a sinking diet supplemented with 300g Pell-Gel-Dry®/ ton. This may be due to the ability of the increased levels of AAs to improve the nutritional value of the experimental diets, as shown in Table (3), and modify the fish-associated gut EAA community. Additionally, these positive effects on growth performance parameters may also be attributed to the dietary supplementation of Pell-Gel-Dry® as a novel feed binder during the manufacturing of the experimental diet, which led to the highest water stability of pellets, which plays an important role in improving the digestibility and consequently leads to enhancing the growth performance of fish. In this respect, **Aziz (2013)** reported that prepared diets can be characterized by a potential nutritional impoverishment due to nutrient loss from the pelleted ration through leaching. These current results agree with those obtained by **Oke *et al*. (2021)**, who reported that *O. niloticus* fed bounded diets with *Corchorus olitorius* leaf had significantly increased growth performance parameters compared to corn starch-bounded diets. The same positive findings were recently reported by **Veronica *et al*. (2022)** for *O. niloticus* fed a bounded diet by mixing *Abelmoschus esculentus* leaves powder with maize (MAA), which led to significantly increased growth performance parameters among all treatments.

In the present study, *O. niloticus* fed the floating diet supplemented with Pell-Gel-Dry® at a level of 400g/ ton significantly increased FI, PER, PPV, and EU, as well as significantly improved FCR among all treatments. In the case of a sinking diet, an unclear trend was observed in a fish-fed supplemented diet with different levels of Pell-Gel-Dry®. Similar to the current findings, **Oke *et al*. (2021)** recently reported that *O. niloticus* fed bounded diets with *C. olitorius* leaf had significantly increased feed efficiency parameters compared to corn starch-bounded diets. Coinciding with the current results, **Veronica *et al*. (2022)** recently postulated that mixing *A. esculentus* leaves powder with MAA as binders in the diet of *O. niloticus* leads to significantly increased feed efficiency among all treatments of treated fish. In addition, the previous authors confirmed the positive relationship with this improvement in growth and feed utilization of fish due to the tested binders and their effects on the physical properties and water stability percentage of the bounded pellets; thus, they deduced to use it as a binder agent during the manufacturing of *O. niloticus* diet. Additionally, improving feeding and feed utilization stands as the only option to minimize the ecological threats to fish farming **(Craig & Helfrich, 2009)**. Inversely to the current findings, **Gao *et al*. (2020**) elucidated that growth performance and feed utilization parameters of the gibel carp, *C. gibelio,* were not significantly different in the gelatin or carboxymethyl cellulose (CMC)-supplemented groups compared to the control. The same findings were recently reported by **Liu *et al*. (2022)** in juvenile largemouth bass, *Micropterus salmoides*, fed guar gum as a feed binder. The differences between the current findings and those obtained in previous studies may be mainly related to factors like the type of tested binders, levels, structure, fish species, age, and experimental design or management.

Regarding the chemical composition of the fish's whole body and their muscles, the obtained results showed that the chemical composition of the body and muscles was significantly improved in *O. niloticus* fed floating or sinking diets bounded by the different levels of Pell-Gel-Dry® compared to those fed the control diet. The addition of binder improves pellet quality and stability and retains nutrients for easy ingestion. Nutrient availability by farmed aquatic organisms is key to improving growth and digestibility. Some binders promote nutrient accessibility since there is a balance between water absorption and nutrient retention **(Argüello-Guevara & Molina-Poveda, 2013)**. Other binders may hinder nutrient accessibility since they form very hard pellets that retain nutrients through gel matrix formation **(Dominy *et al*., 2004)**. Thus, **Aksoy *et al*. (2022)** concluded that at 28°C, diets with soy hull binders lost about 64– 74% of dietary CP, 45– 60% EE, and 32– 45% ash content, which is in stark contrast to the total loss exhibited by each of the diets with control binders at the end of the 48h of water immersion. The CP content of all leached pellets after immersion in the 28°C water decreased compared to the initial percentage. The current findings match with those of **Veronica *et al*. (2022)** who recently reported that mixing *Abelmoschus esculentus* leaves powder with MAA as binders in the diet of *O. niloticus* leads to significantly improved carcass composition in treated fish among all treatments. In the same line as the current findings, they also suggested that the highest carcass CP content of fish, which is an indication of the high nutritional value of the diet, leads to positive effects on growth performance and feed efficiency parameters of treated fish, as well as being mainly related to the high AAs content in the tested binder agent Pell-Gel-Dry®, as shown in Table (3). In addition, **Mariotti *et al.* (2008)** reported that dietary CP concentration is rarely a true reflection of the protein and AAs composition of the diet. The same trend was confirmed for *O. niloticus* by **Kareem *et al*. (2015)** and **Oke *et al*. (2021)**.

Feed production and quality are considered to be one of the major factors influencing the success of the production of fish in aquaculture **(Abowei & Ekubo, 2011)**. Thus, there is a need for technology that will reduce costs and produce a quality fish feed. Quality feed, when fed at the recommended rate under optimal water quality conditions, leads to profitability. However, the bioavailability of the nutrients and the physical quality of the feed, which can be affected by processing technology, are the most important factors in maximizing feed utilization **(Oke *et al*., 2021)**. From an economic point of view, in the present study, the addition of graded levels of Pell-Gel-Dry® (as a novel binder agent) in *O. niloticus* diet has the highest economic efficiency, especially at levels of 400 and 300g/ ton of floating and sinking diets, respectively, compared to those fed the control diet. In addition, feed binder prices may significantly affect the cost of the diet; therefore, the utilization of natural, biodegradable, and renewable binder agents may be advantageous from an environmental and economic point of view **(Paolucci *et al*., 2012)**. In this respect, **Aksoy *et al*. (2022)** recently concluded that the soybean hull-bound pellets (as a natural control binder) were more water-stable than the control binders and could produce better and less expensive natural alternatives to conventional binders. Generally, it could be noticed that the addition of feed binders like Pell-Gel-Dry® (in the present study) has significantly improved the growth and feed efficiency parameters and carcass composition of the experimental fish, as well as increased the water stability of pellets, without any drastic effects on water quality parameters. All these benefits from the addition of Pell-Gel-Dry® as a binder agent to the experimental diets (floating or sinking) consequently lead to increased profitability and sustainability properties that are suitable for fish farmers.

CONCLUSION

Based on the current findings, it could be concluded that, the use of 400 and 300g of Pell-Gel-Dry®/ ton (a novel feed binder) for floating diet and sinking diet, respectively, are recommended as the most efficient and cost-effective levels for improving the growth performance, feed efficiency, chemical composition of the body and muscles, as well as enhancing the water stability of *O. niloticus* floating diet. In addition, these levels of the tested binder agent are a more simply applicable technology that is economically effective and acceptable to achieve more profitability for fish farmers.

REFERENCES

Abowei, J.F.N. and Ekubo, A.T. (2011). A Review of Conventional and Unconventional Feeds in Fish Nutrition. Br. J. Pharmacol. Toxicol., 2(4): 179- 191.

Aksoy, B.; Yildirim-Aksoy, M.; Jiang, Z. and Beck, B. (2022). Novel animal feed binder from soybean hulls-evaluation of binding properties. Anim. Feed. Sci. Technol., 288:115292. https://doi.org/10.1016/j.anifeedsci.2022.115292.

AOAC. (2016). Official Methods of Analyses. 20th Edn. Association of Official Analytical Chemist, Washington, DC., USA.

Argüello-Guevara, W. and Molina-Poveda, C. (2013). Effect of binder type and concentration on prepared feed stability, feed ingestion and digestibility of

Litopenaeus vannamei broodstock diets. Aquac. Nutr., 19(4): 515-522. Doi:10.1111/anu.12003.

Aziz, A. (2013). Water stability and nutrient leaching of different levels of maltose formulated fish pellets. Glob. Vet., 10: 638-642. Doi: 10.5829/idosi.gv.2013.10.6.7278.

Boyd, C.E. and Tucker, C.S. (1998). Pond Aquaculture Water Quality Management. 1st Edition, Springer New York, NY., USA, pp. 700. Doi: https://doi.org/10.1007/978-1-4615-5407-3.

Bureau, D.P. and Encarnação, P.M. (2006). Adequately determining the amino acid requirements of fish: the case example of lysine. pp.: 29-54. In: Advances in Nutrition, Acicula, VIII Symposium International de Nutrition. Acuicola, L.E. Cruz-Suarez, D. Ricoque-Marie, M. TapiaSalazar, M.G. Nieto-Lopez, D.A. Villareal Cavazos, A.C. Pueblo Cruz, and A. Garcia-Ortega, eds. Mazatlán, Sinaloa, Mexico, 15-17 November, 2006.

Calicioglu, O.; Flammini, A.; Bracco, S.; Bellù, L. and Sims, R. (2019). The future challenges of food and agriculture: an integrated analysis of trends and solutions. Sustainability, 11(1): 222. https://doi.org/10.3390/su11010222.

Craig, S. and Helfrich, L.A. (2009). Understanding Fish Nutrition, Feeds, and Feeding. Virginia Cooperative Extension, Virginia State University, USA, pp.:420-256.

Dominy, W.G.; Cody, J.J.; Terpstra, J.H.; Obaldo, L.G.; Chai, M.K.; Takamori, T.I.; Larsen, B. and Forster, I.P. (2004). A comparative study of the physical and biological properties of commercially available binders for shrimp feeds. J. Appl. Aquacult., (14): 81–99. https://doi.org/10.1300/J028v14n03_07.

Encarnação, P.M.; de Lang, F.M. and Bureau, D.P. (2006). Diet energy source affects lysine utilization for protein deposition in rainbow trout. Aquaculture, 261:1371-1381.

Encarnação, P.M.; de Lange, C.; Rodehustscord, F.M.; Hoehler, D.; Bureau, W. and Dureau, D.P. (2004). Diet digestible energy content affects lysine utilization, but not dietary lysine requirements of rainbow trout for maximum growth. Aquaculture, 235:569-586.

FAO. (2020). The State of World Fisheries and Aquaculture. Sustainability in Action. Italy: Food and Agriculture Organization of the United Nations; 244pp. https://doi.org/10.4060/ca9229en.

Gao, S.; Han, D.; Zhu, X.; Yang, Y.; Liu, H.; Xie, S. and Jin, J. (2020). Effects of gelatin or carboxymethyl cellulose supplementation during pelleting processing on feed quality, intestinal ultrastructure and growth performance in gibel carp (*Carassius gibelio*). Aquac. Nutr., 26:1244–1254. Doi: 10.1111/anu.13080.

Glencross, B.; Blyth, D.; Tabrett, S.; Bourne, N.; Irvin, S.; Anderson, M.; Fox- Smith, T. and Smullen, R. (2012). An assessment of cereal grains and other starch sources in diets for barramundi (*Lates calcarifer*)–implications for nutritional and functional qualities of extruded feeds. Aquac. Nutr., 18: 388-399. https://doi.org/10.1111/j.1365-2095.2011.00903.x.

Haetami, K.; Iskandar, J. and Abun, R. (2017). Durability and water stability of pellet fish supplementation results pairing coconut oils and hazlenut oil. IJEAB., 2(3):1336-1340. Doi: https://doi.org/10.22161/ijeab/2.3.40.

Hardy, R.W. and Barrows, F.T. (2003). Diet formulation and manufacture. In: Fish Nutrition; pp.: 505-600, Academic Press.

Hixson, S.M. (2014). Fish nutrition and current issues in aquaculture: The balance in providing safe and nutritious seafood, in an environmentally sustainable manner. J. Aquac. Res. Dev., 5(3): 1000234. Doi: 10.4172/2155-9546.1000234.

Hunter, M.C.; Smith, R.G.; Schipanski, M.E.; Atwood, L.W. and Mortensen, D.A. (2017). Agriculture in 2050: recalibrating targets for sustainable intensification. BioScience, 67(4): 386-391. Doi:10.1093/biosci/bix010.

Islam, M.J.; Kunzmann, A. and Slater, M.J. (2022). Responses of aquaculture fish to climate change-induced extreme temperatures: a review. J. World. Aquac. Soc., 53:314–366. https://doi.org/10.1111/jwas.12853.

Kareem, Z.H.; Abdehadi Y.M.; Christianus A.; Karim M. and Romano M. (2015). Effects of some dietary crude plant extracts on the growth and gonadal maturity of Nile tilapia (*Oreochromis niloticus*) and their resistance to *Streptococcus agalactiae* infection. Fish. Physiol. Biochem., 42: 757-769. Doi: 10.1007/s10695-015-0173-3.

Liu, Y.; Zhang, Y.; Fan, J.; Zhou, H.; Huang, H.; Cao, Y.; Jiang, W.; Zhang, W.; Deng, J. and Tan, B. (2022). Effects of different viscous guar gums on growth, apparent nutrient digestibility, intestinal development and morphology in juvenile largemouth bass, *Micropterus salmoides*. Front. Physiol., 13: 927819. Doi: 10.3389/fphys.2022.927819.

Lovell, R.T. (2001). Nutrition and Feeding of Fish, 3rd edition, Haworth Press, New York, USA.

Ma, S.; Wang, H.; Li, J.; Xue, M.; Cheng, H.; Yuchang Qin, Y. and Blecker, C. (2021). Effect of the ratio of wheat flour and cassava and process parameters on the pellet qualities in low starch feed recipe extrusion, Anim. Feed. Sci. Technol., 271:114714. https://doi.org/10.1016/j.anifeedsci.2020.114714.

Maharsih, I.K.; Tarmidzi, F.M.; Pusfitasari, M.D.; Alviany, R.; Yuli, A. and Asnawi, I. (2023). Water stability characteristic of Nile tilapia (*Oreochromis niloticus*) feed coated with tapioca flour-beeswax-based edible coating. Indo. J. Chem. Res., 11(2): 72-77. Doi: 10.30598//ijcr.2023.11-ing.

Marioti, F.; Tome, D. and Mariand, P.P. (2008). Converting nitrogen into protein – beyond 6.25 and Jones' Factors. Crit. Rev. Food. Sci. Nut., 48:177-184. Doi: 10.1080/10408390701279749.

Momoh, A.T.; Abubakar, M.Y. and Ipinjolu, J.K. (2016). Effect of ingredients substitution on binding, water stability, and floatation of farm-made fish feed. Int. J. Fish. Aquac., 4(3):92-97.

NRC, National Research Council (2011). Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington DC

Oke, I.O.; Adeparusi, E.O. and Dada, A.A. (2021). Utilization of *Corchorus olitorius* leaf as binder in the diet of *Oreochromis niloticus* fingerlings. J. Agric. Sci. Technol., 13(1): 34-39. Doi: 10.15547/ast.2021.01.006.

Olugbade, T.; Ojo, O. and Mohammed, T. (2019). Influence of binders on combustion properties of biomass briquettes: A Recent Review. Bioenerg. Res., 12: 241–259. https://doi.org/10.1007/s12155-019-09973-w.

Onada, O.A. and Ogunola, O.S. (2020). Comparative analysis of sinking time index and water stability of different inclusion levels of cassava flour and brewer yeast in a test diet. J. Aquac. Feed. Sci. Nutr., 11(2):15-18. Doi:10.36478/joafsnu.2019.15.18.

Orire, A.M. and Emine, G.I. (2019). Effects of crude protein levels and binders on feed buoyancy. J. Aquac. Res. Dev., 10: 565. doi: 10.4172/2155-9546.1000565.

Orire, A.M.; Sadiku, S.O.E. and Tiamiyu, L.O. (2010). Evaluation of yam starch (*Discorea rotundata*) as aquatic feed binder. Pak. J. Nutr., 9: 668-671. Doi:10.3923/pjn.2010.668.671.

Paolucci, M.; Fabbrocini, A.; Volpe, M.G.; Varricchio, E. and Coccia, E. (2012). In: Muchlisin, Z.A. (Ed.), Development of biopolymers as binders for feed for farmed aquatic organisms. Aquaculture. Rijeka, Croatia: InTech, pp.: 4–34. https://doi.org/10.5772/28116.

Ruscoe, I.M.; Jones, C.M.; Jones, P.L. and Caley, P. (2005). The effects of various binders and moisture content on pellet stability of research diet for freshwater crayfish. Aquac.Nutr.,11:87-93. https://doi.org/10.1111/j.1365-2095.2004.00324. x.

SAS. (2006). SAS/STAT® 9.1.3 User's Guide. SAS Institute Inc., Cary, NC., USA.

Secci, M.; Ferranti, M.; Giglioli, A.; Pasquini, V.; Sicurelli, D.; Fanelli, G. and Prato, E. (2020). Effect of temperature and duration of immersion on the stability of prepared feeds in echinoculture. J. App. Aqua., 33(2): 1-15. Doi:10.1080/10454438.2020.1724845.

Veronica, O.O.; Olaemy, O.M. and Opeyemi, O.I. (2022). Physical properties and dietary effects of *Abelmoschus esculentus* leaves used as binders in fish feed. Agric. Sci. Technol., 14(2): 62-68. Doi: 10.15547/ast.2022.02.020.

Yadav, M.K.; Ojha, M.L.; Keer, N.R. and Yadav, A. (2019). An overview on the use of oil in fish diet. J. Entomol. Zool. Stud.,7(1): 883-885.

Zaabwe, T.; Singini, W.; Kang'ombe, J.; Kapute, F. and Mbamba, D. (2020). An evaluation of water pellet stability of two iso-proteineous floating and sinking diets. Egypt. J. Aquat. Biol. & Fish., 24(7): 211 – 218. Doi: 10.21608/EJABF. 2020.120615.