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Impact of Distiller's Dried Grains with Solubles on Health and Performance of Nile Tilapia (*Oreochromis niloticus*) Fish

[1]*Mohamed Mostafa Khadr,* [2]*Khaled M. Al-Marakby,* [2]*Sabry, A. Shehata and* [1]*Mohamed S. Ebrahim*

[1]Central Laboratory of Aquaculture Research, Abbasa, Abo-Hammad, Sharkia, Egypt
[2]Animal Production Department, Faculty of Agriculture, Zagazig University, Egypt

Abstract: This work was conducted to study the impact of dried distiller grain with solubles (DDGS) incorporation in Nile tilapia (*Oreochromis niloticus*) diets on performance, health and economic efficiency. An aggregate number of 200 Nile tilapia fingerlings (Average body weight 12.86 g ± 0.14) were randomly distributed into five experimental groups, with 4 replicates (Glass aquaria) contain 10 fingerlings per each. Five diets were formulated to contain 0 (Control diet based on fish meal, soybean meal and yellow corn) 5, 10, 20 and 30% DDGS (T1, T2, T3, T4 and T5, respectively). The fingerlings received the experimental diets twice daily to apparent satiation for 16 weeks. All inclusion levels of DDGS (5, 10, 20 and 30%) had no significant ($P > 0.05$) effects on final body weight, total body weight gain, feed input, feed conversion ratio, relative growth rate and protein productive value. The findings of blood hematological and biochemical parameters did not significantly differed among the experimental groups ($P > 0.05$). The mortality rate was considerably decreased in the T5 group when compared with the control group. The parameters of health conditions were homogeneous in all dietary groups. The relative profit was significantly increased with all inclusion levels of DDGS compared with the control diet (T1). In conclusion, incorporation of DDGS instead of the mixture of fish meal, soybean meal and yellow corn up to the level 30 % had no negative effects on productive performance, and health condition, with a considerable improvement in the economic efficiency of Nile tilapia fish diets.

Key words: Aquafeed · DDGS · *Oreochromis niloticus* · Health · Performance

INTRODUCTION

Aquafeed represents about 50-80% of the operational cost in fish farms. The sustainability of the aquaculture sector is linked to the sustained supply of terrestrial animal and plant proteins, oils and carbohydrate sources for aquafeeds. The aquaculture sector should therefore strive to ensure sustainable supplies of terrestrial and plant feed ingredients [1]. The Washington State Department of Agriculture defines feed as a mix of whole or processed grains, concentrates, feeds for all species of animals to include customer formula and pet feed these feed are now commercially produced for the fish industries [2]. The Food and Drug Administration (FDA) defines Hazard Analysis Critical Control Point (HACCP) as a management system in which food security is directed out of the analysis and control of biological, chemical, and physical risks from consumption of the finished product [3]. The FDA regulates human food and animal feed for fish. Additionally, the FDA regulates pet food. Similar to human foods, animal feeds must be pure and wholesome, ready under good sanitary conditions, and truthfully be classified to provide the required information to the consumer [4].

In Egypt, 50% of maize, 53% wheat, 67% of sunflower meal and 99% of soybean meal used in aquafeeds were imported [5]. Therefore, seeking for unconventional, available and low priced feeds, especially protein sources will reduce the cost of fish production. The fermentation process of cereal grains, especially maize to produce ethanol resulted in a co-product is DDGS [6]. The DDGS is moderately high in protein (30% or higher) and is readily available and competitively priced relative to other alternative protein sources [7]. DDGS, which are rich in energy and protein, have been used in instead of corn and soybean meal in some livestock and poultry feeds, and

Corresponding Author: Mohamed Mostafa Khadr, Central Laboratory of Aquaculture Research, Abbasa, Abo-Hammad, Sharkia, Egypt.

corn DDGS have become the most popular, economical, and widely available alternative feed ingredient for use in U.S [8]. The U.S. Grain Council reported that corn DDGS is used primarily as an energy source in pigs diets because it contains approximately the same amount of digestible energy (DE) and metabolizable energy (ME) as corn, although the ME content may be a bit reduced when feeding reduced-oil DDGS [9]. A 2007 study highlighted the recent trends in the use of DDGS, as many producers are including 20% DDGS up to 35% DDGS has been used in diets fed to incubation swine and finishing pigs [10]. The recent research suggested that Carnivorous fish feed contains 30-50% fish meal and oil, but finding alternatives to fish meal in aquaculture diets [11].

The nutritive values of DDGS are varied, which due to the source and quality of grains, fermentation time and efficiency, drying process and the quantity of distiller's solubles added. Maize DDGS could be incorporated with different levels into Nile tilapia fish (*Oreochromis niloticus*) diets without any adverse effects [12]. This work was carried out to investigate the effect of different inclusion levels (5, 10, 20 and 30%) of DDGS instead of mixture of fish meal, soybean meal and yellow corn (FSC) on the performance of Nile tilapia fish.

MATERIALS AND METHODS

Study Area: This study was conducted at the Animal Production Department, Faculty of Agriculture, Zagazig University, Egypt. The experimental work was carried out at the Central Laboratory for Aquaculture Research, Abbasa, Abo Hammad, Sharkia Governorate, Egypt, during the summer of the year 2015.

Aquaria and Water: Fish groups were located in glass aquaria. Each replicate of fish groups was stoked in a glass aquarium (60×60×40 cm). Fish were kept for two weeks to be acclimatized before the start of the main experimental period. Aquaria were supplied with dechlorinated water from the storage tank. The air was supplied by aquarium air pumps. Fish wastes were drained by siphoning method with the third of water volume every day. Glass aquaria were cleaned biweekly to avoid any natural food formation as algal growth. The water samples were collected periodically from each aquarium to determine the dissolved oxygen and pH at the Central Laboratory of Aquaculture Research. The average dissolved oxygen concentration was 5.5 - 6.5 mg/l and the pH value was 7.5. Water temperature was measured by using a thermometer, which its mean value was 27 ± 2°C during the whole experimental period. The photoperiod was 12 hours approximately.

Experimental Fish: Nile tilapia (*Oreochromis niloticus*) fingerlings were obtained from Central Laboratory of Aquaculture Research in Abbasa (Longitude 31° 44' 16.0" E and latitude 30° 32' 37.0" N), Abo-Hammad, Sharkia, Egypt. Two-hundred apparently healthy fingerlings were randomly allotted to five experimental groups (40 fish /group). Each group had four equal replicates (10 fish /replicate). The initial average body weight of fingerlings was 12.86 g ± 0.14.

Experimental Diets: All feed ingredients and DDGS of yellow corn were procured from Zagazig feed mill (Sharkia, Egypt). Approximately, five isonitrogenous and isocaloric experimental diets (T_1, T_2, T_3, T_4 and $T_{5)}$ were formulated. The control (T_1) diet is formulated to be similar to the commercial tilapia diet, which produced by the Zagazig feed mill. The other four experimental diets were formulated to contain 5, 10, 20 and 30% DDGS (T_2, T_3, T_4 and T_5, respectively), as a replacer of fish meal, soybean meal and yellow corn mixture (FSC) in the control diet. The dietary ingredients were ground, mixed, pelleted (2 mm in diameter) and dried in a hot air drying oven overnight at 65°C. The proximate composition of DDGS and tested diets were determined according to the standard methods of AOAC [13]. The formulation and proximate composition of experimental diets are shown in Table 1.

Feeding Trail: Fish fed two times every day to apparent satiation at 10:00 and 14:00 hours at a rate of 3% of the total body weight. Fish were weighed fortnightly. Diets did not offer on the weighing day. Feed quantities were readjusted according to the change in live body weight. The feeding trial lasted for 16 weeks.

Fish Performance: Feed input was calculated as the total quantity of the offered diet during the trail divided by the number of surviving fish. Body weight gain was calculated as: final average body weight g - initial average body weight g. The relative growth rate was calculated by the following formula: [(Final average body weight - initial average body weight) / (Initial average body weight)] × 100. The feed conversion ratio (FCR) was calculated as (Air dry feed g / weight gain g). the protein productive value (PPV) was calculated as:

[protein retention g / protein intake g] × 100, where protein retention was calculated as: [Final fish body protein in g - initial fish body protein in g] / 100.

Proximate Chemical Analyses: The proximate chemical composition of diets, DDGS and the crude protein content in fish body (Initial and final) were adopted according to the standard protocol of AOAC [13].

Mortality Rate: The mortality rate is calculated as a percentage of the difference between the average initial and the final fish number per each fish group.

Evaluation of The Health Condition: Escape, defensive, tail and ocular reflexes of the experimental fish were regularly observed during the whole experimental period [7].

Blood Parameters: At the end of the experiment, blood samples were randomly taken from the caudal vein of non-anesthetized fish (12 fish per treatment; 3 fish per replicate) by using a sterile heparinized syringe. Plasma was obtained by centrifugation at 1006 g for 10 minutes. The plasma was collected in Eppendorf tubes and stored at -20°C until analyses. Plasma total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), Cholesterol and Triglycerides were analyzed by using commercial kits (Diamond Diagnostics Company, Egypt). Blood hematology was analyzed according to the usual standard protocols. Plasma globulin was calculated by subtracting the values of albumin from total protein. Albumin-to-globulin (A/G) ratio was calculated by dividing the values of albumin by values of globulin.

Statistical Analyses and Economic Study: Data of the experiment were statistically analyzed using the general linear model program of the SAS statistical system package [15]. The multiple comparisons among different means were performed by the Duncan's Multiple Range Test [16]. The inputs and outputs of the economic study were estimated according to the prices of the year 2015. The relative profit (RP) was calculated as follows: RP = (Profit of DDGS diets / profit of control diet) × 100.

RESULTS AND DISCUSSION

Chemical Composition of the Experimental Diets: Inclusion of DDGS in the tested diets increased their contents of the CP, CF, EE, ash and the digestible energy in comparison with the control diet (Table 1). On the contrary, the dietary content of NFE was decreased with raising the level of DDGS in diets. These results may attribute to the differences in nutrient concentration between a mixture of FSC and DDGS.

Growth Parameters: All of the tested levels of DDGS (5, 10, 20 and 30%) as replacers of FSC mixture had no significant ($P > 0.05$) effects on final body weight, total body weight gain and relative growth rate (Table 2). Concurred with our findings, some authors stated that corn DDGS could be incorporated up to 30% in tilapia diets without requiring lysine supplementation [12]. Moreover, the mean weight gain and growth rate in fingerlings did not significantly differed in Nile tilapia fish fed 20% DDGS diet as a substitute for soybean meal and corn meal mixture [17, 18]. In another field trial, the replacement of the dietary soybean meal (55%) by plant protein mixture (DDGS mixed with canola meal by ratio 1:1) had no significant effects on the growth response of Nile tilapia fingerlings [19]. In the hybrid tilapia, the different protein sources (fish meal, soybean meal and meat and bone meal) in combination with 30% DDGS had no significant influence on growth performance in the fingerlings fed the experimental diets [20].

Feed Utilization: The average feed input and feed conversion ratio (FCR) during the whole experimental period did not significantly differed among all fish groups (Table 2). It has been reported that yellow corn DDGS are palatable to Nile tilapia [21]. The mean values of protein utilization as protein productive value (PPV) were slightly improved in all diets contained DDGS in comparison with the control diet (Table 2). The protein utilization values explained as a protein efficiency ratio did not significantly differed among Nile tilapia groups fed diets containing DDGS up to 40% [17]. Moreover, others noticed that incorporation of DDGS (30%) can be incorporated into hybrid catfish diets without adverse effect on protein retention [23]. Similarly, the substitution of the dietary fish meal (15%) with DDGS in Nile tilapia fry diets did not reflect any significant effects on PPV values [24].

Mortality Rate: The mortality rate through the whole feeding period did not differ significantly among the experimental groups (Table 2). Moreover, the lowest mortality rates were recorded with T_3 and T_5, which received the diets containing 10% and 30% of DDGS, respectively. Some previous trials observed that the dietary levels up to 30% of maize DDGS did not

Table 1: Formulation and chemical composition of Nile tilapia fish diets

Parameters	Experimental diets (%) T_1	T_2	T_3	T_4	T_5
Fish meal	8.50	8.08	7.65	6.80	5.95
Soy bean meal	33.00	31.35	29.70	26.40	23.10
Yellow corn	53.00	50.35	47.70	42.40	37.10
DDGS	0.00	4.72	9.45	18.90	28.35
Vegetable oil	3.00	3.00	3.00	3.00	3.00
Premix	0.50	0.50	0.50	0.50	0.50
Molasses	2.00	2.00	2.00	2.00	2.00
Total	100	100	100	100	100
Proximate composition %					
Dry matter	100	100	100	100	100
Organic matter	96.08	96.03	95.95	95.86	95.69
Crude protein	24.20	24.40	24.56	24.91	25.65
Crude fiber	7.16	7.25	7.35	7.53	7.69
Ether extract	3.75	4.33	4.89	6.02	7.16
Nitrogen free extract	60.97	60.05	59.15	57.40	55.19
Ash	3.92	3.97	4.05	4.14	4.31
DE (Kcal / kg diet)	2675	2706	2735	2795	2857

Note. T_1, T_2, T_3, T_4 and T_5, diets contained 0, 5, 10, 20 and 30% DDGS respectively, in place of the mixture of fish meal, soybean meal and yellow corn in the control diet. The chemical composition of DDGS was 100% DM, 94.77% OM, 27.90% CP, 9.10% CF, 15.51% EE, 42.16% NFE and 5.33% Ash. DE, Digestible energy calculation based on values of 3.5 kcal / g CP, 8.1 kcal/g EE and 2.5 kcal/g NFE [27].

Table 2: Effect of DDGS dietary levels on growth performance, feed utilization and mortality of Nile tilapia fish during whole experimental period (16 weeks)

Items	Diets T_1	T_2	T_3	T_4	T_5	*P*-value
Initial body weight g/fish	12.86 ± 0.33	12.85 ± 0.12	12.82±0.11	12.84±0.09	12.95±0.05	0.271
Final body weight g/fish	36.95 ± 1.48	37.11 ± 0.96	36.21 ± 1.95	35.90 ± 3.93	35.13 ± 1.38	0.731
Total weight gain g/fish	24.09 ± 1.51	24.26± 0 .95	23.39±2.02	23.06±3.92	22.18±1.42	0.693
Relative growth rate %	187.33 ± 11.81	188.79±7.46	182.45±19.67	179.60±30.74	171.27±11.45	0.731
Total feed input g /fish	75.68±2.07	73.92±1.41	73.68±2.70	71.44±5.60	70.96±1.93	0.056
Feed conversion ratio	3.14±0.09	3.05±0.06	3.15±0.14	3.10±0.28	3.20±0.11	0.627
Protein productive value %	19.60±0.25	19.87±0.43	19.74±0.17	19.86±0.09	19.72±0.09	0.928
Mortality rate %	10 ± 4.08	10 ±5.77	5 ±5.00	10 ±4.08	5 ±2.89	0.823

Note. T_1, T_2, T_3, T_4 and T_5, diets contained 0, 5, 10, 20 and 30% DDGS respectively, in place of the mixture of fish meal, soybean meal and yellow corn in the control diet. Results are presented as means ± standard error. Body weight gain = (final average body weight g - initial average body weight g). Relative growth = [(final average body weight - initial average body weight) / (initial average body weight)] × 100.Feed conversion ratio = (air dry feed g) / (gain g). Protein productive value = [protein retention g / protein intake g] × 100, where protein retention was calculated as [final fish body protein g - initial fish body protein g] / 100.

Table 3: Effect of DDGS dietary levels on some hematological and biochemical parameters of Nile tilapia fish blood.

Item	Diets T_1	T_2	T_3	T_4	T_5	*P*-value
Hemoglobin (g/dl)	10.13±0.23	10.19±0.20	9.67±0.20	9.97±0.15	9.73±0.15	0.055
Leucocytes (10^3/il)	57.93±1.17	57.65±1.07	57.65±1.79	59.40±3.10	56.87±1.07	0.896
Lymphocytes (%)	74.60±0.81	75.05±0.23	75.38±1.10	74.20±1.04	74.63±1.86	0.954
Monocytes (%)	1.20 ±0.05	1.22±0.06	1.23±0.03	1.37±0.06	1.20±0.05	0.188
Granulocytes (%)	24.20 ±0.85	23.73±0.18	23.39±1.07	24.43±0.98	24.17±1.80	0.962
Total protein (g/dl)	2.04±0.06	1.94±0.08	2.03±0.06	1.98±0.03	1.90±0.06	0.514
Albumin (g/dl)	0.65±0.02	0.62±0.02	0.65±0.02	0.63±0.02	0.62±0.03	0.790
Globulin (g/dl)	1.39±0.04	1.32±0.06	1.38±0.04	1.35±0.02	1.30±0.03	0.354
A/G ratio	0.468±0.003	0.470±0.004	0.471±0.001	0.467±0.010	0.477±0.006	0.053
AST(U/L)	42.33±1.20	43.82±2.17	40.33±1.45	42.00±1.16	43.26±1.54	0.580
ALT(U/L)	37.33±0.77	39.23±0.38	37.29±1.34	36.53±0.84	37.61±1.79	0.566
Cholesterol (mg/dl)	72.33±0.67	72.30±1.19	75.83±3.90	71.80±1.91	77.53±2.72	0.380
Triglyceride (mg/dl)	31.99±1.15	31.72±1.16	33.69±0.97	32.06±0.91	31.35±0.92	0.572

Note. T_1, T_2, T_3, T_4 and T_5, diets contained 0, 5, 10, 20 and 30% DDGS, respectively, in place of the mixture of fish meal, soybean meal and yellow corn in the control diet. A/G ratio, albumin-to-globulin ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Results are presented as means ± standard error.

Table 4: Effect of DDGS dietary levels on economic efficiency of Nile tilapia fish diets during the whole experimental period (16 weeks).

	Diets					
Items	T_1	T_2	T_3	T_4	T_5	*P*-value
Total feed input g/ fish ^{A}	75.68 ± 2.07	73.92 ± 1.41	73.68 ± 2.70	71.44 ± 5.60	70.96 ± 1.93	0.056
Total feed cost E£/ fish ^{B}	0.53 ^{a} ± 0.006	0.50 ^{b} ± 0.006	0.49 ^{b} ± 0.006	0.45 ^{c} ± 0.006	0.42 ^{d} ± 0.003	<0.0001
Total weight gain g/fish ^{C}	24.09 ± 1.51	24.26 ± 0 .95	23.39 ± 2.02	23.06 ± 3.92	22.18 ± 1.42	0.693
Total gain E£/fish ^{D}	0.72 ^{a} ± 0.006	0.73 ^{a} ± 0.006	0.70 ^{b} ± 0.006	0.69 ^{b} ± 0.006	0.67 ^{c} ± 0.006	<0.0001
Profit E£/fish ^{E}	0.19 ^{c} ± 0.000	0.22 ^{b} ± 0.003	0.21 ^{b} ± 0.003	0.24 ^{a} ± 0.003	0.24 ^{a} ± 0.003	<0.0001
Relative profit %	100 ^{c} ± 0.00	118 ^{b} ± 1.67	113 ^{b} ± 1.67	128 ^{a} ± 2.00	128 ^{a} ± 2.00	<0.0001

Note. T_1, T_2, T_3, T_4 and T_5, diets contained 0, 5, 10, 20 and 30% DDGS, respectively, in place of the mixture of fish meal, soybean meal and yellow corn in the control diet. E£, Egyptian pound. B = A × cost of diets (7, 6.83, 6.65, 6.30 and 5.95 E£ / kg diet T_1, T_2, T_3, T_4 and T_5, respectively. The cost of one kg DDGS is 3.50 E£. D = C × 30 E£ / kg fish. E = D - B. Relative profit (%) = (profit of DDGS diets / profit of control diet) × 100. Results are presented as means ± standard error. Means within the same row with different superscripts are significantly different at $P \leq 0.05$.

significantly affect the mortality rate of Nile tilapia fish [18, 21]. Also, other researchers reported that DDGS incorporated up to the level 30% in catfish diets without any negative effect on the mortality rate [22, 23].

Health Condition: Experimental fish were able to respond positively to all tested reflexes (Escape, defensive, tail and ocular reflexes), in aquaria. Fish reacted rapidly to external agitation, so they swim away from the water surface. Generally, escape movements of fish were energetic and their general healthy condition was good without any changes of the fish color. The sick fish did not react to the external agitation and were not able to exhibit normal behavioral responses (Loss of reflexes) and therefore can be easily caught by dip net or by hand be course sick fish are not energetic as reported previously [14]. There were no marked differences in the behavioral responses among the experimental fish groups.

Hematological and Biochemical Parameters: Results of blood hematological and biochemical parameters are illustrated in Table 3. All inclusion levels of DDGS (5, 10, 20 and 30%) had no significant (P=0.05) effect on all of measured hematological and biochemical parameters. In accordance with these findings, the dietary levels of DDGS (10 up to 40%) with or without supplemental lysine, did not affect the hematology of Nile tilapia [21]. In Nile tilapia (*Oreochromis niloticus*) fingerlings, no significant differences of the serological measurements among all fish groups. On an equal protein basis, the inclusion of DDGS as partial substitutes (Up to 60 %) for a combination of soy and corn meals had no significant effects on blood chemistry [18]. The replacement of fish meal and yellow corn in tilapia fingerlings diets with DDGS (4 up to 20%) had not any adverse effect on both of hemoglobin concentration and total count of leucocytes [25]. Values of plasma total protein concentration have not significantly changed among all fish groups received the tested diets. Similar results revealed that inclusion of corn DDGS, up to 40 % did not affect serum protein of Nile tilapia, *Oreochromis niloticus* [21] and Channel Catfish, *Ictalurus punctatus* [26].

The Economic Efficiency Measurements: Economic efficiency showed a significant increase of the profit with all dietary levels of DDGS. Generally, the relative profit was 118, 113, 128 and 128 %, respectively, for diets contained 5, 10, 20 and 30% DDGS compared with 100% for the control diet (Table 4). The improvement in the profit could be attributed to the inclusion of DDGS, which reduced the diet costs. The cost of DDGS is currently readily available and less expensive than other conventional protein sources [12]. Moreover, some previous trials reported that using of DDGS improved the economic efficiency of Nile tilapia fingerlings diets [19, 25].

CONCLUSION

Replacing the dietary FSC mixture with DDGS (5, 10, 20 and 30%) had no adverse effects on the growth performance and health condition. Moreover, this approach improved the economical efficiency of Nile tilapia fish.

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