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EFFECT OF DIETARY GRADED LEVEL OF SUBSTITUTING DISTILLERS DRIED GRAINS WITH SOLUBLES INSTEAD OF FISH MEAL IN TILAPIA DIET

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ABSTRACT

This study was carried out at El-Manzala Lake, Dakahlia Governorate, Egypt in order to investigate the effect of substitution of fish meal (FM) by graded levels of distillers dried grains with solubles (DDGS, being 25.0, 50.0, 75.0 and 100%) in fish diets on performanceof Nile tilapia (*Oreochromisniloticus*, initial body weight was 27.072 g/fish) reared in five floating net cages (12m×24m×3m) representing the dietary treatments and stocked into the experimental cages at a rate of 35 fish/m^3 (about 30.000 fish/cage). All the experimental groups were fed the experimental diets at a rate of 3% of the live body weight of the fish.The experimental period lasted 123 days.Conclusively, it is safe and economic to feed Nile tilapia fish diets containing DDGS (cheaper than fish meal, FM) to replace up to 100 % of the FM (which is locally not enough available) in fish diets. So, the results of the present field (i.e. its results are reproducible) study recommend using DDGS in Nile tilapia diets at replacing rate of 25 % for the best growth performance or 100 % for the best economic efficiency.

Keywords: Nile tilapia, DDGS, performance, chemical composition, blood, economy.

INTRODUCTION

Aquaculture continues to develop rapidly; world aquaculture production is increasing more rapidly than animal husbandry and capture fisheries. It is recognized that seafood production from capture fisheries is at its peak, and that aquaculture will become increasingly important as a source of seafood production. Therefore, there is widespread interest in aquaculture (Lucas, 2012).

To bridging the gap between the animal protein requirements and the actual production, it was necessary to produce a high quality of animal protein with low prices; the aquaculture can do that due to the fast growth and the high reproduction of fishes. In the year 2012, the aquaculture production reached 42.2 %, up from 25.7% in 2000 of the total production of capture and aquaculture (FAO, 2014). It is clear that the aquaculture production has steadily increased from 445 thousand tons in 2003 to 1.018 million tons in 2012; this increase appears the development of land area, the projects, and the technology of the aquaculture production in the last 10 years. (GAFRD, 2014).

Tilapia (Family *Cochleae*) has been an important source of food for man at least since recorded history began. Tilapia is potentially valuable fish in Egypt and is pausing well for semi-intensive and intensive culture. According to GAFRD (2014) the total production of tilapia culture in Egypt was 640058 tons representing about 47% of the total inland aquaculture income in 2012. Male Nile tilapia (*Oreochromis niloticus*) was therefore, chosen to carry out this study.

Including distillers dried grains with solubles (DDGS) in a cost-optimized formulation causes the levels of most other ingredients to change. The net effect is that increasing DDGS use by one tone reduces the use of other feeds by one tone. Diets are also formulated regionally to capture the differing feedstuff prices in different markets (Klasing, 2013). The DDGS contains all the nutrients of pre-processed corn but a reduced starch concentration. DDGS has at least three fold the nutrients as the raw grain. Each bushel of corn (25.4 kg) is converted into approximately 7.7 kg of DDGS, 8.2 kg of ethanol, and 8.2 kg of CO_2 (Jacques *et al.,* 2003). DDGS consists of non-fermented starch and sugars, and non-fermentable proteins, fibers, lipids, and minerals (Rosentrater and Tulbek, 2010).

The present study aimed to investigate water quality traits, growth performance, feed utilization, body chemical composition, blood profile, besides economic evaluation of fingerlings monosex Nile tilapia (*Oreochromis niloticus*) fed formulated diets containing different levels of distillers dried grains with solubles (DDGS) which partially and totally replace fish meal in order to reduce feed cost of Nile tilapia cultured in fish cages.

MATERIALS AND METHODS

The present experiment was carried out at one site in the project of fish cage culture of young farmers in El-Manzala Lake, Dakahlia Governorate, Egypt. in order to evaluate the effect of substitution of fish meal (FM) by graded levels of distillers dried grains with solubles (DDGS) in fish diets on growth performance, feed utilization, body chemical composition, blood profile, and economic efficiency of Nile tilapia reared in floating net cages. The experimental period lasted 123 days. The experiment started at third of July 2012 and finished at the 3rd of November 2012.

Experimental fish and stocking density:

Nile tilapia (*Oreochromis niloticus*) all male fingerlings sex reversed with hormonal treatment was used. The finger length fishes were purchased from a private hatchery in Damietta governorate, Egypt and transported at early morning using a special fish-transporting car with aeration facilities. The fishes were adapted to the experimental system for 10 days in a big fish cage with dimensions 24m×24m×3m with nets 90/50 cm (mesh size ≈ 0.5 cm) and fed the control diet as an acclimatization period before starting the experiment. Thereafter, the fishes were redistributed randomly into five experimental cages,each cage with dimension 12m×24m×3m representing one of the dietary treatments and stocked into the experimental cages at a rate of 35 fish/m^3 (about 30.000 fish/cage). To evaluate growth rate, the fishes were individually weighed biweekly to the nearest 0.1g and the total length was measured to the nearest 0.1 cm. The experimental fishes received the tested diets twice daily at 9.00 a.m. and 3.00 p.m. daily. All the experimental groups were fed the experimental diets at a rate of 3% of the live body weight of the fish. The feed quantity was revalued biweekly on the basis of the actual average biomass of the fish in each treatment. Average initial body weight was 27.072±3.272 g/fish, average initial length was 9.92±0.92 cm/fish and average standard length was 7.67±1.5 cm/fish. The nets of the cages were changed twice, the first one after one month to 50/50 cm (mesh size ≈ 1 cm) then after another one month to 25/50 cm (mesh size ≈ 2 cm).

The experimental fish groups:

The fishes under experiment were distributed randomly into five cages as follow:

The first cage: fishes were considered as control and fed the basal diet with fishmeal, FM (without DDGS).

The second cage: fishes were fed the basal diet with quantitatively replacement of 25% FM by the same weight of DDGS.

The third cage: fishes were fed the basal diet with quantitatively replacement of 50% FM by the same weight of DDGS.

The forth cage: fishes were fed the basal diet with quantitatively replacement of 75% FM by the same weight of DDGS.

The fifth cage: fishes were fed the basal diet with quantitatively replacement all FM (100%) by the same weight of DDGS.

Experimental diets preparation:

Local dry ingredients of the experimental diets were thoroughly grinded, mixed and pelleted in a private feed factory in Al-Asafra industrial zone, Dakahlia governorate, Egypt in 1.5 mm diameter for small fish and 2.5 mm for bigger ones and stored in plastic bags till be used.

Growth performance and feed utilization determination:

At the beginning of the experiment, randomly samples of fishes were measured for its weight and total and standard length. At the end of the experiment, samples of each cages' fish were weighed to determine or calculate the growth performance and feed utilization parameters according toAbdelhamid (2009).

Internal organs indices:

At the end of the experiment, three fishes per treatment were randomly taken, individually weighed and total and standard lengths were measured; then, the liver and the gonads were individually removed and weighed to calculate the condition factors (K_t and K_s), the hepatosomatic index (HSI) and the gonadosomatic index (GSI), respectively,according to Abdelhamid (2009).

Proximate analysis of fish and diets:

At the start of the experiment, 10 fishes were taken and kept frozen until carrying its chemical analysis. At the end of the experiment, the experimental diets and a random sample of five fish collected from each treatment were weighed and grinded, then dried at 70°C for 24 hours and grinded again to be a meal, then assayed to determine the moisture, crude protein, crude fiber, ether extract and ash contents by using Standers Methods (AOAC, 1990). Gross energy was calculated according toNRC (1993).

Economic evaluation:

Economic evaluation (New, 1987) of the experimental diets has been calculated by evaluation the feed cost in Egyptian pound (L.E) needed to produce 1 kg of live bodyweight gain of each experimental fish group.

Statistical analysis:

Data obtained were analyzed using one-way analysis of variance according to statistical analysis system software (SAS, 2006) for windows. Multiple range test (Duncan's, 1955) was used to compare between the parameters of the different nutritional groups. The differences were significant at 0.05 levels.

RESULTS AND DISCUSSION

Water quality criteria:

Table 1 presents means of some quality criteria measured for the rearing water of the experimented fish throughout the experimental period. These means are very suitable for rearing Nile tilapia as mentioned by Abd El-Hakim *et al*. (2002) and Abdelhamid (2009).

Table 1: Water quality along experiment time

Parameter	3 Jul	26 Jul	16 Aug	6 Sep	27 Sep	18 Oct
Temperature, ° C	27	29	28	28	27	24
Salinity, ‰	1.7	1.8	1.6	1.6	1.6	1.6
pH-value	7.8	8.1	7.8	7.7	8	8.1
DO, mg/l	7.8	6.5	7	6.5	7.5	8
Transparency, cm	28	26.5	27	27	28	28
Alkalinity, mg/l	145	165	150	148	155	162
Hardness, mg/l	187	194	185	187	186	190

Composition and chemical analysis of the experimental diets:

The determined chemical composition of the DDGS used herein is 12.90 % dry matter, 44.06 % crude protein, 14.09 % ether extract, 11.00 % crude fiber, 5.61 % ash, and 256.7 Kcal/100g energy content.The composition and proximate analysis of the experimental diets are shown in Table 2. The increase of DDGS level in the diets was associated with lower diet's cost. So, the most expensive diet (4250 LE/ton) was T_1 (control, 1^{st} cage). The price was gradually decreased thereafter by increasing the DDGS level from T_2 till T_5 which was the cheapest diet (2850 LE/ton) that contained 100% replacement of DDGS instead of FM. The decreased diets' costs (from T_1 to T_5) may be due to lowering the crude protein contents (from 27.86 to 24.32 %). However, T.U.S.G.C. (2008) mentioned that DDGS is a by-product of ethanol production from grains. It contains 1044-2639 kcal / kg digestible energy, 3378-3827 kcal / kg metabolizable energy, 0.89-0.91 % available phosphorus, 29 % crude protein, 44-78 % (of the crude protein) lysine, 74-89 % methionine, and 62-87 % threonine.

Table 2: Composition and chemical analysis of the experimental diets.

Ingredients (kg/ton)	T_1 Control	T_2 25%	T_3 50%	T_4 75%	T_5 100%
Rice bran	240	240	240	240	240
Yellow corn	150	150	150	150	150
Fine wheat bran	200	200	200	200	200
Corn Gluten	50	50	50	50	50
Soybean meal	200	200	200	200	200
Fish meal, FM	150	112.5	75	37.5	0.00
DDGS	0	37.5	75	112.5	150
Vit. & Min. premix(1)	10	10	10	10	10
Total	1000	1000	1000	1000	1000
Total cost (LE)(2)	4250	3900	3550	3200	2850
Chemical composition, % on dry matter basis:					
DM	89.33	89.15	89.59	89.45	88.47
OM	10.67	10.85	10.41	10.55	11.53
CP	27.86	27.10	26.05	25.01	24.32
EE	8.31	8.12	9.29	8.14	8.41
CF	6.5	7.5	5.5	8.5	6.5
Ash	8.92	8.77	9.16	8.92	9.02
NFE(3)	48.41	48.51	50	49.43	51.75
GE, Kcal/100 g DM(4)	462.2	460.7	463.84	456.82	456.87
Protein/Energy (P/E ratio) mg CP/Kcal GE(5)	60.19	58.82	56.16	54.74	53.23

(1)Vit& Min. premix each 1 kg contains: Vit. A, 12.000.000 IU; Vit. D3, 3000.000 IU; Vit. E, 10.000 mg; Vit ptK3, 3000 mg;Vit. B1, 200 mg; Vit. B2, 5000 mg; Vit B6, 3000 mg; Vit. B12, 15 mg; Biotin, 50 mg; Folic acid, 1000 mg; Nicotinic acid,35000 mg; Pantothenic acid, 10.000 mg; Mn 80 mg; Cu 8.8 g; Zn 70 g; Fe 35 g; Co 0.15 g and Se 0.3 g.

(2)The total cost (LE=Egyptian pound) is continuous changed and this cost is the average cost of the local dietary ingredients along the experiment time, July- November 2012.

(3)Nitrogen free extract (carbohydrate) content was calculated by subtraction the total percentages of CP, EE, CF and ash from 100.

(4)The gross energy contents of the experimental diets and fish samples were calculated by using factors of 5.65, 9.45 and 4.2 Kcal/g of protein, lipid and carbohydrate, respectively (NRC, 1993).

(5)P/E ratio (mg protein/Kcal gross energy) = CP/GE × 1000

Growth performance:

Data of growth performance parameters and survival rate of the tested fish are given in Table 3. All the tested growth performance parameters reflected significant ($P<0.0001$) differences among treatments (DDGS levels) in favor of the first two treatments (diets). That means that it could replace up to 25 % of the dietary FM by DDGS without any adverse effects on growth performance parameters of the treated fish. There was no significant ($P\geq0.05$) effect of DDGS levels on survival rate among fish groups.

Similar results were obtained by other researchers that confirmed the present findings, since the DDGS could replace 15-50 % of the dietary fish meal in salmon's diet without any adverse effect on growth and feed utilization **(T.U.S.G.C., 2008).**Ethanol production is expected to increase by three fold and hence the increased DDGS availability. Therefore, incorporation of DDGS in various animal feeds becomes necessary. Hence, Tidwell *et al*. (1990) partially replaced corn and soybean meals by DDGS in fish diets. They observed similar weight gain (WG), survival, feed conversion ratio (FCR), and protein efficiency ratio (PER) for fish fed a diet containing 40% DDGS compared to the control diet that have 0% DDGS. Another study indicated that diets using a combination of 35% DDGS and varying concentrations of soybean meal could totally replace fish meal in channel catfish diets without adversely affecting growth or survival (Webster *et al*., 1992). A study on channel catfish using DDGS found that 90% DDGS was sufficient for growth without the addition of supplemental amino acids (Webster *et al*., 1993).

Li *et al*. (2010) examined the use of DDGS in diets and the effects of additional dietary fat on channel catfish performance and found that fish fed the 30 % DDGS diet consumed more diet and gained more weight, but had a similar feed efficiency ratio (FER) compared with fish fed the control diet with additional fat. Yet, Salama*et al*. (2011) registered that using DDGS to replace 50 or 100 % of the dietary fish meal significantly reduced bodyweight gain of tilapia by about 32 and 58 %, respectively comparing with the control.

However, Barnes*et al.* (2012) suggested that DDGS decreased juvenile rainbow trout growth at dietary concentrations of at 10% or greater. Additionally, Gabr*et al.* (2013) and Khalil *et al*. (2013) concluded that DDGS could replace 10-16 % of fishmeal (or soybean, respectively) and yellow corn in diets of Nile tilapia fingerlings without any adverse effect on growth performance.

Table 3: Means of effect of feeding different levels of DDGS on final body weight (FW, g/ fish), total bodyweight gain (TWG, g/fish), average weight gain (AWG, g/fish/day), relative growth rate (RGR, %), specific growth rate, SGR, %/day), and survival rate (SR, %) of Nile tilapia reared in cages.

Treatment	FW	TWG	AWG	RGR	SGR	Survival (%)
Control	285.6[a]	258.5[a]	2.12[a]	954.9[a]	1.93[a]	68.57
25% DDGS	284.5[a]	257.4[a]	2.11[a]	950.8[a]	1.92[a]	68.74
50% DDGS	268.9[b]	241.8[b]	1.98[b]	893.1[b]	1.88[b]	68.19
75% DDGS	270.9[b]	243.8[b]	1.99[b]	900.3[b]	1.89[b]	67.68
100% DDGS	269.4[b]	242.3[b]	1.99[b]	895.2[b]	1.88[b]	67.76
± SEM	1.634	1.634	0.013	6.036	0.005	-
P - value	0.0001	0.0001	0.0001	0.0001	0.0001	-

a and b: Means in each column have different letters are significantly different (P<0.05).

Feed utilization:

Data of feed utilization parameters were calculated as means and given in Table 4. All of these parameters differed significantly (P<0.0001) among treatments (DDGS levels) in favor of the fifth treatment (100 % DDGS instead of FM). This means that it could replace up to 100 % of the dietary FM by DDGS without adverse effects on feed utilization parameters by the treated fish. Nearly similar results were obtained by other researchers but with variable DDGS recommended levels. In this respect, Wu *et al.* (1996) found that tilapia fed with the feed that contained DDGS had comparable weight gain, feed conversion efficiency, and protein efficiency ratio with control diets. Also, T.U.S.G.C. (2008) recommended replacing 15-50 % of the dietary fish meal in salmon's diet without any adverse effect on feed utilization.Moreover, DDGS could replace 20 % of fish meal and soybean meal of the Japanese trout feed (Anon., 2010). However,Goda*et al.* (2011) concluded that replacing up to 60 % of soybean meal by DDGS for 84 days enhanced feed utilization.

Table 4: Means of effect of feeding dietary different levels of DDGS on feed utilization of Nile tilapia reared in cages, in form of feed intake (FI, g/fish), Feed conversion ratio (FCR), Protein efficiency ratio (PER), protein productive value (PPV,%), and energy utilization (EU, %).

Treatment	FI	FCR	PER	PPV	EU
Control	326.5^{a}	1.26^{c}	2.84^{d}	44.77^{a}	28.51^{b}
25% DDGS	323.9^{b}	1.26^{c}	2.93^{c}	31.66^{c}	23.19^{c}
50% DDGS	315.9^{c}	1.31^{a}	2.94^{c}	44.87^{a}	31.07^{a}
75% DDGS	313.9^{d}	1.29^{ab}	3.11^{b}	45.55^{a}	30.69^{a}
100% DDGS	308.8^{e}	1.28^{bc}	3.23^{a}	43.86^{b}	30.74^{a}
± SEM	0.692	0.009	0.022	0.307	0.208
P - value	0.0001	0.0011	0.0001	0.0001	0.0001

a-d: Means in each column have different letters are significantly different (P<0.05).

Gabr*et al.* (2013) and Khalil *et al*. (2013) concluded that DDGS could replace 10-16 % of FM (or soybean, respectively) and yellow corn in diets of Nile tilapia fingerlings without any adverse effect on feed utilization. Øverland*et al.* (2013)had evaluated DDGS in diets for rainbow trout. Feeding the 50% DDGS diet resulted in higher feed intake and weight gain, and lower feed conversion ratio (FCR) than in trout fed the control diet, while feeding the 100% DDGS diet resulted in a lower FCR compared with the control and the 50% DDGS diets. Fish fed the 100% DDGS diet had higher energy retention than those fed the control diet, and had higher nitrogen retention than those fed the control and DDGS 50% diets.

Juvenile Nile tilapia were fed diets containing 0, 10, 20, 40% DDGS, and 40% DDGS with supplemental synthetic lysine, as partial replacements for soybean meal and corn meal, for 10 weeks, and challenged with *Streptococcusiniae* (Lim *et al*., 2007). Fish fed the 40% DDGS diet had the lowest weight gain, protein efficiency ratio, whole body protein, and poorest feed conversion, but supplementing the 40% DDGS diet with synthetic lysine improved weight gain and protein efficiency ratio.Feeding diets containing DDGS had no effect on number of days to first mortality, cumulative mortality 14 days post-challenge, or on hematological and immunological parameters. The authors concluded that up to 20% DDGS can be added to the diet as a partial substitute for soybean meal and corn meal without affecting growth performance, body composition, hematology, immune response, and resistance to a *Streptococcus iniae* infection.

Abo-State *et al.* (2009) replaced soybean in increments between 0 and 100% with DDGS in diets, with or without phytase, and fed them to Nile tilapia (2 g initial body weight). They observed the best growth rate and feed conversion in diets containing 0, 25, and 50% DDGS with phytase. Yet, Schaeffer *et al.* (2009) evaluate the use of DDGS in diets of tilapia. Feeding diets containing 0,17.5,20,22.5, 25, and 27.5% DDGS to partially replace fishmeal resulted in the highest weight gain, feed conversion, and protein efficiency ratio(PER) for fish fed the 0% DDGS diet, except the 17.5% DDGS diet which provided better feed conversion and PER. Nile tilapia were fed 20, 25, and 30%DDGS diets with or without a probiotic, and no differences were found for weight gain, feed conversion, or PER among dietary treatments. The DDGS can be a highly economical feeding ingredient in tilapia diets, and can successfully be used at relatively high dietary inclusion rates if appropriate supplementation of amino acids is done.

Chatvijitkul (2013) evaluated the substitution of soybean meal with lipid-extracted distillers dried grains with solubles (LE-DDGS) in practical diets for the hybrid tilapia (*O. niloticus*× *O. aureus*). The diets contained 0, 20, 30, 40, and 50 % as substitutes for soybean meal on an isonitrogenous basis. Results indicated that formulated diets containing increasing percentages of LE-DDGS without lysine supplementation led to reduced growth of hybrid tilapia. However, positive performances including growth, feed conversion ratio, survival, protein retention, and energy retention were demonstrated in fish fed diet using 40 % LE-DDGS of diet with lysine supplementation.

Some indices of fish organs:

Data calculated for some indices are given as means in Table 5. The significant effect was on condition factor calculated on standard length basis(Ks) ($P<0.001$) andhepato-somatic index(HIS) ($P<0.01$), whereas both of condition factor calculated on total length basis(Kt) andgonado-somatic index(GSI) did not differ significantly ($P\geq0.05$) among treatments due to DDGS levels. Lim and Webster (2006) confirmed that DDGS can be included in Nile tilapia diets by 20% without significant effect on growth performance. However, the fish fed diets containing 30% DDGS had similar WG, PER and FER as those fed the control diet, while fish fed 40% DDGS had significantly lower WG, PER and PER than the control. Likewise, hepato-somatic index indicated that the use of 20% DDGS in the diets showed no negative effects on Nile tilapia condition; while the use of higher levels of DDGS may to negatively affect condition and growth.

Schaeffer *et al.* (2009) used three isocaloric and isonitrogenous diets for juvenile Nile tilapia, the diets contain 20, 30 and 40% DDGS compared with the control diet which contain FM. Weight gains, conversion ratios and hepatosomatic indices did not significantly differ between fish fed 20% DDGS and the control diet. No difference occurred in protein efficiency ratios among all diets, the results indicate that aquaculture diets incorporating 20% DDGS may gain the some performance like the commercials diets of the similar energy content.

Barnes *et al.* (2012) examined the responses of juvenile rainbow trout to diets containing DDGS supplemented with phytase and amino acid and found that the fishes receiving diets containing concentrations of DDGS weighed significantly less and had significantly poorer FCR than of fish fed the control.Also the hepatosomatic index was less than the control diets but the body fat was significantly greater in the fish receiving 20% DDGS compared to fish fed either of the other two diets and there is no significant differences in individual fish weight, condition factor, or any fish health measurements among diet treatments. Also fillet composition was not significantly different among fish reared on any of the diets.

Table 5: Means of effect of feeding dietary different levels of DDGS on body indexes of Nile tilapia reared in cages.

Treatment	K_t	K_s	GSI	HSI
Control	1.91	3.14^b	0.69	2.07
25% DDGS	1.90	3.22^b	0.69	2.21
50% DDGS	1.93	3.41^a	0.72	2.25
75% DDGS	1.92	3.37^a	0.66	2.17
100% DDGS	1.92	3.37^a	0.66	2.32
± SEM	0.013	0.0332	0.0301	0.172
P - value	0.5819	0.001	0.6827	0.0096

a and b: Means in each column have different letters are significantly different (P<0.05).

Chemical composition of fish:

The proximate chemical composition of whole fish body was carried out at the start (Table 6) and at the end (Table 7) of the experiment. It was noticed that ether extract percentage (and hence, the energy content) was higher and ash percent was lower in the aged fish (at the end of the experiment) than in the younger ones (at the beginning of the experiment). That is a fact as mentioned by **Abdelhamid (2009).**

Table 6: Means ± SE of chemical composition (% on dry matter basis) of fishes at the start of the experiment.

Dry matter, DM	Crude protein, CP	Ether extract, EE	Ash	Gross energy, GE, kcal/100 g
23.97±0.52	60.57±0.65	20.90±0.70	18.53±0.20	538.89±1.30

However, fish groups fed DDGS-containing dies reflected lower crude protein and ash but higher ether extract and energy contents than the control fish group (Table 7). All chemical composition and energy content were significantly (P<0.0001, except ash at P<0.05) affected by dietary DDGS inclusion. The 50 % DDGS containing diet caused the highest dry matter and the lowest ash percentages in the whole fish body. Yet, the control group reflected the highest crude protein and ash but the lowest ether extract and energy contents. As a fact also, Table 7 confirm the positive correlations among dry matter, crude protein and ash contents on one side and the negative correlation between crude protein and ether extract contents on the other hand.

Hung (2007) fed common carp by DDGS and result that common carp can be fed to growing to 15%. He indicated also that there is no significant different between the treatments in survival rate, the feeding diets up to 15% DDGS for 3 months didn't affect the chemical composition of fish.

The decreased performance of the 40% DDGS-diet was attributed to a deficiency of lysine since supplementation with 0.4% lysine hydrochloride improved weight gain and feed efficiency to levels comparable to those of the SBM-based control diet (Lim *et al.,* 2007). They observed that, except for protein content in fish fed the 40% DDGS-diet without lysine supplementation that was significantly lower than that fed the SBM-based diet, whole proximate body composition was not affected by dietary levels of DDGS. The decreased protein content in fish fed the 40% DDGS diet without lysine supplementation was attributed to smaller size fish which has less flesh and/or the imbalance of dietary essential amino acids such as deficiency of lysine, which may contribute to reduced protein synthesis.

Whole-body protein and ash % of fish were not influenced by dietary levels of DDGS; however, fish fed diets containing DDGS had increased body fat, may be due to improved diet digestibility leading to higher available energy (Lim *et al.,* 2008). There was no significance in diet consumption and survival rate between the various experimental diets, fish fed the SBM + DDGS diet had a higher percentage of fillet fat and lower moisture compared with fish fed the other diets. There were no other significant differences in fillet proximate composition. FCR was the lowest for fish fed the SBM + DDGS diets (**Robinson and Li, 2008**).

Table 7: Means of effect of feeding dietary different levels of DDGS on chemical composition (% on dry matter basis) and energy content (Kcal./100g) of Nile tilapia reared in cages.

Treatment	DM	CP	EE	Ash	EC
Control	25.47^{b}	61.38^{a}	25.01^{c}	13.61^{a}	582.27^{b}
25% DDGS	20.15^{c}	55.34^{c}	32.16^{ab}	12.50^{b}	615.70^{a}
50% DDGS	27.08^{a}	56.04^{bc}	31.57^{b}	12.33^{b}	614.47^{a}
75% DDGS	25.87^{b}	56.62^{b}	30.93^{b}	12.44^{b}	611.37^{a}
100% DDGS	25.40^{b}	53.86^{d}	33.69^{a}	12.50^{b}	621.33^{a}
± SEM	0.369	0.354	0.503	0.256	3.144
P – value	0.0001	0.0001	0.0001	0.0293	0.0001

a - c: Means in each column have different letters are significantly different (P<0.05).

Blood hematology of fish:

Table 8 presents mean values of hematological parameters measured or calculated for fish blood at the end of the experiment. Hemoglobin concentration (Hb, g/dl), red blood cells count (RBC_s, x $10^6/mm^3$), hematocrit (Hct, vol. %), and white blood cells count (WBC_s, x $10^3/mm^3$) were significantly affected by the dietary inclusion of DDGS; whereas, mean corpuscular volume (MCV, μ^3), mean corpuscular hemoglobin (MCH, µ µ), mean corpuscular hemoglobin concentration (MCHC, %), platelets count (PLT, No./mm^3) were not significantly ($P \geq 0.05$) affected by the dietary inclusion of DDGS.

A study was conducted to examine the effect of dietary levels of DDGS on hematology, immune response, and resistance of channel catfish, *Ictalurus punctatus*, to *Edwardsiella ictaluri* challenge. Dietary treatment had no effect on red and white blood cell counts. Hemoglobin and hematocrit were significantly higher in fish fed diets containing DDGS than in those fed the control diet. Fish fed 20–40% DDGS diets had increased serum total immunoglobulin (Lim*et al*., 2009).

Table 8: Means of effect of feeding dietary different levels of DDGS on some blood hematological parameters of Nile tilapia reared in cages.

Treatment	HGB	RBC	HCT	MCV	MCH	MCHC	PLT	WBC
Control	6.27^{a}	2.36^{a}	35.77^{a}	154.13^{b}	31.23	19.87	37.00	77.93^{a}
25% DDGS	3.97^{b}	1.67^{c}	25.23^{b}	159.57^{ab}	34.57	21.07	58.00	36.90^{c}
50% DDGS	6.47^{a}	1.87^{bc}	33.27^{a}	163.83^{a}	30.93	18.57	52.30	74.30^{ab}
75% DDGS	6.60^{a}	2.28^{ab}	34.93^{a}	159.30^{ab}	32.43	19.67	26.67	58.93^{b}
100% DDGS	6.23^{a}	3.32^{ab}	33.77^{a}	157.30^{ab}	30.13	19.10	23.00	83.70^{a}
± SEM	0.0003	0.0208	0.0023	0.1926	0.33	0.5912	0.1983	0.0010
P - value	0.278	0.142	1.1	2.59	1.504	1.11	11.37	5.64

a-c: Means in each column have different letters are significantly different ($P<0.05$).

Hematology and immunity were not affected by DDGS source. It is concluded that DDGS from all the sources examined can be included in the diet of juvenile hybrid tilapia at about 30% as a replacement of one-third protein from SBM-CM mixture without adverse effects (Welker *et al*., 2014).

Blood plasma biochemical parameters:

Table 9 showed the mean concentrations of plasma total proteins, albumin (AL) and globulin (GL) as well as the AL/GL ratio. Total proteins and globulin concentrate on differ significantly ($P<0.05$) but albumin and AL/GL ratio did not significantly ($P \geq 0.05$) affected by the dietary treatments (DDGS levels). Total proteins concentration as well as globulin values in fish groups fed the control or 50, 75, and 100 % DDGS containing diets were significantly similar, i.e. even up to 100 % substituting of DDGS instead of FM can preserve fish health and immunity through increasing γ-globulin which is produced by lymphocytes and is concerned with antibody formation or immunity, i.e., is responsible for resistance to infection (Soliman and Abd El-Moty, 1974).

Table 9: Means of effect of feeding dietary different levels of DDGS on plasma proteins (g/dl) and AL/GL ratio of Nile tilapia reared in cages.

Treatment	Total protein	AL	GL	ALGL
Control	5.60^{a}	4.23^{a}	1.36^{a}	3.09^{a}
25% DDGS	3.00^{b}	2.13^{b}	0.87^{b}	2.77
50% DDGS	4.67^{a}	3.47^{ab}	1.20^{ab}	2.97
75% DDGS	5.27^{a}	3.83^{ab}	1.43^{a}	2.75
100% DDGS	4.80^{a}	3.60^{ab}	1.20^{ab}	3.02
± SEM	0.479	0.516	0.106	0.605
P - value	0.0256	01231	0.029	0.9917

a-b: Means in each column have the different litter are significantly different (P<0.05).

Moreover, Table 10 illustrates mean concentrations of blood plasma creatinine and triglycerides and activity of both enzymes (ALT and AST). All these criteria were significantly affected by the dietary inclusion of DDGS. Up to 100 % substitution of FM by DDGS did not negatively affect kidney nor liver functions of the tested fish. The evaluation of hematological and biochemical characteristics in fish has become an important means of understanding the immune response. Hematological parameters of fish blood are useful tools that aid in the study immuno potentiators. Such tests are general but not conclusive and must be correlated with biochemical tests of the subject.

Lim *et al.* (2007) conducted a feeding study to evaluate the effect of dietary levels of corn DDGS on hematology and resistance of Nile tilapia, *Oreochromis niloticus*, to *Streptococcus iniae* challenge. Hematological and immunological parameters were not affected by the dietary treatments.

Table 10: Means of effect of feeding dietary different levels of DDGS on plasma creatinine (mg/dl), total lipids (mg/dl) and liver function parameters (alanine aminotransferase, ALT and aspartate aminotransferase, AST in u/L) of Nile tilapia reared in cages.

Treatment	Creatinine	Triglycerides	ALT	AST
Control	0.33^{a}	250.30^{c}	60.33^{b}	660.30^{a}
25% DDGS	0.30^{ab}	378.30^{a}	13.33^{d}	88.00^{d}
50% DDGS	0.13^{c}	319.00^{b}	300.00^{a}	95.67^{cd}
75% DDGS	0.13^{c}	249.00^{c}	31.00^{c}	112.00^{c}
100% DDGS	0.20^{bc}	274.67^{c}	40.67^{c}	188.00^{b}
± SEM	0.0369	9.19	4.267	6.33
P - value	0.0076	0.0001	0.0001	0.0001

a-d: Means in each column have different letter are significantly different (P<0.05).

Economic efficiency:

Tables 11 and 12 include calculation of the economic efficiency by feeding fish DDGS containing diets. The fifth diet (100 % DDGS) was the cheapest one to produce one Kg body weight gain of fish (Table 11). Feeding the experimental fish the 100 % DDGS containing diet resulted in the highest economic efficiency (199.99 %, Table 12).

Table 11: Cost of feed needed to produce 1 kg of body weight gain

Treat.	Total production (kg)	Price (LE)	Total income (LE)	Feed price (LE)	Gain (LE)	EE
Control	5873	12	70476	40035	30441	176.04
25%	5842	12	70104	36738	33366	190.82
50%	5396	10	53960	33441	20519	161.36
75%	5391	10	53910	30144	23766	178.84
100%	5369	10	53690	26847	26843	199.99

The costs of gain in fish fed unpelleted DDGS was significantly lower than in fish fed the pelleted DDGS (Tidwell *et* al., 2000).In another study which performed in aquaria with juvenile hybrid tilapia (*Oreochromis* niloticus ×*Oreochromis* aureus) to evaluate the use of different protein sources in combination with distillers dried grains with solubles (DDGS) compared with the control diet, the control diet based on fish meal (12%) and soybean meal (41%) protein and the other three experimental diets contained 30% DDGS by weight in combination with: 8% fish meal and 34% soybean meal (Diet 2), 26% meat and bone meal (MBM) and 16% soybean meal (Diet 3), and 46% soybean meal alone (Diet 4). The unit cost of Diet 4 was higher than Diets 1, 2, 3 which were the similar cost unit (Coyle *et al.* 2004).

Table 12: Data of calculating the economic efficiency (EE, %) by feeding fish DDGS containing diets.

Treatment	FCR	Feed cost (LE/kg)	Cost of feed needed
Control	1.26	4.25	5.355
25%DDGS	1.26	3.9	4.914
50% DDGS	1.31	3.55	4.6505
75% DDGS	1.29	3.2	4.128
100% DDGS	1.28	2.85	3.648

Schaeffer *et al*. (2010) used DDGS as a fishmeal replacer for juvenile Nile tilapia. On a dry matter basis, five isocaloric-isonitrogenous diets were formulated to contain 17.5, 20, 22.5, 25, and 27.5% DDGS and compared against the reference diet without DDGS. The authors found that fishmeal may be replaced with low levels of DDGS to reduce feeding cost.

Goda*et al.* (2011) studied the effect of partial substitution (0, 20, 40, 60, 80 and 100 %) of dietary soybean meal (SBM) with DDGS on Nile tilapia, *Oreochromis niloticus*. Results indicated that diets in which up to 60% of the SBM are replaced by DDGS are as cost effective, as the control diet.

Conclusively, the obtained results recommend the replacement of 25 % of FM by DDGS for the best growth performance or 100 % for the best economic efficiency.

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تأثير احلال مستويات غذائية متدرجة من نواتج تقطير الحبوب الجافة وذائباتها محل مسحوق السمك فى عليقة البلطى

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أجرى هذا البحث فببحيرة المنزلة بمحافظة الدقهلية. واستهدفت الدراسة بحث تأثير إحلال مستويات متدرجة (٢٥، ٥٠، ٧٥، ١٠٠ %) من نواتج تقطير الحبوب الجافة وذائباتها (DDGS) محل مسحوق السمك فى علائق أسماك البلطى النيلى (٢٧.٠٧٢ جم) المرباة فى أقفاص شبكية (بأبعاد ١٢ x ٢٤x٣ م) بكثافة ٣٥ سمكة/م٣(٣٠.٠٠٠ سمكة/قفص)، وقُدمت العلائق لمدة ١٢٣ يوما على وجبتين يوميا بمعدل ٣ % من وزن الجسم. واستخلص من هذه الدراسة أن تغذية أسماك البلطى النيلى على علائق تحتوى ألـDDGS (أرخص من مسحوق السمك، غير المتاح بوفرة محليا) حتى نسبة احلال ١٠٠ % هو أمر آمن واقتصادى، لذا تُوصى نتائج الدراسة الحقلية (أى التى يمكن تكرار الحصول على نفس نتائجها) الحالية باستخدام ألـ DDGS فى علائق أسماك البلطى النيلى بنسب إحلال ٢٥ % للحصول على أفضل أداء نمو أو ١٠٠ % للحصول على أقصى كفاءة اقتصادية.