Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
ISSN NO. 2320-5407
Article DOI:10.21474/IJAR01/8017
DOI URL: http://dx.doi.org/10.21474/IJAR01/8017

RESEARCH ARTICLE

INCLUSION OF A HIGH PROTEIN DISTILLERS DRIED GRAINS PRODUCT IN NILE TILAPIA DIETS.

Khaled Mohamed Wasly[1] , Ashraf H. Gomaa[1] and Wafai Z. A. Mikhail[2].
1. Regional Center for Food and Feed, Agriculture Research Center, Giza, Egypt.
2. Dept. Natural Resources, Inst. African Research & Studies, Cairo University.

Manuscript Info

Manuscript History
Received: 05 September 2018
Final Accepted: 07 October 2018
Published: November 2018

Keywords:-
Nile tilapia, DDGS, HPDDGS, Protein concentrate.

Abstract

The present study was carried out in the fish laboratory in the Regional Center for Food and Feed (RCFF), Agricultural Research Center, Giza, Egypt. And designed to evaluate the nutritional value of corn co-products from ethanol production, corn distillers dried grains with soluble (DDGS) and a high protein of corn distillers dried grains product (HPDDGS) (by reducing the percent of soybean meal and yellow corn in tested diets), on growth performance, and feed utilization, of Nile tilapia (*Oreochromis niloticus*). Seven experimental diets were formulated Control (Diet 1) doesn't contains both DDGS and HPDDGS, (Diet2 – Diet4) to replace DDGS at the rate of 15, 30, and 45% instead of soybean meal and yellow corn. (Diet5 – Diet7) to replace HPDDGS with supplementation lysine at the rate of 15, 30, and 45% instead of soybean meal, The all experimental diets were isonitrogenus (30% crude protein) and isocaloric (430 kcal /100 g diet), the seven diets were fed to 7 tilapia fingerling groups with initial weight of 2.25 g/ fish. Each group contained of 3 aquaria. The experiment duration was 12 weeks and the fish were fed at a rate of 4% of fish body weight daily. The daily allowances were fed at 5 meals. The results showed that, weight gains and feed utilization of tilapia fingerlings fed (DDGS)15% and (DDGS)30% diets were not different compared to (control) diets, but (*Oreochromis niloticus*) fed (DDGS) 45% diets gained less weight than those fed control ($P < 0.05$). The results of this experiment suggest that DDGS is a good replacement for yellow corn and soybean meal, and can be used up to 30% in the diet to maintain the growth performance and also fish group given HPDDGS diet levels (15%HPDDGS, 30%HPDDGS, 45%HPDDGS), with lysine supplementation showed better in the Growth performance and feed utilization when compare with the control diet. HPDDGS is a good alternative protein source and can be used up to 45% or maybe more with lysine supplementation. No effects were observed of both DDGS and HPDDGS levels on water quality.

Corresponding Author:-Wafai Z. A. Mikhail.
Address:-Dept. Natural Resources, Inst. African Research & Studies, Cairo University.

Introduction:-

Aquaculture might be known in ancient Egypt. There are paintings in some of the tombs of the pharaoh's which appear fish, probably tilapia fish, in man-made pools indicating some type of fish culture **(Mcvey, 1994).** Even though, tilapia are among the easiest and most profitable fish produced in aquaculture, partially due to their low trophic level (Omnivorous diet). Feed cost still represents the highest expense in its production (up to 50% of total cost production (**Lim *et al*., 2011).** Protein fraction is usually the most expensive component of the diet **(Coyle *et al*., 2004),** traditionally provided by the incorporation of fish meal. However, due to economic, environmental and social constrains, fish meal has been replaced by more economical protein source, as soybean meal, in order to improve cost-effectiveness of feeds. Soybean meal is less expensive than fish meal, has a good nutritional value with high crude protein content and a reasonably balanced amino acid profile and high availability **(Gatlin *et al*., 2007).**

DDGS are a concentrated source of non fermentable components from the original grain and are relatively high in protein, fiber, lipid, and ash. In fish diets, the use of DDGS is still limited, however, recent research have shown that DDGS is a potential alternative ingredient for Omnivores fish, such as tilapia **(Lim *et al*., 2011; Schaeffer *et al*., 2011).** This co-product is readily available and is less expensive than most conventional plant ingredients, such as soybean meal and corn on a protein-cost basis **(Welker *et al*., 2014).** The fiber content of DDGS is one factor that limits its inclusions in non-ruminant diets. Variation of nutrients in DDGS has reduced its quality as animal food and thus limited its application (**Liu, 2011).** Improvement of DDGS quality is critical for increasing DDGS value and further improving corn ethanol plant profitability. Protein is an important nutrient and indicator of DDGS quality as an animal feed. Typically, the animal food price has a linear correlation to the protein content of the food ingredient (**Srinivasan *et al*., 2006**). Increasing DDGS protein is a credible way to improve DDGS quality.

Soybeans have a long-term history as a dietary protein source. In addition to wet and dry fractionation processes, chemical processes adapted from the soybean industry can be applied to further enrich protein and reduce structural carbohydrates (fiber) from DDGS. Increasing protein will increase the price of DDGS and reducing fiber content may increase the share of DDGS as a food ingredient for non-ruminant animals. Recovered or removed fiber can be used as feedstock for making other products (**Doner *et al*., 1998; Moreau *et al*., 1999; Dien *et al*., 2005**) or fed to ruminants. The aim of the present study was influence of feeding different levels of DDGS and HPDDGS (by reduce the percent of soybean meal and yellow corn in tested diets) on growth performance and feed utilization, of Nile tilapia *(Oreochromis niloticus)*.

Material and Methods:-

The present study was carried out during 21st April and lasted till the 13th July 2016 in the Regional Center for Food and Feed (RCFF), Agricultural Research Center, Giza, Egypt.

Culture conditions:-

Nile tilapia fingerlings were obtained from a local farm of Ismailia, Egypt. The fish were acclimated to laboratory conditions for 4 weeks in fiberglass tanks. At the beginning of the experiment, 21 glass aquaria (60 l) were stocked with 15 fish, each with average initial weight of 2.25 g. The aquaria were supplied with continues flow of fresh water free of chlorine, at a rate of 2 l/min and were provided with supplemental aeration. The aquaria were illuminated using an overhead fluorescent lightning set on a 14:00 hour light : 10:00 hour dark cycle **(EL-Saidy and Gaber, 2003)**. A thermo-controlled electric heater was used to adjust water temperature at the range of 24-28°C. The dissolved oxygen and the nitrate values were found to be at the range of 5.1-6.2 mg/l and 29.0-40.2 mg/l respectively, while ammonia was not detectable. pH value were in the range of 6.4-7.7.

Diets and feeding regime:-

Seven experimental diets were formulated to be iso-nitrogenous and iso-caloric in terms of crude protein (30%) and gross energy (430 kcal/100 g diet). The energy value was calculated using the gross energy values for the macronutrients (5.6 kcal/g protein, 9.2 kcal/g fat and 4.1 kcal/g carbohydrate; fiber was not included in the calculation). A mixture of soybean meal (44% crude protein, CP), herring fish meal (72% CP), yellow corn (8 % CP), distillers dried grains with soluble DDGS (27 % CP), and a high protein distillers dried grains HPDDGS (44 % CP. The HPDDG used in this experiment was obtained as a new co-product of corn DDGS was performed according to **Cookman and Glatz** (**2009**) and **Bandara *et al.*** (**2011**)**,** with slight modifications. Corn oil and fish oil were purchased from the local market. Vitamin-mineral premix was obtained from *P*fizer Company, Egypt. The proximate analysis of the feed ingredients are listed in **Table (1)**. These experimental diets were designed and formulated to contain different levels of either DDGS or HPDDGS as follows:

Control (Diet 1): Doesn't contains neither DDGS nor HPDDGS.
15% DDGS. (Diet 2): Contains 15 % corn distillers dried grains with solubles (DDGS).
30% DDGS. (Diet 3): Contains 30 % corn distillers dried grains with solubles (DDGS).
45% DDGS. (Diet 4): Contains 45 % corn distillers dried grains with solubles (DDGS).
15% HPDDGS. (Diet 5): Contains 15 % high protein corn distillers dried grains with solubles (HPDDGS).
30% HPDDGS. (Diet 6): Contains 30 % high protein corn distillers dried grains with solubles (HPDDGS).
45% HPDDGS. (Diet 7): Contains 45 % high protein corn distillers dried grains with solubles (HPDDGS).

Chemical analysis:-

Analysis of different experimental feed ingredients, formulated diets were carried out for moisture, nitrogen, ether extract, crude fiber and nitrogen free extract according to the procedures of Association of Official Analytical Chemists **(AOAC, 2005)** using triplicate samples for each determination, Crude protein was calculated as nitrogen content x 6.25.

Amino acid determination:-

Amino acids except tryptophan and tyrosine were individually determined according to the method of **Official Journal of European Communities (1998)**.

Table 1:- Proximate chemical analysis of experimental dietary ingredients as fed basis (%).

Ingredient	Moisture	Crude Protein	Ether Extract	Crude Fiber	Ash	NFE*
Fish Meal	6.4	70.0	10.2	1.08	11.4	0.92
Soybean Meal	9.8	44.5	2.4	4.9	6.2	32.2
Yellow Corn	9.3	7.8	2.18	2.3	1.85	76.57
DDGS	9.0	26.5	8.2	10.0	5.1	41.2
HPDDGS	8.9	44.0	6.7	3.2	4.1	33.1

*NFE (nitrogen free extract) = 100 - (moisture + crude protein + ether extract + Crude Fiber + Ash)

The composition of the experimental diets used in the feeding trials is presented in **Table (2)**. The proximate chemical analysis and the energy content of the diets are presented in **Table (3)**, while their amino acid analysis is presented in **Table (4)**. Feed ingredients were finely grinded and mixed manually. Water was added to each diet till paste was formed, and then passed through a meat mincer machine to convert the mixture into pellets. The wet pellets were then sun dried and stored at -20°C until used. Each diet was tested by three groups of 15 fish each. Fish fed five times a day, at a daily rate of 4% of fish body weight for 12 weeks. Every two weeks fish in each aquarium were weighed and the ration offered was altered according to their body weight.

At the beginning of the experiment, one hundred fish were used as an initial control group. They were killed and kept frozen for chemical analysis. At the end of the experiment all fish in all aquaria were killed and kept frozen for chemical analysis.

Table 2:-Ingredient Composition of the experimental diets used in the feeding trial

Ingredient		**Diet DDGS%**			**Diet HPDDGS%**		
	Control	**15**	**30**	**45**	**15**	**30**	**45**
Fish meal	6	6	6	6	6	6	6
Soybean	54	47	39	31	39	24	9
Yellow corn	30	23	17	11	30.15	30.85	31.05
DDGS	-	15	30	45	-	-	-
HPDDGS	-	-	-	-	15	30	45
Fish oil	2	2	2	2	2	2	2
Corn oil	4	3	2	1	3.5	2.5	2
Premix (Vit. & Mins.)	3	3	3	3	3	3	3
Calcium mono phosphate	1	1	1	1	1	1	1
Lycine-HCl	-	-	-	-	.35	.65	.95
Total	100	100	100	100	100	100	100

Table 3:-Proximate chemical analysis and calculated energy content (Kcal DE/l00g DM) of the experimental diets used in the feeding trial.

Item	Control	Diet DDGS%			Diet HPDDGS%		
		15	30	45	15	30	45
Moisture %	8.5	8.25	8.10	8.00	8.35	8.42	8.75
Crude Protein %	30.65	30.88	30.75	31.00	30.55	30.78	30.82
Ether Extract %	8.22	8.41	8.31	8.55	8.35	8.25	8.35
Crude Fiber %	3.34	3.82	4.10	4.80	3.20	2.97	2.8
Ash %	4.61	4.44	4.32	3.95	4.40	4.35	4.45
NFE*	44.68	44.20	44.42	43.70	45.15	45.23	44.83
Energy**	430.45	431.52	430.77	430.41	433.01	433.71	433.21

NFE* = 100 - (Moisture + crude protein + ether extract + crude fiber + Ash).
Energy** Kcal DE/100g DM

Table 4:-Essential amino acid analysis of the experimental diets (g/ 100 g crude protein), used in the feeding trial, and amino acid requirements for Nile tilapia after (Santiago and Lovell, 1988).

Amino acid	Control	Diet DDGS%			Diet HPDDGS%			Requirements
		15	30	45	15	30	45	
Therionine	4.27	4.15	4.20	4.32	4.06	3.93	3.86	3.75
Methionine	2.74	2.72	2.64	2.50	2.78	2.72	2.66	2.68
Isoleucine	3.36	3.11	3.22	3.23	3.37	3.54	3.15	3.11
Leucine	7.37	7.61	8.36	8.22	8.84	9.68	10.32	3.39
Phenylalanine	4.89	4.70	4.91	4.83	5.07	5.30	5.03	3.75
Valine	4.21	4.05	4.29	4.51	4.39	4.45	4.28	2.80
Lysine	5.51	5.18	5.14	4.22	5.34	5.39	5.42	5.12
Histdine	2.64	2.56	2.57	2.59	2.55	2.44	2.27	1.72
Arginine	6.62	6.35	6.11	5.95	5.99	4.91	4.32	4.20

Measurements of growth performance, and feed utilization parameters:-

Means of weight gain, percentage weight gain, average daily gain (ADG) and specific growth rate (SGR % day) were calculated according **to (Wu *et al*., 1996)**, as the following equations:

1. Weight gain = W1- W_0.
2. Percentage weight gain (WG %) = (W1 - W_0) / W_0 x 100.
3. Average daily gain (ADG) = weight gain/experimental period (d).
4. Specific growth rate (SGR %) = (In W1 - In W_0/T) x 100.

Where:

W_0: Mean initial weight (g).
W1: Mean final weight (g).
d: Experimental period in days.
T: Time in days between weightings.

Means of feed conversion ratio (FCR), protein efficiency ratio (PER) and Protein Productive value (PPV %) were calculated according to **(Tahoun *et al*., 2008)** as the following equations:

1. Feed conversion ratio = Feed intake (g) / Weight gain (g).
2. Protein efficiency ratio = Weight gain (g). / Protein intake (g).
3. Protein Productive Value = B – B_0/BI x 100.

Where:
B_0: Initial body protein content (g)
B : Final body protein content (g)
BI: Protein intake (g)

Water quality:-

Water temperature, pH, dissolved oxygen, ammonia NH_3 and nitrate NO_3 were all periodically measured during the feeding trials. Water temperature (°C) was measured by using a thermometer while pH was measured using ORION pH/ISE meter; model EA 940 EXPANDAPLE IONANALYZER according to **Official Methods of Analysis (1993)**. Ammonia (NH_3) and dissolved oxygen were measured according to **Standard Methods for the Examination of Water and Wastewater (1995)**. ATI Orion ion meter was used with ammonium electrode model 95-12 and 97-80 for ammonia and oxygen measurements, respectively.

Statistical Analysis:-

Each sample was analyzed in triplicate and the replicates were then averaged. The statistical analysis was performed with SAS program (**SAS, 2007**) using analysis of variance (ANOVA) and means were separated by Duncan's multiple range test **(Duncan, 1955)** with a probability $P \leq 0.05$.

Results and Discussion:-

Data representing means for initial weights, final weights, weight gain, average daily gain and specific growth rates of fish given diets containing different levels of both DDGS and HPDDGS are shown in **Table (5)**. There were no significant differences ($P \leq 0.05$) between the initial individual weights of the all experimental groups indicating that fish at stocking were homogeneously distributed among treatments and replicates. The growth performances of Nile tilapia fingerlings in each experimental diets group are presented in **Table (5)**. Weight gains of Nile tilapia fingerlings fed 15 %DDGS and 30% DDGS diets were no significant different when compared to the control diets, but Nile tilapia fingerlings fed 45% DDGS diets gained less weight than those fed control diets ($P \leq 0.05$).

Table 5:-Growth performance of tilapia fish (*Oreochromis niloticus)* fed on experimental diets.

Item	Control	Diet DDGS%			Diet HPDDGS%		
		15	30	45	15	30	45
Initial weight (g/fish)	2.25^{a}	2.24^{a}	2.25^{a}	2.26^{a}	2.24^{a}	2.26^{a}	2.25^{a}
Final weight (g/fish)	16.36^{c}	16.49^{c}	16.33^{c}	12.50^{d}	16.83^{b}	17.17^{a}	16.54^{c}
Weight gain (g/fish)	14.11^{c}	14.25^{c}	14.07^{c}	10.24^{d}	14.58^{b}	14.91^{a}	14.29^{c}
Average daily gain (g/fish/day)	0.168^{a}	0.170^{a}	0.168^{a}	0.122^{a}	0.174^{a}	0.178^{a}	0.170^{a}
Specific growth rate (%/day)	$2.36a^{b}$	2.380^{ab}	2.35^{b}	2.04^{c}	2.40^{ab}	2.42^{a}	2.37^{ab}

Different superscript letters for each row indicate statistically significant difference ($P \leq 0.05$).

The present results of this study indicate that dietary supplementation of DDGS up to 30% did not affect the growth performance of Nile tilapia fingerlings ($P \leq 0.05$). While Nile tilapia fingerlings fed 45% DDGS diets showed the lowest growth performance values ($P \leq 0.05$), these may be due to the lower contents of lysine amino acid and increased fiber in diets.

Our results are in agreement with previous studies **(Tidwell *et al.*, 1990; Robinson and Li, 2008; Li *et al.*, 2010)** have also reported that corn-based DDGS can be integrated into channel catfish diets without negative effects on growth performance, and is suitable to replace soybean meal and corn meal in hybrid catfish diets **(Zhou *et al.*, 2010)**. The effectiveness of a diet containing DDGS on the growth of freshwater fishes is related to several factors such as improved digestibility **(Randall and Drew, 2010)** and decreased exposure to anti nutritional factors **(Borgeson *et al.*, 2006).** Nile tilapia grew bad at low levels of lysine amino acid, when fish fed 40% DDGS diet without lysine supplement **(Lim *et al.*, 2007)**.

Fish group given HPDDGS diet levels 15% HPDDGS, 30% HPDDGS, and 45% HPDDGS, with lysine supplementation showed better in the growth performance in terms of final mean weight, weight gain and average daily gain when compare with the control diet ($P \leq 0.05$).

These results are in agreement with those of **Kim *et al*. (2009)** and **Jacela *et al*., (2010)** who demonstrated that DDGS and HPDDG are well suited for use in terrestrial animal feeds, such as swine. Nevertheless, there are very few studies on the suitability of HPDDG for use in aquaculture. **Barnes *et al*. (2012 a, b)** showed that the inclusion of up to 200 g kg-1dietary DDGG or HPDDG had no adverse effects on growth and feed intake in rainbow trout.

These results also are in agreement with those of **Wu *et al.* (1994** and **1995)**,who reported that diets containing corn distiller's grains with solubles 29% and 32% or 36% protein resulted in higher weights of tilapia. (**Thompson *et al.*, 2008)** observed that corn-based DDGS might be improved palatability in Sunshine Bass diet. On the other hand, **Lim *et al.* (2009)** showed that up to 40% corn-based DDGS could be included in the diet of channel catfish as a replacement of soybean meal and corn meal without affecting weight gain.

In the present study, decreased growth performance and feed utilization at high dietary inclusion levels of DDGS (45%DDGS) may be associated with various factors including increased fiber levels, reduced feed palatability and an imbalanced dietary amino acid profile (lysine). Fibers and anti-nutrients are related to reduce digestibility in catfish (**Francis *et al.*, 2001).**

Reductions in growth and feed utilization of fish fed plant protein due to imbalanced dietary amino acids reduced mineral content, increased fiber, reduced palatability, and the presence of anti-nutrient factors **(Lim and Lee, 2009).**

Data representing means for feed conversion ratio (FCR), protein efficiency ratio (PER) and Protein Productive Value (PPV %) for fish given diets containing different levels of both DDGS and HPDDGS are presented in **Table (6)**.

Table 6:- Feed utilization parameters of *Oreochromis niloticus* fish fed the 7 experimental diets.

Item	Control	Diets DDGS%			Diets HPDDGS%		
		15	30	45	15	30	45
Feed conversion ratio (FCR)	1.65^{b}	1.60^{bc}	1.63^{bc}	1.94^{a}	1.59^{c}	1.58^{c}	1.60^{bc}
Protein efficiency ratio (PER)	1.98^{b}	2.02^{ab}	2.00^{b}	1.66^{c}	2.06^{a}	2.05^{a}	2.02^{ab}
Protein productive value -PPV%	29.05^{ab}	29.73^{ab}	30.71^{ab}	26.43^{b}	34.9^{a}	32.15^{ab}	33.48^{a}

Different superscript letters for each row indicate statistically significant difference ($P \leq 0.05$).

Feed utilization efficiencies in terms of food conversion ratio (FCR), protein efficiency ratio (PER) and Protein Productive Value were also significantly affected by the treatments ($P \leq 0.05$). The Feed conversion ratio (FCR) of fingerlings Nile tilapia fed 15 %DDGS and 30% DDGS diets, were slight different compared to the control diets, but fingerlings Nile tilapia fed 45% DDGS diets worst FCR when compared to other treatments. The highest FCR, lowest PER and the lowest (PPV %) representing the worst findings were achieved when fish was fed on diet containing 45% DDGS diets.

These results are in agreement with those of **Lim and Webster (2006)** who found that 20% fuel-based DDGS can be included in Nile tilapia diets without a significant effect on overall growth performance. However, fish fed diets containing 30% DDGS had similar weight gain (WG), PER and feed efficiency ratio (FER) as those fed the control diet, while fish fed 40% DDGS had significantly lower WG, PER and FER than those on the control diet. Similar results were obtained by **Li *et al*. (2011)**, who found significant decrease ($P \leq 0.05$) in growth rate and FCR when 40% soybean meal and corn without addition lysine was replaced with wheat DDGS in tilapia diets. Also, these results are in agreement with previous studies in DDGS inclusion in tilapia diet, which showed that 40% DDGS level in fish diet resulted in lower growth and feeding performance according **Li *et al*. (2011) who** pointed out that, 40% inclusion level of DDGS resulted in lower tilapia growth and feed efficiency than control diet, and **Schaeffer *et al.* (2009)** found that the WG and FCR of fish fed the 20% DDGS diet were similar to that of the control diet. These values were significantly better than those fed diets with 30 or 40% DDGS **(Salama *et al*., 2010).**

Generally when alternative plant ingredients are used in diets with the same concentration of energy and are able to meet the nutritional requirements of the animal being fed, similar performance may be expected **(Cruz-Suarez *et al*., 2001).** Many scientific studies have revealed that fermented plant ingredients at a proper incorporation level may be of great nutrient resources pertaining to fish **(Sun *et al*., 2007; Seo *et al*., 2011)** and shrimp (**Molina-Poveda and Morales, 2004**). In a laboratory study of Nile tilapia *(Oreochromis niloticus)*, **Lim *et al.* (2007)** reported that increasing dietary levels of DDGS to 40% without addition of lysine significantly reduced WG and PER compare to those obtained with diets containing lower DDGS levels (0, 10, and 20%), FCR of this diet (40% DDGS) was also significantly worst than that of the control diet. Results of earlier studies have shown that based on growth performance and feed utilization efficiency, DDGS is a promising feed ingredient for several fish species, including rainbow trout **(Cheng and Hardy, 2004),** tilapia **(Wu *et al.*, 1996),** and channel catfish **(Tidwell *et al.*, 1990; Webster *et al.*, 1993).** The lowest FCR, highest PER and the highest PPV% were achieved when fish was fed on the

HPDDGS diet levels 15%HPDDGS, 30%HPDDGS and 45%HPDDGS, with lysine supplementation. This is an agreement with the findings of **Barnes *et al.* (2012a)** and **Cheng and Hardy (2004),** who showed that the, final weight, FCR, PRE, P retention, and SGR of fish fed HPDDG were greater than those of fish fed the control diet.

From this study, the nutritive value of HPDDGS was investigated and showed very high digestibility of protein and amino acid, this feed ingredient appeared to be a good protein source in the low fishmeal diet for rainbow trout **(Prachom *et al.*, 2013).** The results of this experiment suggest that DDGS is a good substitute for plant materials such as Yellow corn and soybean meal, and can be used up to 30% in feed to maintain the growth performance of Nile tilapia, and also fish group given HPDDGS diet levels (15%HPDDGS, 30%HPDDGS, 45%HPDDGS), with lysine supplementation showed better in the Growth performance and feed utilization. HPDDGS is a good alternative protein source and can be used up to 45% or may be more with lysine supplementation.

References:-

1. AOAC (2005). Official Methods of Analysis of the Association of official Analytical Chemists, 18th. ED .Vol.1, published by the A.O.A.C., Benjamin Francklin station, Washington, DC.
2. Bandara N, Chen L, Wu J. (2011) Protein Extraction from Triticale Distillers Grains.Cereal Chem. 88(6):553–559.
3. Barnes, M.E., Brown, M.L. and Rosentrater, K.A. (2012a). Initial observations on the inclusion of high protein distillersdried grain into rainbow trout diets. The Open Fish ScienceJournal.,5, 21–29.
4. Barnes, M.E., Brown, M.L. and Rosentrater, K.A. (2012b). Juvenile rainbow trout responses to diets containing distillers dried grain with solubles, phytase and amino acid supplements. Open Journalof AnimalSciences., 2, 69 – 77.
5. Borgeson, T.L., Racz, V.J., Wilkie, D.C., White, L.J. and Drew, M.D. (2006). Effect of replacing fishmeal and oil with simple or complex mixtures of vegetable ingredients in diets fed to Nile tilapia (*Oreochromis niloticus*). Aquaculture Nutrition, 12:141–149.
6. Cheng, Z.J. and Hardy, R.W., (2004). Nutritional value of diets containing distiller's dried grain with solubles for rainbow trout, *Oncorhynchus mykiss*.Journal of Applied Aquaculture, 15:101-113.
7. Cookman, D. J., and Glatz, C. E. (2009). Extraction of protein from distillers grain. Bioresour. Technol. 100:2012-2017.
8. Coyle SD, Mengel GJ, Tidwell JH, Webster CD (2004) Evaluation of growth, feed utilization and economics of hybrid tilapia, *Oreochromis niloticus Oreochromis aureus*, fed diets containing different protein sources in combination with distillers dried grains with solubles. Aquaculture Research 35:1–6.
9. Cruz-Suarez, L.E., Ricque-Marie, D., Tapia-Salazar, M., McCallum, I.M. and Hickling, D. (2001). Assessment of differently processed feed pea (*Pisum sativum*) meals and canola meal (*Brassica sp.)* in diets for blue shrimp (*Litopenaues stylirostris*). Aquaculture, 196:87–104.
10. Dien BS, Nagle N, Singh V, Moreau RA, Tucker MP, Nichols NN, Johnston DB, Cotta MA, Hicks KB, Nguyen Q, Bothast RJ (2005) Review of process for producing corn fiber oil and ethanol from ‘‘quick fiber’’. Int Sugar J 107:187–191.
11. Duncan DB (1955). Multiple range and multiple F test. *Biometrics*, 11: 1-42.
12. Doner LW, Chau HK, Fishman ML, Hicks KB (1998). An improved process for isolation of corn fiber gum. Cereal Chem 75(4):408–411.
13. El-Saidy, D.M.S.D. and Gaber, M.M.A. (2003). Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia, *Oreochromis niloticus* (L.) diets. Aquaculture Research, *34*, 1119-1127.
14. Francis, G. Makkar H.P.S. and Becker, K. (2001). Antinutritional factors present in plant derived alternate fish feed ingredients and their effects in fish. Aquaculture 199:197-227.
15. Gatlin III, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, A, Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R.,Wurtele, E.(2007). Expanding the utilization of sustainable plant products in aquafeed: a review. Aquac.Res. 38, 551–579.
16. Jacela, J.Y., Frobose, H.L., DeRouchey, J.M., Tokach, M.D., Dritz, S.S. (2010). Amino acid digestibility and energy concentration of high-protein corn dried distillers grains and high- sorghum dried distillers grains with soluble for swine. J. Anim. Sci., 88, 3617– 3623.
17. Kim, B.G., Pedersen, G.I., Hinson, R.B., Allee, G.L. Stein, H.H. (2009). Amino acid digestibility and energy concentration in a novel source of high-protein distillers dried grains and their effects on growth performance of pigs. J. Anim. Sci. 87, 4013-4021

18. Li E, Lim C, Cai C and Klesius PH. (2011). Growth response and resistance to *Streptococcus iniae* of Nile tilapia, *Oreochromis niloticus*, fed diets containing different levels of wheat distillers's dried grains with solubles with or without lysine supplementation. Anim Feed Sci Technol 170, 246-255.
19. Li, M.H., Robinson, E.H., Oberle, D.F., and Lucus, P.M. (2010). Effects of various corn distillers by-products on growth, feed efficiency, and body composition of channel catfish, Ictalurus punctatus. Aquac.Nutr. 16,188-193.
20. Lim SJ and Lee KJ. (2009). Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*. Aquaculture 290, 283-289.
21. Lim, C., Garcia, J.C., Yildirim-Aksoy, M., Klesius, P.H., Shoemaker, C.A. and Evans, J.J. (2007). Growth response and resistance to streptococcus iniae of Nile tilapia, *Oreochromis niloticus*, fed diets containing distiller's dried grains with solubles. Journal of the World Aquaculture Society,38:231–237.
22. Lim, C., Li, E., and Klesius, P. H. (2011). Distiller's dried grains with solubles as an alternative protein source in diets of tilapia. Reviews in Aquaculture, 3(4), 172–178. http://doi.org/10.1111/j.1753-5131.2011.01054.x
23. Lim, C., and Webster, C.D. (2006). Nutrient requirements. In: Lim, C., Webster, C.D. (Eds.), Tilapia: Biology, Culture and Nutrition. The Haworth Press, Inc., Binghamton, New York, New York, pp. 469–501.
24. Lim, C., Yildirim-Aksoy, M. and Klesius, P.H. (2009). Growth response and resistance to Edwardsiella ictaluri Channel Catfish, Ictalurus punctatus, fed diets containing distiller's dried grains with solubles. Journal of the World Aquaculture Society, 40:182–193.
25. Liu K (2011) Chemical composition of distillers grains, a review.J Agric Food Chem 59:1508–1526.
26. Mcvey EM (1994). Aquaculture for youth and youth educators. http://www.cyfernet.org/curicul/aquacul.htm.
27. Molina-Poveda, C. and Morales, M.E. (2004). Use of a mixture of barley based fermented grains and wheat gluten as an alternative protein source in practical diets for Litopenaeus vannamei (Boone). Aquaculture Research, 35:1158–1165.
28. Moreau RA, Norton RA, and Hicks KB (1999) Phytosterols and phytostanols lower cholesterol. Inform 10:572–577. Official Journal of the European Communities, (1998). L 257 / 14-28.
29. Official Journal of the European Communities, (1998). L 257, 14 – 28
30. Official Methods of Analysis of AOAC International (1993), 16th ed., 5th revision.
31. Prachom, N., Haga, Y. and Satoh, S. (2013). Impact of dietary high protein distillers dried grains on amino acid utilization, growth response, nutritional health status and waste output in juvenile rainbow (*Oncorhynchus mykiss*). *Aquacult. Nutr.*(doi: 10.1111/anu. 12049).
32. Randall, K.M. and Drew, M.D. (2010). Fractionation of wheat distiller's dried grains and solubles using sieving increases digestible nutrient content in rainbow trout. Animal Feed Science and Technology, 159:138–142.
33. Robinson, E. H. and LI. M. H. (2008). Replacement of Soybean Meal in Channel Catfish, Ictalurus punctatus, Diets with Cottonseed Meal and Distiller's Dried Grains with Solubles. Journal of the World Aquaculture Society 39:521-527.
34. Salama, F.A.; Tonsy, H.D.; Labib, E.M.; Mohmoud, S.H. and Zaki, M.A. (2010). Nutritional studies on partial and total replacment of soybean meal by distillers dried grains with soluble (DDGS) in diets for Nile tilapia (Oreochromis niloticus). Egyptian J. Nutrition and Feeds, 13 (1); 165 – 176.
35. Santiago, C.B. and Lovell. R.T. (1988). Amino acid requirement for growth of Nile tilapia, J. of Nutr., *118*, 1540-1546.
36. SAS (2007). Statistical Analysis System. SAS Users' guide: Statistics SAS Institute Inc. Editors, Cary, NC.
37. Schaeffer, T. W., Brown, M. L. and Rosentrater, K. A. (2009). Performance characteristics of Nile tilapia (Oreochromis nitoticus) fed diets containing graded levels of fuel-based distifiers dried grains with solubles. Journal of Aquaculture Feed Science and Nutrition 1: 7 8-83.
38. Schaeffer, T. W., Brown, M. L. and Rosentrater, K. a. (2011). Effects of Dietary Distillers Dried Grains with Solubles and Soybean Meal on Extruded Pellet Characteristics and Growth Responses of Juvenile Yellow Perch. North American Journal of Aquaculture, 73(3), 270–278. http://doi.org/10.1080/15222055.2011.593461.
39. Seo, J.-Y., Shin, I.-S, and Lee, S.-M. (2011). Effect of dietary inclusion of various plant ingredients as an alternative for Sargassum thunbergii on growth and body composition of juvenile sea cucumber Apostochopus japonicas. Aquaculture Nutrition, 17:549–556.
40. Srinivasan R, Singh V, Belyea RL, Rausch KD, Moreau RA and Tumbleson ME (2006). Economics of fiber separation from distillers dried grains with solubles (DDGS) using sieving and elutriation. Cereal Chem 83:324–330.
41. Standard methods for the examination of water and wastewater. (1995). In: A.E. Greenberd, R. R. Trussell and L. S. Clesceri (Editors). Nitrate electrode screening method. American Public Health, Association, Washington, 16th edition, pp: 393 - 394.

42. Sun, M., Kim, Y.C., Okorie, O.E., Lee, S., Devnath, S., Yoo, G., Bai, S.C. and Jo, Y.K. (2007). Evaluation of fermented soybean curd residues as an e nergy source in diets for juvenile olive flounder, *Paralichthys olivaceus.* Journal of the World Aquaculture Society, 38:536–542.
43. Tahoun, A.M., Ibrahim, M.R., Hammouda, Y.F., Eid, M.S., Zaki El-din, M.M.A. and Magouz, F.I. (2008). Effects of age and stocking density on spawning performance of Nile tilpia, Oreochromis niloticus (l.) broodstock reared in hapas. 8th International Symposium on Tilapia in Aquaculture, 329-343.
44. Thompson, K.R., Rawles, S.D., Metts, L.S., Smith, R.G., Wimsatt, A. Gannam, A.L., Twibell, R.G., Johnson, R.B., Brady, Y.J. and Webster, C.D.(2008). Digestibility of dry matter, protein, lipid, and organic matter of two fish meals, two poultry by-product meals, soybean meal, and distiller‘s dried grains with solubles in practical diets for sunshine bass, *Morone chrysops* × *M. saxatilis*. Journal of the World Aquaculture Society, 39:352–363.
45. Tidwell JH, Webster CD and Yancey DH.(1990). Evaluation of distiller's grains with solubles in prepared channel catfish diets. Tran Kentucky Acad Sci 52, 135-138.
46. Webster CD, Tidwell JH, Goodgame LS and Johnsen PB. (1993). Growth, body composition, and organoleptic evaluation of channel catfish fed diets containing different percentages of distillers' grains with solubles. Prog Fish-Cult 55, 95-100.
47. Welker, T. L., Lim, C., Barrows, F. T., & Liu, K. (2014). Use of distiller's dried grains with solubles (DDGS) in rainbow trout feeds. Animal Feed Science and Technology, 195, 47–57. http://doi.org/10.1016/j.anifeedsci.2014.05.011.
48. Wu, V. W., Rosati, R. R., and Brown, P. B. (1996).Effects of diets containing various levels of protein and ethanol co-products from corn on growth of tilapia fry. Journal of Agricultural Food Chemistry, 44: 1491-1493.
49. Wu, Y. V.; Rosati, R. R.; Sessa, D. J. and Brown, P. B. (1995). Evaluation of corn gluten meal as a protein source in tilapia diets. J. Agric. Food Chem., 43: 1585-1588. Received for review November 6, 1995. Revised manuscript.
50. Wu, Y. V.; Rosati, R.; Sessa, D. J. and Brown, P. (1994). Utilization of protein- rich ethanol co-products from corn in tilapia feed. J. Am. Oil Chem. Soc., 71:1041-1043.
51. Zhou, P., Zhang, W., Davis, D.A. and Lim, C. (2010). Growth response and feed utilization of juvenile hybrid catfish fed diets containing distillers's dried grains with solubles to replace a combination of soybean meal and corn meal. North American Journal of Aquaculture, 72: 298–303.