Nutritional studies on brewer dried grains as an alternative protein source for Nile tilapia (*Oreochromis niloticus*) diets.

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ABSTRACT

Brewer dried grain (BDG) was tested to replace soybean meal in diets of mono sex Nile tilapia (*Oreochromis niloticus*). Five isonitrogenous (30%), isocaloric (442 kcal/100g) diets were formulated to contain BDG as an alternative protein source instead of soybean meal at different levels (0, 25, 50, 75 and 100%). A total number of 225 of Nile tilapia (0.45g)were randomly distributed into five experimental groups, each in trireplecates, and were fed daily at a rate of 15% decreased gradually to be 4% of fish live weight through 14weeks experimental period.Data collected were on growth performance, feed utilization and body composition in addition to economic evaluation. Results of growth performance did not show any adverse effects related to the incorporation of BDG up to 75%. FI shows the exact similar trend of growth performance. The incorporation of BDG in the diets improved slightly FCR, FER and PPV. Fish whole body composition of treatments groups which showed higher DM, EE, energy and somewhat protein contents and lower moisture and ash than that recorded at the start of the experiment.

Keywords: Nile tilapia, brewer dried grain, growth performance, feed utilization, body composition, economic efficiency.

INTRODUCTION

According to the UN Food and Agriculture Organization, aquaculture is growing more rapidly than all other animal food-production sectors (FAO, 2006).Tilapias are second only to carps as the most widely farmed freshwater fish in the world; three-quarters of the world's supply comes from aquaculture. World tilapia production has been increased steadily during the last decade, with output more than doubling from 931,000 mt in 1997 to 2.3 million mt in 2006 (FAO, 2007a). Tilapias are the third largest group of farmed finfish species, only after carps and salmonids with an average annual growth rate of about 11.5% (FAO, 1997). About 2.3 million mt. or 99% of farmed tilapia were produced in developing countries in 2006 with Africa producing about 13% of this amount (FAO, 2007b). Egypt is the second biggest tilapia producer (202.60 mt) after China (FAO, 2007b).

The expansion of aquaculture production has been accompanied by rapid growth of aquafeed production. The challenge facing the aquaculture industry is to identify economically viable and environmentally friendly alternatives to fish meal, soybean meal and yellow corn on which many present aquafeeds are largely based.

Soybean (Glycine max) meal (SBM) hasbeen used as a major protein source in diets for aquaticanimals, especially for the replacement of fishmeal (FM), which is costlier, less available, and lesssTable in supply and price than soybean(Hertrampf and Piedad-Pascual, 2000; Chien and Chiu 2003). A significantamount of research has been conducted on the replacement of FM with SBM as protein sources in feedsfor tilapia Oreochromis spp. (Jackson, Capper &Matty 1982;Twibell&Brown 1998;El-

Sayed 1999; Fontainhas Fernandes, *et al.*, 1999) and SBM is now a major ingredient in commercialtilapia feed, and in turn numerous studies suggested good alternative vegeTable protein source instead of SBM such aslupin seed meal (Viola, *et al.*, 1988; Fontainhas-Fernandes *et al.*, 1999) tomato by-product (Soltan, *et al.*, 2005) cucumber leaves, squash leaves and broad bean leaves (Magouz, *et al.*, 2008) brewer's grains with yeast (Muzinic, *et al.*, 2004) distillers dried grains with Solubles (Salama, *et al.*, 2010).

This study aimed to investigate the effects of incorporation of brewer dried grain (BDG) instead of soybean meal on growth performance, feed utilization, body composition and economic efficiency of Nile tilapia.

MATERIALS AND METHODS

Experimental fish and treatments:

This study was conducted at central laboratory for fish aquaculture research, Abbassa, Abo-Hammad, Sharkia, Egypt. The experimental rearing system consisted of series 15th glass aquaria containing 90 L of de-chlorinated tap water, (Five treatments in trireplecates). Fish were acclimated indoor tank for 2 weeks to laboratory conditions. Nile tilapia fry (all male) was distributed randomly, each aquaria was stocked with 15 fish with an average initial weight 0.45 g/fish. Each aquarium was supplied with compressed air via air-stones from air pumps, settled fish wastes were cleaned daily by siphoning of quarter of the volume, which was replaced by aerated water. The photoperiod was set on a 12-12 hour light-dark cycle using fluorescent tubes as a light source. Fish in each aquarium were weighed every two weeks.

Diet preparation and feeding:

Brewer dried grains (BDG) samples used in this study were granted fromBeer division, Al-Ahram Company of drinks, Abo-Hammad, Sharkia. The proximate composition of the ingredients used in the diets formulated is presented in Table (1). The experimental diets were prepared by fine grinding of the dietary ingredients. Thereafter, all ingredients of each experimental diet were thoroughly mixed together to get homogenous mixture. After that, the mixture was pelleted in a modified paste extruder provided with 1mm die to form five experimental diets.

Table 1: Composition of the ingredients used in the experimental diet.

Ingredients	FM	SBM	YC	PBP	BDG
Dry matter (%)	92.10	90.80	89.20	88.20	91.58
Crude protein (%)	72.00	44.00	8.50	12.50	23.50
Lipid (%)	8.70	1.20	3.60	3.00	3.40
Crude fiber (%)	-	5.80	3.20	11.00	16.30
NFE (%)	6.7	42.50	82.70	69.10	52.60
Ash (%)	12.60	6.50	2.00	5.00	4.20
Calculated composition					
Gross energy (kcal/kg)[1]	5158.15	4299.4	4128.45	3753.05	3452.95
Digestible protein (%)[2]	59.69	34.85	5.81	10.25	19.34
Digestible energy (kcal/kg)[3]	3392.2	2699.7	2656.6	2408	2412.9

1- Gross energy was calculated using gross calorific values of 5.65, 4 and 9.45 kcal/gfor protein, carbohydrate and fat, respectively, according to Jobling (1983).

2- Digestible proteins were calculated using values of 82.90% for FM and 68.3% for yellow corn according to Wilson and Robinson (1982). Values of 79.2 and 82.00 were used for SBM and WB, respectively, according to Hepher (1988). Brewer dried grain was calculated using value of 82.3 according to Cheng, *et al*., (2004).

3- Digestible energy was calculated using values 3.5, 8.1 and 2.5kcal/gfor protein, fat and carbohydrate, respectively according to NRC (1993).

The five diets were approximately similar in all the nutrient contents (almost 30% CP) but containing different levels (0, 25, 50, 75 and 100%) of BDG to replace SBM (Tables 2&3). Feed was given to experimental fish at a rate of 15% of the body weight; this rate was decreased gradually until to be 4% of body weight. The feeding rate was adjusted every two weeks according to the development of fish weight. The tested diets were provided two times daily at 9.00 and 15.00 hr.

Table 2: Composition of the experimental diets.

Ingredients	Diets				
	T_0	T_1	T_2	T_3	T_4
Fish meal	19.00	19.00	19.00	19.00	19.00
Soybean meal	28.00	21.00	14.00	7.00	00
BDG	00.00	13.11	26.21	39.32	52.43
Yellow corn	34.00	30.00	26.00	22.00	17.00
Wheat bran	13.00	10.00	8.00	6.00	4.00
Oil	3.00	3.89	3.79	3.68	4.57
Vit& Min*	3.00	3.00	3.00	3.00	3.00
Sum	100	100	100	100	100

*Vitamin & mineral mixture/kg premix: Vitamin D , 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g,3B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin, 20 mg, Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.

Table 3: Chemical composition of the experimental diets.

ingredients	diets				
	T_0	T_1	T_2	T_3	T_4
DM	90.95	90.97	90.99	90.96	90.98
CP	30.95	30.5	30.25	29.96	29.5
EE	6.69	7.53	8.11	8.75	9.25
CF	4.50	5.47	6.82	8.15	9.5
ASH	6.8	6.73	6.68	6.52	6.39
NFE	51.06	49.77	48.14	46.62	45.36
Calculated values					
GE (kcal/100g)[1]	442.33	442.56	440.11	438.44	435.53
DE (kcal/100g)[2]	331.75	331.92	330.08	328.83	326.65
ME (kcal/100g)[3]	371.66	371.94	369.82	368.40	365.98
P/E ratio [4]	69.97	68.92	68.73	68.33	67.73

1- Gross energy, according to Jobling (1983).
2- Digestible energy calculated as 75% of the gross energy Jobling, (1983).
3- Metabolizable energy calculated using values 4.5, 8.1 and 3.49kcal/gfor protein, fat and carbohydrate, respectively according to Pantha, (1982).
4- P/E ratio =mg crude protein/ kcal GE.

Growth performance and feed utilization parameters:

At the termination of the experimental period final body weight (FBW), body weight gain (BWG), specific growth rate (SGR), relative growth rate (RGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV) and energy utilization (EU) were calculated according to the following equations:-

1- Total weight gain = final weight (g)- initial weight (g).
2- Relative growth rate = final weight (g)- initial wt (g)/initial wt (g)
3- SGR = Ln W2- Ln W1 / T X 100, Where: W2=final weight, W1 = Initial weight, T = periods in days, Ln = Natural logarithm.
4- FCR = Feed intake / Weight gain.

5- PER= W gain (g)/protein consumed (intake) (g) x100.
6- PPV% = 100[final fish body protein (g)- initial fish body protein (g)/ crude protein intake (g)].
7- EU % = 100[final body energy (K Cal) - initial body energy (K Cal)/ energy intake (K Cal)]

Chemical and statistical analysis:

Diets and fish samples were analyzed according to AOAC (1990) for moisture, protein, fat and ash. Metbolizable energy calculated according to Jauncey (1982). The obtained numerical data were statistically analyzed using SPSS (1997) for one-way analysis of variance. When F-test was significant, least significant difference was calculated (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical composition:

Compared with SBM, BDG (Table1) have the following disadvantages: lower protein content, lower gross energy and higher fibre. As described in this Table therelatively high protein content of BDG indicate the possibility of incorporation of this cheap industrial by-product in fish diets as a replacement of the coasty SBM, moreover EE content in BDG is higher than that of SBM. BDG fiber content is higher than that of SBM, so it may be act as a negative factor on both growth performance and feed utilization (De Silva and Anderson, 1995). BDG has admirable essential amino acids profile comparing to that of both SBM and the profile concluded by Santiago and Lovell (1988) for tilapia except for lysine, methionine and somewhat threonine and tryptophan.

Growth performance:

The growth performance parameters of Nile tilapia fed the experimental diets are shown in Table (4). The initial weight of fish groups are equal indicating the homogeneity of distribution. By the termination of the feeding period (98 days), fish fed tested diets revealed insignificant differences (P>0.05) for final body weight, body weight gain up to 75% replacement level, in spiteof veryminor reduction in FBW and BWG. Also, SGR and RGR values however recorded some decreases but it still insignificant (P>0.05) for all treatment groups compared to the control.

Table 4: Effect of replacement of soybean meal protein with BDG on growth performance parameters of Nile tilapia.

	Experimental diets				
Parameters	T_0	T_1	T_2	T_3	T_4
IW (g/fish)	0.44 ±0.03	0.45^{a} ±0.01	0.45^{a} ±0.00	0.45^{a} ±0.02	0.45^{a} ±0.03
FW (g/fish)	16.52^{a} ±0.08	16.23^{ab} ±0.05	15.89^{ab} ±0.79	16.23^{ab} ±0.09	14.65^{b} ±0.09
BWG (g/fish)	16.05^{a} ±0.8	15.78^{ab} ±0.04	15.44^{ab} ±0.79	15.78^{ab} ±0.01	14.2^{b} ±0.06
SGR (%)	3.67^{a} ±0.03	3.66^{a} ±0.03	3.64^{a} ±0.05	3.66^{a} ±0.04	3.56^{a} ±0.06
RGR (%)	3624^{a} ±44	3509^{a} ±82	3436^{a} ±175	3516^{a} ±137	3180^{a} ±192

a, b, …etc.: Means within row with different superscripts are significantly different (P < 0.05)

Unfortunately, Published data on the use of brewer's grains in aquaculture diets are limitedhowever Brewer's grains doesnot have any known anti-nutritional factors, such as gossypol Muzinica, *et al.* (2004), this property may be due to the proceduresto which barley were subjected (steeping, heating and enzyme treats) during the production of beer (Hough, 1985; El-Boushy and Van der Poel, 1994) these procedures collectively the common recommended processing techniques for removing anti-nutritional factors as reported by Francis, *et al.*, (2001). So, the cause of the minor reduction in the growth performance concurrent with low substitution levels and more pronounced in the high one (100%), may be constrained by both the higher fiber content and the lower gross energy content compared to SBM. This is in agreement with Grant (1985) who evaluated the potential brewer's grain meal (BGM) as a diet ingredient for rainbow trout, Ingredient levels were varied in isonitrogenous and isocaloric diets containing 0, 9.3%, 18.7%, 28.0%, and 37.3% BGM, respectively (up to 100% replacement of plant protein namely, soybean and cottonseed meals). After the 66-day feeding trial, rainbow trout had no differences in weight gains, feed conversion ratios, and percentage survival among treatments, thereafterhe concluded that BGM was a good diet ingredient for rainbow trout that could replace soybean and cottonseed meals at relatively high levels (37%). Salama, *et al.*, (2010) used distillers dried grains with soluble (DDGS) instead of soybean in diets for Nile tilapia. They found that growth performance recorded significant improvements by different degrees up to 80% replacement level compared with the control. Moreover, Muzinica, *et al.* (2004) found that a combination of soybean meal and brewer's grains with yeast (BGY) could be totally replacing fish meal in red claw crayfish diets without any significant effects on final weight, weight gain, or survival.

Feed utilization:

As described in Table (5) FI recorded insignificant difference (P>0.05) between treatments up to 75% replacement level, T_2 (25%) recorded the best FI value while T_4 (100%) recorded the poorest one. Concerning to FCR, PER and PPV results exhibited insignificant differences (P>0.05) for all treatments comparing to the T_0 (control) with slight improvement for T_1 (25%), T_2 (50%) and T_4 (100%) respectively. Energy utilization exhibited fluctuated results collectively positive, T_2 and T_4 recorded significant improvement (P<0.05), while T_1 and T_3 did not reveal any adverse effects compared to the control.

Table 5: Effects of replacement of soybean meal protein with BDG on feed utilization parameters of Nile tilapia.

Treatment		BDG			
	T_0	T_1	T_2	T_3	T_4
FI (g/fish)	$28.28±0.08^a$	$27.74±1.43^{ab}$	$26.02±0.56^{ab}$	$28.63±1.43^a$	$24.32±0.21^b$
FCR	$1.77±0.12^a$	$1.76±0.05^a$	$1.68±0.04^a$	$1.81±0.04^a$	$1.71±0.06^a$
PER	$2.01±0.05^a$	$2.05±0.09^a$	$2.17±0.06^a$	$2.02±0.08^a$	$2.17±0.06^a$
PPV%	$33.07±1.0^a$	$33.14±1.93^a$	$35.58±0.97^a$	$34.13±0.8^a$	$36.60±1.35^a$
EU%	$21.10±0.71^b$	$19.20±0.76^b$	$23.57±0.38^a$	$21.17±0.13^b$	$23.41±0.88^a$

a, b, …etc.: Means within row with different superscripts are significantly different (P < 0.05)

The previous results are in accordance with Salama, *et al.*, (2010) detected that feed intake increased gradually and significantly with increasing (DDGS) up to 100%. Also they found that the lower substitution levels 20, 40% recorded the best FCR, PPV and EU values than the higher ones (60, 80 and 100%) but the opposite trend was observed with PER. Soltan (2002) and Soltan, *et al.*, (2005) reported that increasing the levels of TBM as a replacement of SBM in tilapia and common carp diets respectively up to 50% had no significant effects on either FCR or PER.

Whole body chemical composition:

The chemical composition of the whole body of fish fed the experimental diets is presented in Table (6). Dry matter showed no hassignificant differences between the treatments associated with replacement process. Protein content demonstrated an improvement for all treatments; these improvements are significant only for T_1 and are not for the others. Fat content revealed improvements with the increase of BDG levels, except for T_1 which recorded significant decrease. Ash content tends to decrease significantly with theincrease of substitution levels except for T_1.

Table 6: Chemical composition and energy contents of tilapia carcass as affected by diets with BDG.

parameters	Diets					
	initial	T_0	T_1	T_2	T_3	T_4
DM	20.16	26.39±0.7^a	25.29±0.73^a	26.60±0.84^a	26.21±0.18^a	26.82±0.5^a
CP	58.71	61.66±1.21^b	65.18±0.41^a	61.23±0.74^b	63.78±0.68^{ab}	62.07±0.57^b
EE	11.32	22.45±0.11^b	18.41±0.5^c	25.58±0.74^a	22.94±0.85^{ab}	24.62±0.42^{ab}
Ash	29.97	15.86±1.26^a	16.51±0.1^a	13.21±0.14^b	13.30±0.16^b	13.31±0.17^b
Energy*	437.99	559.70±7.37^b	541.35±2.48^c	586.77±2.98^a	576.30±4.16^a	582.49±0.96^a

a, b, c…etc.: Means within row with different superscripts are significantly different ($P < 0.05$)
*Gross energy (kcal/100g) calculated according to Jobling (1983).

Energy content recorded significant improvement compared to the control (T_0) for T_2, T_4 and T_3 respectively, while T_1 recorded the least energy content with significant decease with all other treatments. These results are partially in agreement with the results of Salama, *et al.*, (2010) who reported that the gradual increase in the replacement level of DDGS instead of SBM protein up to 40%resulted in gradual increase in fish body content of dry matter, crude protein, ether extract and energy content while, ash content decreased. Also, Lim, *et al.*, (2008) and Tahoun, *et al.*, (2009), moreover, Carl *et al.*, (1993) found that the increase in the replacement level of DDGS instead of SBM protein up to 30% increased EE content. Teles, and Gonçalves, (2001) evaluated the effects of partial replacement of fishmeal by brewer's yeast. They reported that, no significant differences in whole body composition among experimental groups except for the protein content which was significantly higher in fish fed the brewer's yeast containing diets than the control diet.

Economic evaluation:

Feed cost is considered to be the highest recurrent cost in aquaculture, often ranging from 50 % to 60% depending on the intensity of the operation. Any reduction in feed costs either through diet development, improved husbandry or other direct or indirect means is therefore crucial to the development and well-being of the industry.

The economic parameters of the tested diets are presented in Table (7).

The calculated figures showed that the cost of one ton of feed mixture was reduced in all levels of SBM substituted by BDG.

Table 7: Economical study of feeding of Nile tilapia fed diets containing graded levels of BDG.

Item	diets				
	T_0	T_1	T_2	T_3	T_4
Costs, LE/ton	4395	4198	3947	3695	3488
Decrease in feed cost (LE).	00	4.48	10.19	15.93	20.64
Consumed feed to produce 1kg fish (kg).	1.71	1.71	1.64	1.76	1.66
Feed cost/Kg gain.	7.78	7.39	6.63	6.68	5.96

However, the control diet recorded the highest price being 4395L.E/ ton, while, for BDG the cost of feed mixture was 4198, 3947, 3695 and 3488 L.E/ ton for 25, 50, 75 and 100% BDG substitution level respectively. By calculation, T_0 showed the high feed cost needed for producing one Kg fish gain (7.78 L.E) while the lowest feed cost (5.96 L.E) for one Kg fish gain was obtained when SBM was completely replaced by BDG. Relatively, the decreasing rate in feed cost to obtain one Kg fish gain increased gradually as the substitution level of BDG increased from 25 to 100%. This confirms the previous obtained finding that BDG could be included safely and economically in fish diets to replace up till 75% of SBM, These results was confirmed by the findings of Soltan (2002), Soltan *et al.*, (2005) and Salama, *et al.*, (2010).

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ARABIC SUMMARY

دراسات غذائيه على تفلة البيره كمصدر بروتين بديل في علائق البلطى النيلى

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اجريت هذه التجربه لدراسة تأثير الاحلال الكلي لكسب فول الصويا بمخلفات صناعة البيره في علائق البلطي النيلي وحيد الجنس على اداء النمو والكفاءه الغذائيه وتحليل جسم الأسماك والكفاءه الاقتصاديه للعلائق.تم تكوين خمس علائق لتحتوي على ٣٠% بروتين خام و ٤,٤٥كيلو كالوري/جم ماده جافه، لتحل فيها تفلة البيره محل٠،٢٥،٥٠، ٧٥ و١٠٠% من بروتين فول الصويا. تم استخدام ٢٢٥ اصبعيه بلطي نيلي وحيد الجنس (0.45جم) وزعت عشوائيا في خمس مجموعات تجريبيه كل منها في ثلاث مكررات. معدل التغذيه اليومي بدأ ب١٥% ليتناقص تدريجيا حتى ٤% من وزن الجسم خلال ١٤ اسبوع هى فترة التجربه.

وقد اظهرت النتائج ان عملية الاستبدال لم يكن لها اي تاثير ضار على كل من آداء النمو او الغذاء المستهلك مع تحسن في كل من معامل التحويل الغذائي، كفاءة استخدام الغذاء والكفاءة النسبيه للبروتين. بالنسبه لتحليل جسم الاسماك فقد اظهرت كل المجموعات ارتفاع في محتواها من المده الجافه، الدهن والطاقه مع انخفاض في نسبة الرطوبه والرماد مقارنة بما كانت عليه في بداية التجربه.