

Effects of Radish Root Extract on Improving the Utilization of Corn Dried Distillers Grains with Solubles in Nile Tilapia (*Oreochromis niloticus*) Fry Diets

¹Faiza A. Salama, ¹M.N. Ali, ¹S.H. Mahmoud, ¹Hayam D. Tonsy and ²M.M.E. Hassouna

¹Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

²Department of Animal Production, Faculty of Agriculture, El-Fayoum University, Egypt

Abstract: The present study aimed to evaluate the effect of replacing corn dried distillers grains with solubles (DDGS) at 0, 50 or 100 % replacement levels of fish meal (FM) without or with radish root extract (as a source of peroxidase enzyme, RRE) on performance of Nile tilapia (*Oreochromis niloticus*) fry. A total number of 180 mono sex Nile tilapia (*Oreochromis niloticus*) fry averaging 1.7 ± 0.23 g in wet body weight were allotted factorially to 6 dietary treatments. Such treatments were formulated to contain approximately 30% crude protein using fish meal as source of animal protein in treatment one, T₁ (control diet), DDGS (27 % CP) to replace 50 % of fish meal in diet two (T₂) and 100% in diet three (T₃), without using radish root extract (RRE). RRE was added at 1% to the previous diets to obtain the other three treatments T₄, T₅ and T₆, respectively. The fish were stocked at a density of 10 fish/aquarium (70 l each) in triplicate via water recirculating system with exchange water rate approximately 10% of total volume per day. Using DDGS at 50 or 100% instead of fish meal significantly decreased weight gain of fish by 31.65 and 58.05 %, respectively compared to control diet. The addition of RRE to DDGS at 50 or 100% instead of fish meal significantly increased weight gain by 55.7 and 40.83 %, respectively compared to such diets alone. The addition of RRE to control, DDGS at 50 or 100% instead of fish meal improved feed conversion by 4.79, 17.25 and 13.05%, respectively compared to such diets alone. It seemed that RRE was a suitable and natural additive to DDGS diet. Further research is needed for the use of DDGS at higher inclusion levels in the diet to replace soybean meal after using different enhancers.

Key words: Nile tilapia • Corn dried distillers grains with solubles • Radish root extract • Growth performance • Feed utilization

INTRODUCTION

Fish meal (FM) is used as a major protein source for most finfish and crustacean diets [1]. Due to the high cost of fish meal and other considerations, there is interest in the partial or total replacement of these ingredients with less expensive plant protein meals without adverse effect on growth and health of culture species [2]. Distillers dried grains with solubles (DDGS) are the principal by-products of ethanol production from the fermentation of dry milled whole grains [3]. Hilton and Slinger [4] reported that rainbow trout could utilize diets containing 100 g maize DDGS/kg. In a pond study with channel catfish, Robinson and Li [5] reported that up to 300-400 g /kg DDGS with supplemental lysine could be used in fish diets. They also noted that feed efficiency ratio was improved in fish fed diets containing 300-400 g/kg DDGS.

Tidwell *et al.* [6] concluded that growth, survival and pond yield of freshwater prawn (*Macrobrachium rosenbergii*) were unaffected by either 50% or 100% replacement of fish meal with soybean meal and distillers dried grains with soluble (DDGS). Labib *et al.* [7] found that the possibility of partial replacement (40 % about 23.08%DDGS of soybean meal protein by distillers dried grain with soluble protein had no adverse effects on growth performance or feed utilization of Nile tilapia. On the other hand, the high variability among DDGS products within and between individual production plants, due to grain characteristics and processing, which may be a limiting factor on the use of DDGS products in aqua feeds [8-10]. It is clear from previous studies, Parsons *et al.* [9] that excessive heat applied during the drying process of DDGS may cause Maillard reactions between the lysine residues and carbohydrate moieties, subsequently

darkening the color of by-product. Maillard reaction may reduce the digestibility of lysine by competing with absorption of lysine [11] or inhibiting the release of protein bound lysine by inhibition of carboxypeptidases [12]. Also, Maillard reactions cause inhibition of growth, protein and carbohydrate digestion, amino acid absorption and activity of intestinal enzymes including amino-peptidases, proteases and saccharidases [13] but low molecular weight of Maillard reaction products exhibit antioxidant effects after they get absorbed in small intestine [14]. Melanoidins are high molecular weight amino-carbonyl compounds (Maillard reactions) produced by non-enzymatic browning reactions. The enzymatic system responsible for the degradation of melanoidins (high molecular) consists mainly of sugar oxidases and peroxidases, manganese dependent and independent peroxidases [15].

The peroxidase enzyme affects the Maillard reactions, the fiber matrix and phenolic compounds [16]. Compared alkaline treatment and phenoloxidase (Laccase and Horseradish peroxidase) treatment to cellulase-treated lignin-carbohydrate complex. They found that similar amounts and composition of monosaccharide being released from each treatment. Ali [17, 18] described method for improving the utilization of high fiber feedstuffs like wheat bran in broiler [17, 19], laying hens [20,21] and Quail diets [22] using RRE as a source of peroxidase enzyme. They found that RRE either alone or with commercial enzyme preparation improved performance and digestibility of nutrients. Shalash *et al.* [23] found with broilers that fed on diets RRE as a source of peroxidase enzyme is a suitable feed additive for improving the utilization of DDGS.

The aim of the present study is to evaluate the effect of partial (50%) or total replacement of fish meal with DDGS without or with RRE (as a source of peroxidase enzyme) on performance and feed utilization of Nile tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

The present study was carried out at the Utilization By-products Department, Animal Production Research Institute, Agricultural Research Center, Egypt.

Experimental Fish: A total number of 180 mono sex Nile tilapia (*Oreochromis niloticus*) fry of an initial average body weight of 1.7 ± 0.23 g were obtained from commercial fish farm hatchery located in Wady El-Natroun District, El-Behaira Governorate. The fish were weighed in bulk at the start of experiment and they were adapted for 15 days before starting the experiments which lasted for 98 days and the fish were stocked at a density of 10 fish/aquarium (70 Liter each) in triplicate via water recirculation system with water rate exchange of approximately 10% of total volume per day. The daily ration was offered in two equal portions at 9.00 and 13.00 hrs at a level of 6% of body weight for 13 days biweekly. The fish in each triplicate were weighed biweekly at the 14th day i.e., after one day off feeding and the amount of daily diet was adjusted accordingly.

Experimental Diets: Six experimental diets were formulated (Table 2) to contain approximately 30% crude protein using fish meal as source of animal protein in diet one (T₁), fish meal in T₁ (control diet) was replaced by

Table 1: Proximate analysis (DM %) of the feed ingredients used in formulating the experimental diets

Ingredients	Moist.	Crude protein	Ether extract	Crude fiber	Ash	NFE*	GE**
Fish meal	7.10	64.13	5.71	1.02	14.87	2.36	4761
	7.73	63.82	6.43	1.23	16.15	0.07	4904
	8.57	67.05	8.53	0.39	15.45	8.58	4819
Soybean meal	8.85	43.12	1.49	7.19	6.23	42.97	4513
	9.27	42.36	1.03	7.86	5.41	43.34	4473
	9.45	46.52	1.08	6.85	6.30	39.25	4670
Wheat bran	10.78	13.73	3.35	11.62	7.11	64.19	4222
	11.42	14.06	3.81	12.35	7.38	62.40	4250
	10.80	12.44	4.54	12.03	4.11	66.88	4089
Yellow com	11.19	7.21	4.02	2.67	1.27	84.83	4231
	11.36	7.83	3.92	2.55	1.58	84.12	4349
	10.45	7.46	3.46	2.58	1.05	85.45	4260
Corn gluten	9.45	61.42	2.04	1.36	1.28	33.9	5051
	10.13	60.17	2.16	1.93	1.55	34.19	5122
	10.42	58.41	2.40	2.71	1.97	34.51	4965
DDGS*	10.85	26.41	9.50	7.91	6.33	49.85	3855
	11.24	27.32	10.10	7.35	5.74	49.49	3769
	10.97	27.87	11.0	8.2	2.96	49.97	3815

*dried distillers grains with solubles

Table 2: Formulation and proximate analysis of the experimental diets (on %DM) fed to Nile tilapia (*Oreochromis niloticus*) fry

Item	Treatments					
	T1	T2	T3	T4	T5	T6
Ingredients,						
Fish meal (65% CP)	15.0	7.5	-	15.0	7.5	-
Soybean meal (44% CP)	24.0	26.4	25.89	24.0	26.4	25.89
DDGS@	-	18.1	36.11	-	18.1	36.11
Wheat bran	20.0	20.0	11.0	20.0	20.0	11.0
Radish root extract	-	-	-	1.0	1.0	1.0
Yellow corn	25.0	10.0	5.0	24.0	9.0	4.0
Corn gluten	10.0	10.0	12.0	10.0	10.0	12.0
Fish oil	2	4	6	2	4	6
Veg. Oil	2	2	2	2	2	2
Vit. and Min. premix	2	2	2	2	2	2
Total	100	100	100	100	100	100
CP	30.80	30.80	30.19	30.7	30.7	30.09
GE	4529	4457	4424	4529	4457	4424
Proximate analysis (%)						
Moisture	8.5	7.77	6.99	7.84	7.06	7.51
Crude protein (CP)	30.47	31.24	29.76	30.3	29.05	30.57
Ether extract (EE)	6.98	9.25	12.57	6.3	10.8	11.81
Crude fiber (CF)	6.85	7.5	8.12	7.03	8.11	9.56
Ash	7.43	6.74	5.01	8.51	7.51	5.27
NFE*	48.27	45.27	44.54	47.86	44.53	42.79
GE (Kcal/Kg) **	4586	4750	4976	4503	4768	4937

@ Dried distillers grains with solubles

* Calculated by difference.

** Gross energy was calculated from their chemical composition using the factors 5.65, 9.45, 4.0 and 4.0 (Cal GE/g DM) for crude protein, ether extract, crude fiber and nitrogen free extract, respectively [24].

Distiller's dried grain with solubles (DDGS, 27 % CP) at 50 % (PDDGS diet, T₂) and 100% (TDDGS diet, T₃) levels without using radish root extract. One level of radish root extract was added at 1% of diets to T₁, T₂ and T₃ to obtain three treatments T₄, T₅ and T₆, respectively. Radish roots were purchased from local market, Cairo. Radish root extract (RRE) was prepared by cutting the root of radish into chips and put the chips into carrot press and the juice was collected into glass cups. A sample of radish extract was taken to measure peroxidase activity according to method of Amako *et al.* [25]. The peroxidase activity of radish extract is expressed in a unit/milligram protein. Peroxidase activity for RRE was 0.81 U/mg protein. Each diet was mechanically mixed, wetted with water, then mixed thoroughly again and pressure pelleted using meat mixer mincer provided with 2 mm die and sun dried. The experimental diets were freeze-dried and kept in black plastic bags then stored in a refrigerator at 1°C throughout the whole experimental period.

Growth Performance and Feed Utilization Parameters: Growth response parameters were calculated according to Cho and Kaushik [26] as follows:

$$\text{SGR (specific growth rate) \% / day} = 100 (\ln \text{ final weight} - \ln \text{ initial weight}) / \text{days No.}$$

Where: Ln, is the natural log.

$$\text{FCR (feed conversion ratio)} = \text{total dry feed intake (g)} / \text{total wet weight gain (g)}.$$

$$\text{PER (protein efficiency ratio)} = \text{wet weight gain (g)} / \text{protein intake (g)}.$$

$$\text{Feed intake} = \text{total dry feed fed (g/fish)} [27].$$

$$\text{FER (feed efficiency ratio)} = \text{wet weight gain (g)} / \text{dry feed intake (g)}.$$

$$\text{Total gain (g/fish)} = \text{Final weight (g)} - \text{Initial weight (g)}.$$

$$\text{Average daily gain (ADG) (g/fish/day)} = \text{total gain} / \text{duration period in day}$$

$$\text{Protein productive value (PPV \%)} = 100(P_T - P_i) / \text{protein intake (g)}$$

Where: P_T: Protein content in fish carcass at the end and P_i: Protein content at the start.

$$\text{Energy utilization (EU \%)} = 100(E_T - E_i) / \text{Energy intake (kcal)}.$$

Where: E_T: Energy in fish carcass (kcal) at the end and E_i: Energy in fish carcass at the start.

Proximate Analysis of Diet and Fish: Chemical proximate analysis of feed ingredients used in formulating the experimental diets is shown in Table 1. The chemical analysis of ingredients, diets and fish samples were analyzed according to AOAC [28] methods for dry matter, crude protein, ether extract, crude fiber and ash while nitrogen free extract (NFE %) was calculated by difference. Gross energy (GE) contents of the experimental diets and fish samples were calculated using the values 5.65, 9.45 and 4 Kcal/g for CP, fat and Carbohydrates [24]. At the start of the experiment, 15 fish were sampled and stored at -20°C for chemical analysis of whole body composition. After the final weighing, five fish per aquarium were withdrawn and frozen at -20°C till analysis. All carcass traits were calculated as percentage of the whole fish weight.

Statistical Analysis: Biological data obtained from the treatments were subjected to statistical evaluation using two-way analysis of variance (ANOVA) of the general liner model (GLM) using [29] statistical package. Duncan's multiple range test [30] was used to test the significance ($P < 0.05$) of differences among means.

RESULTS

Growth Performance: There were significant differences in final weight and weight gain between different treatments (Table 3). The fish fed control diet plus RRE recorded the highest value while fish fed TDDGS recorded the lowest value. The use of PDDGS or TDDGS significantly decreased weight gain of fish by 31.65 and 58.05 %, respectively compared to control diet. The addition of RRE to control diet significantly increased weight gain by 12.93 % compared to control diet.

The addition of RRE to PDDGS and TDDGS significantly increased weight gain by 55.7 and 40.83 %, respectively compared to PDDGS or TDDGS alone. Significant effects of RRE and DDGS supplements levels or their interactions were found in all growth measurements.

Feed Utilization: The analysis of variance of feed utilization data indicated that there were significant differences between experimental treatments (Table 4). The fish fed control diet plus RRE recorded the highest value of feed intake and the best value of feed conversion ratio while the fish fed TDDGS without RRE recorded the lowest feed intake and the worst feed conversion values. Using PDDGS and TDDGS degrade feed conversion by 17.96 and 60.47% compared to control diet. The addition of RRE to control, PDDGS and TDDGS diet improved feed conversion by 4.79, 17.25 and 13.05%, respectively and the same trends also observed in PER. The addition of RRE to PDDGS increased PPV and EU by 14.18 and 29.84 %, respectively, it gave no response with TDDGS. The effect of RRE on feed utilization measurements was significant except PPV (%). No significant interaction between RRE or DDGS replacement level on feed conversion, PER and PPV (%) were observed. The fish fed control diet plus RRE recorded the highest value of feed intake and the best value of feed conversion ratio while the fish fed TDDGS without RRE recorded the lowest feed intake and the worst feed conversion values. Using PDDGS and TDDGS degrade feed conversion by 17.96 and 60.47% compared to control diet. The addition of RRE to control, PDDGS and TDDGS diet improved feed conversion by 4.79, 17.25 and 13.05%, respectively and the same trends also observed in PER. The addition of RRE to PDDGS increased PPV and EU by 14.18 and 29.84%, respectively, it gave no response with TDDGS.

Table 3: Effect of RRE and DDGS replacement on fish performance

Item	DDGS replacement*	RRE**	Initial weight g	Final Weight G	Weight Gain g	Average daily gain g	Specific growth rate %/day	
T1	0	-	1.75±0.03	26.33±0.62 ^b	24.58±0.58 ^b	0.27±0.006 ^b	2.98 ±0.003 ^a	
T2	50	-	1.71±0.02	18.48±0.90 ^c	16.80±0.90 ^c	0.18±0.008 ^c	2.61±0.03 ^b	
T3	100	-	1.69±0.02	12.04±0.57 ^d	10.31±0.60 ^d	0.11±0.006 ^d	2.13±0.06 ^d	
T4	0	+	1.76±0.02	29.52±0.95 ^a	27.76±0.92 ^a	0.31±0.008 ^a	3.10±0.01 ^a	
T5	50	+	1.76±0.01	27.93±0.82 ^{ab}	26.17±0.83 ^{ab}	0.29±0.006 ^{ab}	3.03±0.03 ^a	
T6	100	+	1.92±0.03	16.44±1.23 ^c	14.52±1.21 ^c	0.16±0.01 ^c	2.36±0.06 ^c	
Source of variation:								
RRE	-	-		NS	0.0001	0.0001	0.0001	0.0001
Level of DDGS replacement	-	-		NS	0.0001	0.0001	0.0001	0.0001
RRE x DDGS	-	-		NS	0.0092	0.0086	0.0161	0.0228

a, b,...etc.: Means within the same column with different superscripts are significantly different ($P < 0.05$).

* dried distillers grains with solubles

**radish root extract

Table 4: Effect of RRE and DDGS replacement on feed utilization.

Item	DDGS replacement	RRE	Feed intake, g	Feed conversion Ratio	PER%	PPV %	EU%
T1	0	-	41.07±1.47 ^a	1.67±0.02 ^a	1.96±0.02 ^{abc}	27.06±0.58 ^{bc}	14.92±0.75 ^b
T2	50	-	33.15±1.16 ^b	1.97±0.04 ^{ab}	1.75±0.14 ^{bcd}	29.75±1.38 ^{ab}	14.65±0.72 ^c
T3	100	-	27.50±1.39 ^c	2.68±0.22 ^c	1.330.11 ^d	21.17±1.87 ^{cd}	13.02±0.42 ^d
T4	0	+	44.221.18 ^a	1.59±0.01 ^a	2.23±0.18 ^a	31.03±1.88 ^{ab}	16.96±0.65 ^a
T5	50	+	42.80±0.46 ^a	1.63±0.04 ^a	2.18±0.08 ^{ab}	33.97±1.51 ^a	16.89±0.69 ^a
T6	100	+	33.40±0.26 ^b	2.33±0.19 ^{bc}	1.52±0.18 ^{cd}	19.72±1.92 ^d	10.25±0.76 ^d
Source of variation:							
RRE	-	-	0.0001	0.0254	0.0134	0.2016	0.0014
Level of DDGS							
replacement	-	-	0.0001	0.0001	0.0011	0.0003	0.0001
RRE*DDGS	-	-	0.0357	NS	NS	NS	0.0067

a, b,...etc.: Means within the same column with different superscripts are significantly different ($P < 0.05$).

* dried distillers grains with solubles

**radish root extract

Table 5: Effect of RRE and DDGS replacement Whole body composition (%DM) of Nile tilapia (*Oreochromis niloticus*).

Item	DDGS	RRE	DM%	CP%	EE%	Ash (%)	Gross energy (Kcal GE/kg)
T1	0	-	24.56±0.80 ^{bc}	55.81±1.03 ^b	15.01±0.38 ^c	12.28±0.15 ^{bc}	5.25±0.01 ^c
T2	50	-	30.00±0.98 ^a	55.03±0.30 ^{bc}	14.05±1.06 ^c	11.61±0.44 ^c	5.22±0.07 ^c
T3	100	-	29.880.69 ^a	51.29±0.72 ^d	26.77±1.78 ^a	9.91±0.96 ^d	5.91±0.12 ^a
T4	0	+	23.10±0.90 ^c	59.96±1.06 ^a	18.28±0.24 ^b	11.37±0.41 ^{cd}	5.53±0.04 ^b
T5	50	+	26.10±0.05 ^b	58.79±0.26 ^a	16.10±0.31 ^{bc}	14.37±0.05 ^a	5.27±0.01 ^c
T6	100	+	24.20±0.50 ^{bc}	53.42±0.46 ^{cd}	18.14±1.00 ^b	13.55±0.30 ^{ab}	5.33±0.07 ^{bc}
Source of variation:							
RRE	-	-	0.0001	0.0001	NS	0.0006	NS
Level of							
DDGS replacement	-	-	0.0002	0.0001	0.0001	0.0427	0.0005
RRE*DDGS	-	-	0.0408	NS	0.0001	0.0013	0.0001

a, b,...etc.: Means within the same column with different superscripts are significantly different ($P < 0.05$).

Fish analysis as zero group was: DM, 22.76%, CP, 53.48%, EE, 20.13%, Ash, 18.92% and GE, 5220 Kcal/Kg.

The effect of RRE on feed utilization measurements was significant except PPV (%). No significant interaction between RRE or DDGS replacement level on feed conversion, PER and PPV (%) were observed.

The interaction between RRE and DDGS was significant in all measurements except protein content.

DISCUSSION

Proximate Analysis of Fish Whole Body: There were significant differences between all values of fish meat recorded by different experimental treatments (Table 5). The fish fed control diet plus RRE recorded the lowest values of dry matter while fish fed TDDGS alone recorded the highest values and conversely with protein content. The fish fed FDDGS recorded the highest value of ether extract while fish fed PDDGS recorded the lowest values. Fish fed PDDGS plus RRE recorded the highest value of ash content while the fish fed TDDGS recorded the lowest value and conversely with gross energy. The main effect of RRE was significant with all measurements except ether extract and gross energy while the DDGS replacement level main effect was significant in all proximate analysis.

Although fish meal has high levels of available energy, excellent amino acid profiles and is very digestible, it is one of the most expensive ingredients in fish diets. One of the economical strategies is to reduce feed costs by substitution of fish meal with alternative plant protein sources. Muzinic *et al.*[31] indicated that fish meal and shrimp meal can be totally replaced with soybean meal and brewer's grains with yeast in diets for juvenile red claw crayfish. This study aimed to evaluate the possibility of improves the utilization of DDGS as a source of protein to replace fish meal by using RRE as a source of peroxidase enzyme. Peroxidase enzyme uses the free radicals in the intestine in oxidation and degradation of Maillard reaction and fiber matrix. The use of PDDGS or

TDDGS significantly decreased weight gain of fish by 31.65 and 58.05 %, respectively compared to control diet. These results disagree with those found by Tidwell *et al.* [6] who concluded that growth, survival and pond yield of freshwater prawn (*Macrobrachium rosenbergii*) were not affected by either 50% or 100% replacement of fish meal with soybean meal and distillers dried grains with solubles (DDGS). Also, Wu *et al.* [32] evaluated the inclusion of DDGS at 30% of the total diet fed to tilapia and reported good growth. The difference between current study and previous studies may be related to the different condition and species. The reduction in growth performance with using DDGS may due to Maillard reactions which may adversely affect protein, mineral and vitamin nutrition [13]. Also, the high content of fiber and / or lower content and availability of amino acid in DDGS compared to fish meal may be the reason of reduction in growth performance. The reduction in lysine digestibility in darker colored DDGS has also been reported by Ergul *et al.* [33], who demonstrated a reduction in true lysine digestibility of approximately 20% from their lightest to darkest DDGS sources. The DDGS used in this study was medium between light and dark. In this respect, Lim *et al.* [34] showed that lysine was a limiting factors in Nile tilapia diet containing 40% DDGS.

The addition of RRE to control diet significantly increased weight gain by 12.93 % compared to control diet. The beneficial effect of RRE with control diet can be explained on the basis that RRE contains factors other than peroxidase that may increase the growth. The RRE contains significant amount of anthocyanins which have radical scavenging [35]. Also the RRE was used as a partial detoxification of aflatoxin B1 with growing chicks [36] or laying hens [37]. The addition of RRE to PDDGS and FDDGS significantly increased weight gain by 55.7 and 40.83 %, respectively compared to such diets alone. These results agree with those obtained by Shalash *et al.* [23] who found with broiler that fed diets containing RRE as a source of peroxidase enzyme is a suitable feed additive for improving the utilization of DDGS. It was observed that the addition of RRE not only improved PDDGS but also increase weight gain, PER, PPV and EU compared to control diet. Chandra *et al.* [14] showed that low molecular weight of Maillard reaction products exhibit antioxidant effects after they get absorbed in small intestine. Shalash *et al.* [23] found that addition of RRE to diet containing 12% DDGS significantly increased plasma antioxidants capacity by 515% compared to birds fed DDGS alone and indicated that peroxidase may degrade the higher Maillard molecules into low molecules weight

which are exhibited as antioxidants affects. It is known that plasma antioxidants capacity is accompanied with the best feed conversion with broiler [19]. So addition of RRE to PDDGS may degrade the higher Maillard molecules into low molecules weight and consequently increased antioxidants capacity. On the other hand, RRE may make another beneficial component in DDGS more available. For example, DDGS may enhance immunity from the yeast component, which may be as much as half the protein in DDGS [38]. The previous discussion can explain the reason of increasing feed utilization when the PDDGS was supplemented with RRE compared to control diet. However,, Abo-State *et al.* [39] reported that *O. niloticus* fingerlings fed on diets containing DDGS with phytase or Lysine had greater growth than the control diet (without phytase or lysine). There were insignificant interaction between DDGS replacement level effect and RRE effect in feed conversion, PER meaning that the level of DDGS only affects feed utilization. It was observed that addition of RRE to control or PDDGS diet increased protein content of fish meat. Putting in mind that RRE contain natural antioxidants which decrease the degradation of protein in the cell by free radical. Tonsy *et al.* [40] indicated that natural antioxidants increase protein deposition in fish meat as a result of decreasing protein lost by free radical. These results are in agreement with those obtained by Zheng *et al.* [41] who found that commercial products containing natural oregano essential oil increase antioxidant activity and enhance muscle protein sedimentation. Also, the whole-body protein contents was increased by myo-inositol (as an antioxidants) supplementation [42]. The increase in ether extract of meat of fish fed FDDGS may be due to higher level of oil in the diet and/ or decrease the utilization of protein and consequently increased fat synthesis. These results agree with previous laboratory and pond studies which shown that the use of 300-350 g /kg DDGS in the diet significantly increases fat deposition in channel catfish [43, 5]. However, Robinson and Li. [44] found that fish body fat deposition increases with increasing dietary fat levels. Increasing ash content in fish fed PDDGS plus RRE can be explained as a result of degradation of Maillard reaction which known adversely affect protein, mineral and vitamin nutrition [13] and / or degrade the fiber matrix and consequently increase ash absorption. It seemed that RRE was a suitable additive to PDDGS diet. From previous results we can hypothesize that RRE succeeded in improving the utilization of PDDGS and to some extent TDDGS diet but the later may have another problem such as lower amino acid content rather than

content of fiber and Maillard reaction. From previous studies, it was observed that higher levels of DDGS used in fish diets did not affect growth indicating that the replacement of soybean by DDGS is not as fish meal. The difference between soybean and fish meal may due to the difference in amino acids contents and nutrients. Further research is needed to use DDGS with higher inclusion rate in the diet to replace soybean meal after supplementation the diet by amino acids and RRE.

CONCLUSION

The use of PDDGS or TDDGS significantly decreased weight gain of fish by 31.65 and 58.05 %, respectively compared to control diet. The addition of RRE to PDDGS and TDDGS significantly increased weight gain by 55.7 and 40.83 %, respectively compared to PDDGS or TDDGS alone. The addition of RRE to control, PDDGS and TDDGS diet improved feed conversion by 4.79, 17.25 and 13.05%, respectively compared to such diets alone. It seemed that RRE was a suitable additive to PDDGS diet. Further research is needed to use DDGS with higher inclusion rate in the diet to replace soybean meal after amino acids and RRE supplementation.

REFERENCES

1. Lovell, R.T., 1989. Nutrition and Feeding of Fish. Van Nostrand Reinhold, New York, USA.
2. Hardy, R.W., 1996. Alternate protein sources for salmon and trout diets. Anim. Feed Sci. Technol., 59: 71-80.
3. Rasco, B.A., F.M. Dong, A.E. Hashisaka, S.S. Gazzaz, S.E. Downey and M.L. San Buenaventura, 1987. Chemical composition of distillers dried grains with solubles (DDGS) from soft white wheat, hard red wheat and corn. J. Food Sci., 52: 236-237.
4. Hilton, J.W. and S.J. Slinger, 1986. Digestibility and utilization of canola meal in practical-type diets for rainbow trout. Can. J. Fish Aquacult. Sci., 43: 149-155.
5. Robinson, E.H. and M.H. Li, 2008. Replacement of soybean meal in channel catfish, *Ictalurus punctatus*, diets with cottonseed meal and distillers dried grains with solubles. J. World Aquacult. Soc., 39: 521-527.
6. Tidwell, J.H., C.D. Webster, D.H. Yancey and L.R. D'Abramo, 1993. Partial and total replacement of fish meal with soybean meal and distillers by-products in diets for pond culture of the freshwater prawn (*Macrobrachium rosenbergii*). Aquaculture, 118: 119-130.
7. Labib, E.M., F.A. Salama, H.D. Tonsy, S.H. Mahmoud and M.A. Zaki, 2010. Nutritional studies on partial and total replacement of soybean meal by distiller's dried grains with soluble (DDGS) in diets for Nile tilapia (*Oreochromis niloticus*). Egyptian J. Nutr. and Feeds, 13(1): 165-176.
8. Belyea, R.L., B.J. Steevens, R.J. Restrepo and A.P. Clubb, 1989. Variation in composition of by-product feeds. J. Dairy Sci., 72: 2339.
9. Parsons, C.M., K. Hashimoto, K.J. Wedekind, Y. Han and D.H. Baker, 1992. Effect of overprocessing on availability of amino acids and energy in soybean meal. Poult. Sci., 7: 133-140.
10. Cromwell, G.L., K.L. Herkelman and T.S. Stahly, 1993. Physical, chemical and nutritional characteristics of distillers' dried grains with solubles for chicks and pigs. J. Anim. Sci., 71: 679-686.
11. Sherr, B., C.M. Lee and C. Jelesciwicz, 1989. Absorption and metabolism of lysine Maillard products in relation to the utilization of l-lysine. J. Agric. Food Chem., 37: 119-122.
12. Hansen, L.P. and R.J. Millington, 1979. Blockage of protein enzymatic digestion (carboxypeptidase-B) by heat-induced sugar lysine reactions. J. Food Sci., 44: 1173-1177.
13. Finot, P.A., 1990. Metabolism and physiological effects of Maillard reaction products. In The Maillard Reaction in Food Processing, Human Nutrition and Physiology Aeschbacher, H.U., R.F. Hurrell, R. Liardon, Eds. Birkhauser: Basel, Switzerland, pp: 259-271.
14. Chandra, R., R.N. Bharagava and V. Rai, 2008. Melanoidins as major colourant in sugarcane molasses based distillery effluent and its degradation. Bioresource Technol., 99: 4648-4660.
15. Watanabe, Y., R. Sugi, Y. Tanaka and S. Hayashida, 1982. Enzymatic decolourisation of melanoidin by *Coriolus* sp. no. 20. Agric. Biol. Chem., 46: 1623-1630.
16. Tsujiyama, S.I., J.I. Azuma and K. Okamura, 1993. Degradation of lignin-carbohydrate complex (LCC) by wood-rotting fungi IV. Enzymic splitting of bondings between hemicellulose and lignin viaquinone methide by coriolus versicolor. Mokuzai. Gakkaishi. J. Japan Wood Res. Society, 39: 1404-1408.
17. Ali, M.N., 2002. Improvement the utilization of some feed ingredients with high contents of fiber in poultry diets. Ph.D. Thesis, Fac. of Agric., Ain Shams Univ., Egypt.

18. Ali, M.N., 2005. Triple powered compound for improving performance and digestion of poultry diets. Patent 23365. Egyptian Patent Office Issue No, 106: 3
19. Ali, M.N., M.S. Abou Sekken and Mostafa, Kout El-Kloub, El., 2008. Incorporation of wheat bran in broilers diets. Int. J. Poultry Sci., 7: 6-13.
20. Abaza, M.I., N.M. Ali and S.M. Hassan, 2004. Nutritional and physiological studies on improving the utilization of wheat bran in laying hen diets. Egypt. Poult. Sci., 24(4): 1015-1031.
21. Ali, M.N., M.S. Hassan and I.M. Abaza, 2006a. Effect of improving the utilization of wheat bran on productive and physiological performance for local laying hens. Egypt. Poult. Sci., 26: 137-158.
22. Ali, M.N., M.M.M. Namra and M. Hala Abdel Wahed, 2006b. Improving the utilization of wheat bran in quail. Egypt. Poult. Sci., 26: 557-577.
23. Shalash, S.M.M., M.N. Ali, M.A.M. Sayed, E. El-Gabry, Hoda and M. Shabaan, 2009. Novel method for improving the utilization of corn dried distiller's grains with solubles in broiler diets. Int. J. Poultry Sci., 8(6): 545-552.
24. Jobling, M., 1983. A short review and critique of methodology used in fish growth and nutrition studies. J. Fish Biol., 23: 685-703.
25. Amako, A., G.X. Ghen and K. Asada, 1994. Separate assays specific for the ascorbate peroxidase and guaiacol peroxidase and for the chloroplastic cytosolic isozyme of ascorbate peroxidase in plants. Plant cell Physiol., 35: 497-507.
26. Cho, C.Y. and S.J. Kaushik, 1985. Effect of protein intake on metabolizable and net energy values of fish diets. In C.B. Cowey, A.M. Mackie and J.G. Bell (Editors), Nutrition and Feeding in Fish. Academic Press, London, pp: 95-117.
27. Richardson, N.I., D.A. Higgs, R.M. Beames and J.R. MC Bride, 1985. Influence of dietary calcium, phosphorus, zinc and sodium phytate levels on cataract incidence, growth and histopathology in juvenile Chinook salmon, *Oncorhynchus tshawytscha*. J. Nutr., 115: 553-567.
28. AOAC., 2000. Association of Official Analytical Chemists. Official Methods of Analysis. 16th Edition, AOAC, Arlington, VG., USA.
29. SAS, 1990. SAS Users Guide, Statistics, SAS Institute, Inc, Cary, NC.
30. Duncan, D.B., 1955. Multiple range tests and multiple F tests. Biometrics, 11: 1-42.
31. Muzinic, L.A., K.R. Thompson, A.C. Morris, D. Webster, D.B. Rouse and L. Manomaitis, 2004. Partial and total replacement of fish meal with soybean meal and brewer's grains with yeast in practical diets for Australian red claw cray fish, *Cherax quadricarinatus*. Aquaculture, 230: 359-376.
32. Wu, Y.V., K. Warner, R. Rosati, D.J. Sessa and P. Brown, 1996. Sensory evaluation and composition of tilapia (*Oreochromis niloticus*) fed diets containing protein-rich ethanol by-products from corn. J. Aquatic Food Product Technol., 5: 7-16.
33. Ergul, T., C. Martinez Amezcua, C. Parsons, B. Walters, J. Brannon and S.L. Noll, 2003. Amino acid digestibility in corn distillers dried grains with solubles. Poult. Sci., 82(Suppl.1): 70.(Abst.).
34. Lim, C., J.C. Garcia, M. Yildirim-Aksoy, P.H. Klesius, C.A. Shoemaker and J.J. Evans, 2007. Growth response and resistance to *Streptococcus iniae* of Nile tilapia fed diets containing distiller's dried grains with solubles. J. World Aquacult. Soc., 38: 231-237.
35. Matsufuji, H., T. Otsuki, T. Takeda, M. Chino and M. Takeda, 2003. Identification of reaction of Acylated Anthocyanins from red radish with peroxy radicals. J. Agric. Food Chem., 51: 3157-3161.
36. Qota, E.M.A., M.N. Ali, R.A. Hassan and M.K. Abou-Elmagd, 2005. Detoxification of aflatoxin contaminated local chicken diets using aluminosilicate, sodium sulphate and peroxidase enzyme. 3rd International Poultry Conference 4-7 Apr. 2005 Hurgada-Egypt.
37. Ali, M.N., F.M.A. Qota, R.A. Hassan and M.K. Abou-Elmagd, 2006c. Novel methods of detoxification of aflatoxin B1 in contaminated local laying hen diets. Egypt. Poult. Sci., 26: 911-940.
38. Belyea, R.L., K.D. Rausch and M.E. Tumbleson, 2004. Composition of corn and distillers dried grains with solubles from dry grind ethanol processing. Bioresource Technol., 94: 293-298.
39. Abo-State, H.A., A.M. Tahoun and Y.A. Hammouda, 2009. Effect of Replacement of soybean meal by DDGS combined with commercial phytase on Nile tilapia (*Oreochromis niloticus*) fingerlings growth performance and feed utilization. American-Eurasian J. Agric. Environ. Sci., 5: 473-479.

40. Tonsy, Hayam D., Faiza A. Salama, S.H. Mahmoud, Kh. F. El-Kholy and M.N. Ali, 2010. Efficiency of using low plant protein diets containing some feed additives on growth performance and body composition of Nile tilapia (*Oreochromis niloticus*) fingerlings. *Egyptian J. Nutrition and Feeds*, 13: 607-621.
41. Zheng, Z.L., Justin Y.W. Tan, H.Y. Liu, X.H. Zhou, X. Xiang and K.Y. Wang, 2009. Evaluation of oregano essential oil (*Origanum heracleoticum* L.) on growth, antioxidant effect and resistance against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*) *Aquaculture*, 292: 214-218.
42. Jiang, W.D., L. Feng, Y. Liu, J. Jiang and X.Q. Zhou, 2009. Growth, digestive capacity and intestinal microflora of juvenile Jian carp (*Cyprinus carpio*) var. Jian) fed graded levels of dietary inositol. *Aquacult. Res.*, 40: 955-962.
43. Webster, C.D., J.H. Tidwell and D.H. Yancey, 1991. Evaluation of distillers' grains with solubles as a protein source in diets for channel catfish. *Aquaculture*, 96: 179-190.
44. Robinson, E.H. and M.H. Li, 2007. Catfish Feeds and Feeding. Mississippi Agricultural and Forestry Experiment Station Bulletin No. 1163, Mississippi State, MS, USA.