

Effects of Different Levels of Potassium Diformate on Growth Performance and Feed Utilization of Monosex Nile Tilapia (*Oreochromis niloticus*) Fingerlings

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Received: 15/1/2014

Abstract: Potassium diformate has demonstrated effectiveness in enhancing growth performance of terrestrial animals and improves the general health status of cultured animals by its stronger antimicrobial effect. Consequently, this study was conducted to determine the effects of dietary organic acid salts as (potassium diformate) on growth promoters for monosex Nile tilapia fingerlings. Four experimental diets with three different inclusion levels of potassium diformate (KDF) (0.1, 0.3 and 0.5%) besides the control diet containing no KDF. A total of 120 tilapia, weighing approximately 5 ± 0.5 g, were divided equally between 4 different treatments with 3 replicates (10 fish / replicate). Fish were fed two times a day at rate 3% of body weight on a pelletized isonitrogenous diet containing 25% crude protein and isocaloric (gross energy 393 Kcal/100g). The results indicated that the addition of 0.3% KDF had a significantly higher growth performance, feed conversion ratio, feed efficiency, feed intake and survival of monosex tilapia fingerlings, compared to the control group. In conclusion, these results revealed that using 0.3% KDF was the best in terms of growth performance and economic analysis under this experimental condition.

Keywords: potassium diformate, growth performance, monosex, Nile tilapia.

INTRODUCTION

The human demand of fish consumption is increasing all over the world. Fish is about to become the main alternative source of animal protein. Nile tilapia, *Oreochromis niloticus*, is the predominant cultured species worldwide (Thodesen *et al.*, 2011, 2012; Gjerde *et al.*, 2012; El-Sayed *et al.*, 2012). Tilapia aquaculture represents about 42% of world total aquaculture fish production, and about 64% of the total fish production in Egypt (GAFRD, 2011).

Potassium diformate (K-diformate, KDF) is odorless, low-corrosive, flow able (Hebeler *et al.*, 2000) and dietary inclusion has demonstrated effectiveness in enhancing growth performance of terrestrial animals (Øverland *et al.*, 2000; Canibe *et al.*, 2001; Mroz *et al.*, 2002). Consequently, KDF was the first substance approved as a possible nonantibiotic growth promoter by the European Union [Commission Reg (EC) number 1334/2001]. potassium diformate (KDF) improves the general health status of cultured animals by its stronger antimicrobial effect towards coliform bacteria than toward Lactobacilli (Lueck, 1980; Kirchgessner *et al.*, 1992; Février *et al.*, 2001), leading to a more favorable microbiota with lower population levels of *Escherichia coli* and *Salmonella* and higher population level and diversity of Lactobacilli (Hebeler *et al.*, 2000). To our knowledge only one preliminary study on the inclusion of KDF in aquatic feed has been reported (Ramli *et al.*, 2005) and its antimicrobial mode of action in aquatic animals is therefore unclear.

The aim of the present study was to investigate the effects of different levels of potassium diformate on growth performance and feed utilization of monosex Nile tilapia fingerlings and its effect on body composition with economic analysis.

MATERIALS AND METHODS

An experiment was conducted at fish Research center in Suez Canal University Ismaillia- Egypt to

investigate the effect of dietary contain three levels of potassium diformate (KDF) (0.1, 0.3 and 0.5%) in addition to control diet on growth performance, chemical body composition and economic analysis of monosex Nile tilapia (*O. niloticus*) fingerlings.

Experimental units

Fish were stocked in 12 plastic tanks (80cm x 60cm x 60cm) randomly divided into four equal experimental treatments (10 fish each tank, three replicate/ treatment). Tanks were supplied with air blowers. Photoperiod was 12h light/ 12h dark regulate. About one third of water tank was exchanged with fresh water 2-3 time/week and totally changed with fresh water every 4 weeks.

Experimental fish

One hundred and twenty monosex Nile tilapia (*O. niloticus*) fingerlings with average initial weight of 5 ± 0.5 g which were obtained from El-Morshedy Brothers Company (Fish Hatchery), Abbassa, Abou Hammad, Sharkia, Egypt. Fish were homogenous in body weights and apparently healthy. Fish were acclimated to laboratory conditions for 2 weeks before the experiment start.

Experimental diets

Four isonitrogenous and isocaloric diets were formulated from practical ingredients (Table 1). The control diet had no addition of KDF while the other three diets were supplemented by 0.1, 0.3 and 0.5% potassium diformate for diets 2, 3 and 4 respectively. The experimental diets were isonitrogenous containing 25% crude protein and isocaloric (gross energy 393 Kcal/100g). The experimental diets were prepared by individually weighing of each component thoroughly mixing the mineral, vitamins and additives with corn. This mixture was added to the components together with oil. Water was added until the mixture became suitable for making pellets. The wet mixture was passed through CBM Pelleting machine with 2mm diameter. The produced pellets were dried at room temperature and kept frozen until experimental start. The composition and proximate analysis of the experimental diets are

presented in (Table 1). The fish were fed handly at rate 3% of body weight, twice/day (10 am and 4 pm) throughout the experimental period (84 days).

Experimental Methodology

At the beginning and the end of the experiment, sample of 5 fish were randomly taken from each experimental group for chemical analysis to determine body composition. The tested diets were analyzed for crude protein (CP %) ether extract (EE %), Crude fiber (CF %), ash (%) and moisture. While whole body composition of fish samples were also analyzed except crud fiber (CF %) according to the procedures described by standard A. O. A. C. methods (1995). The nitrogen free-extract (NFE %) was calculated by differences. Fish were weighed every two weeks and the average of 4 weeks was determined by UWE MJW-300 balance to the nearest 0.01g. The amount of feed was readjusted according to the new weight of fish. Water temperature and dissolved oxygen were measured daily using metteler Toledo, model 128.s/No1242. Other water quality including pH and ammonia were measured every two days by pH meter (Orion model 720A,s/no 13062) and ammonia meter by Hanna ammonia meter. Water quality parameters were within the acceptable range for Nile tilapia (Wilson, 1991). Water temperature ranged from 26.5 to 28.0 °C by using a 250 watt immersion heater with thermostat, DO from 5.7 to 6.3 mg/L, pH from 6.8 to 7.9 and ammonia (NH₃) from 0.23 to 0.29 mg/L.

Growth performance parameters:

The growth performance parameters were calculated according to the following equations:

Average Weight Gain (AWG):

(AWG) = Average final weight (g) - Average initial weight (g)

Specific Growth Rate (SGR %/day):

(SGR % / day) = $100 [\ln Wt1 - \ln Wt 0 / T]$

Where: Ln: natural log

Wt 0: initial weight (g).

Wt 1: final weight (g)

T: time of days.

Feed and protein utilization parameters:

Feed and protein utilization parameters are calculated according to the following equations: -

Feed Conversion Ratio (FCR):

FCR = Total feed consumption (g) / weight gain (g).

Feed Efficiency (FE):

FE = weight gain/ Total feed consumption

Feed Intake (FI):

Amount of consumed feed all period.

Protein Efficiency Ratio (PER):

PER = body weight gain (g)/ protein intake (g).

Protein Retention Efficiency (PRE, %):

PRE (%) = $100 [\text{Retained protein (g)} / \text{protein intake}]$.

Survival (S%):

S (%) = $Nt / N0 \times 100$

Where; Nt = Total number of fish survived in tank at end of experiment.

N0 = Total number of fish survived in tank at beginning of experiment. According to (Harrell *et al.*, 1990).

Economical Evaluation:

Feed cost of kg: Calculated from the price of feed ingredient and the cost per kg gain (FCR × price of kg feed). The changes were calculated as a percentage from the highest value. Cost of 1 kg ingredients used were 3.20 L.E. and 10 L.E. for potassium diformate. Egypt Feed Ingredients Price at start of 2012.

Statistical analysis:

The data obtained in this study were analyzed by one-way ANOVA procedure of Statistical Analysis System (SAS Institute, 1998). Means were compared by Duncan's new multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Averages of initial body weight of fish groups at the beginning of the experiment were within range 5 ± 0.5 g (Table 2). The differences among the groups were insignificant ($P > 0.05$) indicating the homogeneity of the experimental groups at the beginning of the experiment. The group of monosex Nile tilapia fingerlings fed diet 3 which containing 0.3% KDF had a significantly ($P < 0.05$) highest BW compared to other groups throughout the experimental period followed by the group of monosex Nile tilapia fingerlings fed the other diets 4, 2 and 1, respectively. This finding is in agreement with Ringø (1992) in Arctic charr (*Salvelinus alpinus*) and Cuvin-Aralar *et al.* (2011) in juvenile male Nile tilapia (*Oreochromis niloticus*).

Also, it was found that the group of monosex Nile tilapia fingerlings fed on diet 3 had highest feed intake then the rest of experimental groups. While the control group had the lowest feed intake than the rest of experimental group in agreement with Ramli *et al.* (2005) reported that potassium diformate significantly increased feed intake ($P < 0.01$).

There was a trend of increasing growth performance with increasing inclusion level of KDF at 0.3% on the basis of final body weight, weight gain and specific growth rate (SGR). These results indicated that the best BWG values were obtained at the end of experiment for the fish fed diet 3 followed in a decreasing order by those fed on diets 4, 2 and 1 respectively (Table 2). Similar results were obtained by Ramli *et al.* (2005) in Nile tilapia and Zhou *et al.* (2009) in hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*) fingerlings. In addition, same trend was found on the result of SGR which indicated that the highest SGR at the end of the experimental period was recorded in the group of monosex Nile tilapia fingerlings fed diet 3 followed in a decreasing order by diets 4, 2 and 1 respectively indicated that the highest SGR at the end of the experimental period was found in the group of monosex Nile tilapia fingerlings fed diet 3 followed in a decreasing order by diets 4, 2 and 1 respectively. This may be due to that potassium diformate (K-diformate, KDF) is odorless, low-corrosive, flow able (Hebeler *et al.*, 2000) or more palatable to fish and dietary inclusion has demonstrated effectiveness in enhancing growth

performance of terrestrial animals (Øverland *et al.*, 2000; Canibe *et al.*, 2001; Mroz *et al.*, 2002). Similar results were obtained by Christiansen and Lückstädt (2008) in Atlantic salmon (*Salmo salar*).

These results indicated that the best value of FCR values were obtained for group of monosex Nile tilapia fingerlings fed diet 3 followed by diets 4, 2 and 1 respectively (Table 2). These results indicated that the highest FE values were obtained for group of monosex Nile tilapia fingerlings fed diet 3 than diets 4, 2 and 1 respectively. Similar results were obtained by Ramli *et al.* (2005). They reported that potassium diformate improved the feed conversion ratio significantly ($P > 0.05$). Owen *et al.* (2006) tested the sodium salt of butyric acid as a feed additive in the African catfish (*Clarias gariepinus*) at 0.2% in two diets. They reported that FCR was improved in catfish. Also, Christiansen and Lückstädt (2008) reported that the addition of 0.8 and 1.4% KDF in the diets of Atlantic salmon (*Salmo salar*) resulted in significantly ($P > 0.05$) better FCR after 126 days of rearing. Liebert *et al.* (2010) reported that when 0.3% of sodium diformate was added to the diet of the tilapia fingerlings (*Oreochromis niloticus*), feed conversion ratio significantly improved. Cuvin-Aralar *et al.* (2011) reported that the supplementation of either KDF or NDF significantly ($P > 0.05$) improved feed conversion of male Nile tilapia compared to the control group, these results are in agreement with the present study.

The highest PER values were obtained for group of monosex Nile tilapia fingerlings fed diet 3 followed in a decreasing order by diets 4, 2 and 1 respectively. Similar results were obtained by Ramli *et al.* (2005) reported that protein efficiency ratio was also significantly ($P < 0.05$) improved due to the addition of the formic acid salt. The improvement was superior with 0.2% and 0.5% addition of potassium diformate. This result was in agreement with Lückstädt and Schulz (2008) and Storebakken *et al.* (2010) due to higher protein digestibility.

These results indicated that the highest PRE values were obtained for group of monosex Nile tilapia fingerlings fed diet 3 followed in a decreasing order by diets 2, 4 and 1, respectively. A similar result was obtained by Liebert *et al.* (2010) showed that when 0.3% of sodium diformate was added in the diet of the tilapia fingerlings (*Oreochromis niloticus*), protein retention efficiency improved significantly.

There were significant differences ($P < 0.05$) in survival rate among group of monosex Nile tilapia (*O. niloticus*) fingerlings fed different KDF level. The group of monosex Nile tilapia fingerlings fed diet 3 (0.3% KDF) had a highest survival rate 100%. While the group of fingerlings fed diet 1 (Control) and 2 had a lowest survival rate 93% (Table 2). In agreement with Ramli *et al.* (2005) concluded that the use of potassium diformate at 0.2% is an efficient tool to control *V. anguillarum* in tropical tilapia culture.

Table (1): Diet composition and chemical composition (%) used in experiment.

Feed Ingredients	Experimental Diets			
	1 (Control)	2 (0.1% KDF)	3(0.3% KDF)	4(0.5% KDF)
Fish meal	20	20	20	20
Corn gluten	60	60	60	60
Soybean meal	330	330	330	330
Wheat Mill run	130	130	130	130
Rice bran	140	140	140	140
Yellow corn	250	249	247	245
DCP (Di calcium phosphate)1	10	10	10	10
Vitamin & Mineral Mix2	20	20	20	20
C.M.C3	20	20	20	20
Soybean oil	20	20	20	20
Potassium diformate4	-	1	3	5
TOTAL	1000	1000	1000	1000
Chemical Composition (%)				
Moisture	10.62	10.50	10.31	10.44
Crude protein	25.13	25.30	25.00	25.22
Ether extract	7.71	7.82	7.63	7.75
Crude fiber	6.24	6.16	6.1	6.05
Ash	7.05	7.1	7.12	7.16
N.F.E5	43.25	43.12	43.84	43.38
Gross energy Kcal/ 100g6	392.60	394.07	393.54	394.02
P/E ratio CP/GE 7	64.01	64.20	63.53	64.01

DCP (Di calcium phosphate)

Each Kg vitamin & mineral mixture premix contained Vitamin A, 4.8 million IU, D3, 0.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g; B12, 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.

Carboxyl methyl cellulose

AQUAFORM® (KDF) ADDCON, Germany

Nitrogen Free Extract = $100 - (\% \text{Protein} + \% \text{Fat} + \% \text{Fiber} + \% \text{Ash})$.

Gross Energy calculated based on protein (5.65 Kcal/g), fat (9.45 Kcal/g) and carbohydrate (4.11 Kcal/g). According to (NRC, 2011).

Protein Energy Ratio. (crud protein mg / GE Kcal).

Body Composition

The effects of different levels of KDF in dietary monosex Nile tilapia fingerlings on body chemical analysis are presented in (Table 3). It was observed that the best protein content was obtained in group of monosex Nile tilapia fingerlings fed diet 3 (0.3% KDF) than the rest of experimental groups. In agreement with Rahman *et al.* (2008). While the lowest means of ether extract and ash content were found in group of fish fed diet 3. There were no significant differences ($P>0.05$) in moisture contents in all treatments at the end experiment. Similar results were obtained by Ng *et al.* (2009).

Economic Evaluation

Calculations of economical efficiency of the tested diets based on the cost of feed, costs of one Kg gain in weight and its ratio with the control group are shown in Table (4). The lowest Feed cost /1Kg gain (4.68 LE) followed by group of fish fed diets 4, 2 and 1 (control) respectively. The percent of feed cost of Kg gain relative to the control 100% were 83% in group of fish fed diet 3 (0.3% KDF) followed by group of fish fed diets 4 (0.5% KDF) 94%, and 2 (0.1% KDF) 95% respectively.

Table (2): Means growth performance, feed intake, nutrient efficiency and survival rate of monosex Nile tilapia fingerlings (*O. niloticus*) fed different levels of potassium diformate for 84 days.

Parameter	Diet No.			
	1 (Control)	2 (0.1% KDF)	3 (0.3% KDF)	4 (0.5% KDF)
Average Initial Body Weight(g)	5 ^a ± 0.49	5.2 ^a ± 0.54	5.1 ^a ± 0.89	5 ^a ± 0.75
Average Final Body Weight (g).	17 ^c ±0.68	20 ^c ±0.32	29 ^a ±0.65	23 ^b ±0.52
Average Body Weight gain (g)	12 ^c ± 0.46	15 ^b ± 0.35	24 ^a ± 0.66	18 ^b ± 0.55
Specific Growth Rate (SGR) (%/day)	1.46 ^c ± 0.21	1.60 ^b ± 0.44	2.07 ^a ± 0.61	1.82 ^b ± 0.40
Feed Intake (FI) (g)	21.2 ^c ± 0.87	24.8 ^b ± 0.94	34.6 ^a ± 0.86	29.4 ^b ± 0.93
Feed Conversion Ratio (FCR)	1.77 ^c ± 0.52	1.68 ^b ± 0.50	1.45 ^a ± 0.37	1.63 ^b ± 0.43
Feed Efficiency (FE)	0.57 ^c ± 0.29	0.60 ^b ± 0.31	0.69 ^a ± 0.29	0.61 ^b ± 0.31
Protein Efficiency Ratio (PER)	2.52 ^c ± 0.40	2.64 ^b ± 0.37	3.08 ^a ± 0.37	2.71 ^b ± 0.64
Protein Retention Efficiency (PRE)	34.35 ^c ± 0.31	36.36 ^b ± 0.26	40.74 ^a ± 0.53	35.56 ^b ± 0.89
Survival Rate (%)	93 ^c	93 ^c	100 ^a	97 ^b

* Means in the same row having the same superscript letter are not significantly different ($P>0.05$).

Table (3): Effect of different levels of potassium diformate on body composition (% dry weight) of monosex Nile tilapia fingerlings *O. niloticus* at the start and end of the experiment (84 Day, Means±SD).

Item	at Initial	Diet No.			
		1 (Control)	2 (0.1% KDF)	3 (0.3% KDF)	4 (0.5% KDF)
Moisture	77.20	73.4a ± 1.63	72.96 a ± 1.22	73.33 a ± 1.35	73.95 a ± 1.42
at end of the experiment					
Crude Protein	49.31	52.12b ± 0.73	52.72b ± 0.54	56.18a ± 0.62	51.94 b ± 0.26
Ether extract	30.51	31.23b± 0.26	31.12b ± 0.38	30.34a ± 0.51	31.20 b ± 0.37
Ash	20.18	16.65b ± 0.32	16.16b ± 0.24	13.48a ± 0.31	16.86 b ± 0.23

Note: Values are means ± SE of three replications. Means in the same row having different superscripts are significantly different ($P < 0.05$).

Table (4): Economic analysis of Nile tilapia fingerlings (*O. niloticus*) fed different levels of potassium diformate for 84 days.

	Diet No.			
	1 (Control)	2 (0.1% KDF)	3 (0.3% KDF)	4 (0.5% KDF)
Cost of Kg diets (L.E)	3.20	3.21	3.23	3.25
Feed Cost /1 kg fish gain(L.E)	5.65	5.38	4.68	5.31
Relative % of feed cost of Kg gain	100	95	83	94

IN CONCLUSION

It was found that the best group of fish fed KDF (0.3%) in term of growth performance, feed utilization and economic analysis under this experimental condition.

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تأثير التركيزات المختلفة من البوتاسيوم داي فورمات على أداء النمو والاستفادة الغذائية لاصبغيات سمك البلطي النيلي وحيد الجنس

عبد الحميد محمد صلاح عيد - خالد احمد السيد على محمد - بديدة عبد الفتاح على - نهاد محمود سيد
كلية الزراعة - جامعة قناة السويس - ٤١٥٢٢ الإسماعيلية - مصر

أجريت تجربة تغذية لمدة ١٢ أسبوع لتقدير تأثير إضافة أملاح الأحماض العضوية كمنشطات للنمو في علف أصبغيات أسماك البلطي النيلي وحيد الجنس. تم توزيع عدد ١٢٠ إصبغية بلطي نيلي ذات متوسط وزن 5 ± 0.5 جم إلى أربع مجاميع تجريبية احتوت ثلاث منها على ملح البوتاسيوم داي فورمات (KDF) بثلاث تركيزات (٠,١ ، ٠,٣ ، ٠,٥ %) بالإضافة إلى العليقة الكنترول. كل معاملة ٣ مكررات (١٠ إصبغيات / مكررة). غذيت الأسماك مرتين يوميا بمعدل ٣% من وزن الجسم على علائق متساوية في البروتين ٢٥ % بروتين والطاقة الكلية ٣٩٣ كيلو كالوري/ ١٠٠ جم. أشارت النتائج إلى إن إضافة البوتاسيوم داي فورمات بنسبة ٠,٣ % سجلت اعلي زيادة معنوية من حيث أداء النمو ، معامل التحويل الغذائي، الاستفادة الغذائية والغذاء المستهلك و معدل الإعاشة لاصبغيات البلطي النيلي وحيد الجنس مقارنة بالعليقة الكنترول. أظهرت النتائج إن استخدام البوتاسيوم داي فورمات بتركيز ٠,٣ % كان الأفضل من حيث أداء النمو و الاستفادة الغذائية والتحليل الاقتصادي تحت هذه الظروف التجريبية.