Effect of Dietary Levels of Potasium Diformate on Growth Feed Utilization and Resistance to *Streptococcus iniae* of Nile Tilapia, *Oreochromis niloticus*

ABSTRACT

Mixed-sex Nile tilapia juvenile (7.05 ± 0.14 g) in four replicate aquaria were fed each of the diets supplemented with 0 (control), 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 % potassium diformate (KDF) twice daily to apparent satiation for 12 weeks. Survival at the end of week 12 did not differ among fish fed different diets. Weight gain and feed efficiency ratio of fish fed the diet with 1.00 % KDF were significantly higher than those fed diets containing 1.25 and 1-50% KDF but were not different from those fed diets with lower supplemental levels (0, 0.25, and 0.75%) of KDF. Dry matter feed intake was highest and lowest for fish fed diets with 0.75 and 1.5% KDF, respectively. These values were significantly different from those of fish fed other diets. There were no significant differences among hematological parameters (total, red and white blood cell counts, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) of fish fed different diets. Likewise, innate immune responses (serum protein, immunoglobulin, lysozyme and alternative complement) did not differ among treatments. Cumulative mortality 14 days post-challenge with S. iniae and post-challenge antibody titer against the same bacterium were also not affected by dietary treatments.

INTRODUCTION

In intensive aquaculture production, loss due to bacterial diseases has been identified as a major economic loss to producers. Feeding antibiotic-medicated feeds is a common practice to treat bacterial infections. The prophylactic use of antibiotics as growth promoters in aquaculture production has also occurred, although not as extensive as in swine and poultry production. This can lead to the emergence of antibiotic-resistance bacteria, and contamination in food product and the environment. The use of antibiotics in animal production has been banned in EU countries and is increasingly under public scrutiny and criticism in most other countries. Consequently, a wide variety of products ranging from plant extracts, prebiotics, probiotics and organic acids or their salts have been evaluated as alternatives to antibiotics, but results obtained are inconsistent

OBJECTIVES

This study evaluated the effect of various dietary levels of potassium diformate (KDF) on growth performance, feed utilization efficiency, hematology, immune response and resistance of Nile tilapia to Streptococcus iniae challenge

MATERIALS AND METHODS

Mixed sex juvenile Nile tilapia produced at our laboratory were acclimated to the basal diet for 2 weeks to an average weight of 7.05 ± 0.14 g and randomly stocked into 28, 57-L aquaria at a density of 35 fish per aquarium. Aquaria were supplied with flow-through, dechlorinated, heated municipal water at an initial rate of about 0.6 L/min and increased gradually to about 1.0 L/min by the end of week 10. Water was continuously aerated using air stones. During the trial, water temperature averaged 24.6 ± 0.3 C, and dissolved oxygen averaged 6.4 ± 0.2 mg/L. Photoperiod was maintained at a 12:12 h light:dark schedule.

A basal practical diet was formulated to contain approximately 36% crude protein, 6% lipid and 3,200 kcal/kg of digestible energy (Table 1). The basal diet was supplemented with KDF at 0, 0.25, .50, 0.75, 1.00, 1.25 or 1.50% at the expense of celufil. Diets were processed using Hobart mixer and grinder and dried at room temperature to a moisture content of about 10%, ground, sieved to appropriate sizes and stored in plastic bags at -20 C throughout the feeding trial. Fish in 4 random aquaria were fed one of the 7 experimental diets twice daily to apparent satiation for 12 weeks and the amounts of diet consumed was recorded daily. Once a week, aquaria were scrubbed and accumulated wastes siphoned. On cleaning days, fish were fed only in the afternoon. Fish in each aquarium were group-weighed and counted at 3-week intervals. Feed was not offered on sampling days.

At the end of the feeding period (week 12), 3 random fish from each tank were bled with heparinized (100 IU/mL) tuberculin for hematological assays (2 determinations/fish) following the methods described by Lim et al. (2009). An additional 4 fish per tank were bled using non-heparinized tuberculin syringes and serum samples collected and stored at -80 C until used. Serum from each of the four fish per tank was assayed in duplicate for serum total protein, total immunoglobulin, and lysozyme and complement activity using the methods described by Lim et al. (2009).

Twenty (20) remaining fish per aquarium were randomly selected and intra-peritoneally (IP) injected with 0.1 mL of 1x104 cfu/mL of S. iniae (1x103 cfu/fish) using a tuberculin syringe and only the challenged fish were returned to their respective aquaria. They continued to receive their respective diets. Fish were monitored and mortality was recorded twice daily for 15 days following injection and dead fish removed. Fifteen days after challenge, four surviving fish per tank were bled and serum was collected for determination of agglutinating antibody titers against S. iniae as described in Yildirim-Aksoy et al. (2007).
Data were analyzed by one-way analysis of variance using the General Linear Model (GLM) of SAS. Duncan's multiple range test was used to compare treatment means. Differences were considered significant at the 0.05 probability level.

Table 1. Percentage composition of the basal diet

Ingredients	*% in diet*
Menhaden fish meal	8.0
Dehulled soybean meal	45.0
Corn meal	23.5
Wheat middling	13.6
Corn oil	3.4
CMC	3.0
Dicalcium phosphate	1.0
Mineral premix	0.5
Vitamin premix	0.5
Celufil	1.5
Analyzed Nutrients:	
Crude protein (%)	36.7
Crude fat (%)	5.94
Estimated DE (kcal/g)	3.2

Table 2. Growth performance, feed utilization efficiency and survival of Nile tilapia fed various levels of dietary KDF

Levels of KDF (% of diet)	Weight gain (g)	Feed intake (g DM/fish)	FER	Survival
0	48.9ab	69.3b	0.70ab	99.8
0.25	50.0ab	68.6b	0.73a	96.4
0.50	49.9ab	68.4b	0.73a	97.8
0.75	50.9ab	73.9a	0.69abc	95.0
1.00	51.4a	69.6b	0.74a	97.1
1.25	44.6bc	69.3b	0.64c	97.9
1.50	41.7c	63.5c	0.66bc	92.9
Pooled SEM	1.9	1.2	0.02	1.8

Table 3. Hematological parameters of Nile tilapia fed various levels of dietary KDF

Levels of KDF (% of diet)	RBC x106/uL	WBC x105/uL	Ht (%)	Hb (g/dL)	MCHC (%)	MCV (fL)
0	2.08	2.54	28.96	7.74	26.81	141.9
0.25	1.80	2.77	29.21	8.27	28.46	162.7
0.50	2.10	2.86	3150	7.89	25.24	151.1
0.75	1.94	2.70	29.17	7.66	26.60	154.4
1.00	2.08	2.41	30.04	7.73	25.93	145.2
1.25	1.83	2.62	27.88	7.50	26.97	153.2
1.50	1.94	2.86	29.62	7.78	26.43	155.3
Pooled SEM	0.141	0.375	0.300	1.65	1.41	10.1

Table 4. Selected immune responses of Nile tilapia fed various levels of dietary KDF

Levels of KDG (% in diet)	Serum protein (mg/mL)	Total Ig (mg/mL)	Lysozyme activity (µg/mL)	Alternative complement (Units/mL)
0	42.41	3.00	11.77	102.02
0.25	44.75	3.42	13.67	124.20
0.50	43.22	5.54	13.19	123.58
0.75	45.63	5.32	15.96	110.37
1.00	43.96	4.79	12.18	134.35
1.25	43.02	4.19	9.35	128.81
1.50	46.21	7.05	13.30	113.38
Pooled SEM	1.25	2.04	4.73	15.2

RESULTS AND DISCUSSION

Weight gain (WG) and feed efficiency (FE) of fish fed the 1% KDF diet were significantly better than those of fish fed 1.2 or 1.50% KDF diets (Table 2). There were no differences among weight gain of fish fed diets with 1% or lower KDF. Feed intake was significantly highest and lowest in fish fed diets supplemented with 0.75% and 1.50% KDF, respectively. Survival did not differ among treatments. Ramli at al. (2005) obtained significant improvement in WG and feed utilization efficiency in Nile tilapia fed diets containing 0.2 - 0.5% KDF. With the same species, however, Luchstadt (2008; personal communication) obtained a non-significant increase in WG and FE in fish fed the 0.3% KDF. A study with red hybrid tilapia showed no significant improvement in growth, FE and nutrient digestibility in fish fed 0, 0.1, 0.2, 0.3% organic acid blend or 0.2% KDF (Ng et al. 2009). Similarly, the performance of O. niloticus x O. aureus hybrid was not significantly affected by feeding diets containing 0.3 – 1.2% KDF (Zhou et al 2009)

In the present study, hematological parameters (red and white blood cell count, hematocrit, hemoglobin, mean corpuscular hemoglobin concentration and mean corpuscular volume) and innate immune responses (serum protein, total immunoglobulin, lysozyme and complement activity did not differ among treatments. We also observed that supplementation of KDF had no effect on mortality 15 days post challenge with S. iniae and post-challenge antibody titers. An earlier study by Remli et al. (2005) showed decreased mortality 15 days post challenge with Vibrio anguillarum in Nile tilapia fed KDF containing diets but significantly improvement was obtained with the 0.5% KDF diet. Ng et al. (2009) also reported that dietary supplementation of organic acid blend or KDF significantly reduced mortality in red hybrid tilapia 15 days post challenge.

Table 5. Cumulative mortality 15 days post-challenge and antibody production against S. iniae

Levels of KDF (% of diet)	Cumulative mortality (%)	Antibody titer (Log10)
0	20.0	1.32
0.25	25.0	1.30
0.50	20.0	1.24
0.75	28.8	1.45
1.00	32.5	1.19
1.25	25.0	1.28
1.50	18.8	1.43
Pooled SEM	5.3	1.09

CONCLUSION

There was a trend of increased WG of Nile tilapia with increasing dietary levels of KDF from 0.25 to 1.0%, but levels of 1.25% or higher adversely affected WG and FE. However, dietary inclusion of KDF has no effect on hematological parameters , immune responses and the resistance of fish against S. iniae challenge.

The discrepancy among data of published studies on the beneficial effects of inclusion of KDF in tilapia diets may be due to variations among fish species, strain, size or age, levels of inclusion, composition and nutrient content of experimental diets, buffering capacity of dietary ingredients, culture and feeding management, and water quality.

It is suggested that more research be conducted to better understand the mechanism of the potential beneficial effects of this compounds in diets of tilapia as well as of other species.

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