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The Influence of Changing Dietary Cation-Anion Differences and Dietary Na/K Ratios on Growth and Feed Efficiency in Rainbow Trout, *Oncorhynchus mykiss*

[1]Muhammed Arabaci, [2]Huseyin Nursoy and [3]Ibrahim Diler
[1]Department of Fisheries, Faculty of Agriculture,
[2]Department of Animal Nutrition and Disease, Faculty of Veterinary,
Yuzuncu Yil University, Van 65080, Turkey
[3]Department of Aquaculture, Faculty of Fisheries,
Suleyman Demirel University, Egirdir-Isparta, Turkey

Abstract: The effects of diets, regulated in respect of both dietary cation-anion difference and dietary Na^+/K^+ ratio (dietary CAD-Na/K level), on growth, feed efficiency and serum mineral concentration in rainbow trout were investigated. Five dietary CAD-Na/K levels (101-0.4; 352-1.32; 560-2.15; 703-2.63; 981-3.53 meq kg^{-1} mol/mol) were used in the experiment. A total of 500 fish, 2 groups for each treatment consisting of 50 fish with a mean initial body weight of 87±1.75 g were used in the trial. The experiment lasted fifteen weeks. Increasing both dietary CAD level and dietary Na/K ratio has significant positive effects on growth performance and feed efficiency in rainbow trout. It was concluded that weight gain, specific growth rate, thermal-unit growth coefficient and protein efficiency rate increased linearly, feed conversion rate decreased linearly and daily feed intake was not influenced with increasing dietary CAD-Na/K level between 101-0.4 and 981-3.53 (meq kg^{-1} mol/mol). Na, K, Cl and CAD level in the serum remained constant among groups. The dietary CAD-Na/K levels between 560-2.15 and 981-3.53 (meq kg^{-1} mol/mol) produced the best growth for rainbow trout. Use of Na_2CO_3 and $NaHCO_3$ as a cationic supplement and Na source was appropriate for making diet cationic and increasing the CAD level and Na/K ratio of diet.

Key words: Rainbow trout, dietary CAD level, dietary Na/K ratio, growth, feed efficiency

INTRODUCTION

Dietary Cation Anion Difference (CAD) is defined as Na+K-Cl and expressed as meq kg^{-1}. Dietary CAD level has an influence on blood pH, feed intake and growth in dairy cows (Horst *et al*., 1997), dairy calves (Jackson *et al*., 1992), growing steers, lambs (Fauchon *et al*., 1995), chicken (Mongin, 1981), horses (Baker *et al*., 1993) and juvenile African catfish (Dersjant-Li *et al*., 1999). In juvenile African catfish, Dersjant-Li *et al*. (1999) showed that feed intake and growth increased linearly with dietary cation anion difference from -100 to 700 meq kg^{-1} and the level of 700 meq kg^{-1} was optimal. In their later study, Dersjant-Li *et al*. (2001) investigated the effect of dietary Na/K ratios on feed intake, growth and nutrient utilisation of juvenile African catfish at an optimal dietary CAD level of 554-657 meq kg^{-1} and showed that the dietary Na/K ratio of 1.5-2.5 produced the best growth in African catfish. In rainbow trout, Chiu *et al*. (1984, 1987, 1988) investigated the relation between dietary CAD and amino acid metabolism and reported that there was no clear effect of CAD on growth. However, apart from CAD also other factors differed between experiments (Chiu *et al*., 1988). Wilson *et al*. (1985) found no significant effects on growth or feed efficiency in rainbow trout fed a dietary CAD level -90 to 638 meq kg^{-1}. Thus there exists only limited data on the effect of dietary CAD level and dietary Na/K ratio on growth and feed efficiency in rainbow trout.

In this study, rainbow trout diets were regulated in respect of both dietary CAD levels and dietary Na/K ratio (dietary CAD-Na/K level). The objective of the study was therefore to determine the effects of dietary CAD-Na/K levels on growth, feed efficiency and serum mineral concentration in rainbow trout.

MATERIALS AND METHODS

Fish and facilities: The study was conducted at Institute of Mediterranean Fisheries Research, Production and Training, Kepez Unit, Antalya, Turkey. A total of 500 rainbow trout, 2 groups for each treatment consisting of

Corresponding Author: Muhammed Arabaci, Department of Fisheries, Faculty of Agriculture, Yuzuncu Yil University, Van 65080, Turkey

Table 1: Ingredients and chemical composition of the diets used in the experiment

	Dietary measured treatment CAD* level (meq kg^{-1})				
	101	352	560	703	981
	Dietary Na/K (mol mol^{-1})				
Items	0.4	1.32	2.15	2.63	3.53
Ingredients					
Fish meal	45	45	45	45	45
Soybean meal	15	15	15	15	15
Full fat soybean meal	10	10	10	10	10
Blood meal	3	3	3	3	3
Wheat middlings	12.5	10.7	10.7	8.7	8.7
Fish oil	10	10	10	10	10
Additives					
Na_2CO_3			2		4
$NaHCO_3$		2		4	
Choline[4]	0.15	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15	0.15
Pellet binder	0.4	0.4	0.4	0.4	0.4
Antioxidant	0.5	0.3	0.3	0.3	0.3
Analysed composition					
Dry matter (%)	90.20	89.65	89.06	89.33	87.24
Crude ash (%)	10.08	11.50	11.81	11.53	12.74
Crude protein (%)	48.01	47.87	48.73	47.20	47.75
Ether extracts (%)	14.81	11.71	13.23	12.60	12.64
Crude fiber (%)	2.01	2.05	1.82	1.89	2.43
Na (%)	0.26	0.86	1.34	1.67	2.32
K (%)	1.01	1	0.95	0.97	1
Cl (%)	0.96	0.99	0.94	0.97	1
DE** (kcal kg^{-1})	3262	3262	3262	3200	3200
Feed pH	5.96	7.01	8.18	7.38	9.13

[1]Vitamin mixture: Included of per kg; 18.000 IU A, 2.000 IU D3, 200 IU E, 12 mg K, 150 mg C, 30 mg B2, 20 mg B1, 0.05 mg B12, 20 mg pyridoxine, 10 mg panthotenic acid, 220 mg niacin, 210 mg inositol, 5 mg folic acid, 0.5 mg biotine, 2.000 mg choline, [2]Mineral mixture: Included of per kg: 70 mg zinc, 60 mg manganese, 60 mg magnesium, 4 mg ferrum, 2 mg copper, 1.5 mg iodine, 0.5 mg cobalt, 0.05 mg selenium, [3]Vitamin C, Hoffman La-Roche Inc, Istanbul, Turkey, [4]Choline, Ufuk Kimya Ilaç San. ve Tic. Ltd. Sti Istanbul, Turkey, *Calculated from Na, K, Cl content of diet, **DE: Digestible Energy which was calculated with NRC (1993) and New (1987) of tables using for diet ingredients

50 fish with a mean initial body weight of 87±1.75 g (mean±SE) were used in the experiment. Before the commencement of the study, fish were acclimated to rearing conditions for 2 weeks. The study, five dietary CAD-Na/K levels were tested, lasted 15 weeks. Fish were reared at a temperature of 11.2-15°C, under natural light regime. The O_2 concentration was maintained above 6 ppm. Ten concrete ponds were used in the study. Each tank having 40×40×200 cm of dimensions and with 280 L capacity, received a constant flow of water (1.0 l sn^{-1}). Hardness and conductivity of water used in the experiment was 370 mg L^{-1} $CaCO_3$ and 650 μmho cm^{-1}, respectively.

Experimental design, diets and feeding: Five dietary CAD levels (meq kg^{-1}) and dietary Na/K ratio (mol/mol) (101-0.4, 352-1.32, 560-2.15, 703-2.63, 981-3.53) were tested using a randomised experimental design with two replications in each treatment. While increasing the dietary CAD levels, dietary Na/K ratios were also taken into consideration. Dietary CAD levels and dietary Na/K ratios were increased together (r = 0.999). Dietary CAD-Na/K level were varied by supplementation of a basal diet (control) with 2 and 4% $NaHCO_3$, 2 and 4% Na_2CO_3 (Table 1). The basal diet consisted of soybean meal, full fat soybean meal, blood meal, wheat middlings, fish oil, fish meal, choline, methionine, vitamin and mineral premix (Table 1). Diets were pelleted at the size of 3 mm. During 15 weeks, feeding period, meals were supplied to fish manually three times daily (at 09:00, 13:00 and 17:00 h) according to feeding rate. Feeding rate was predetermined according to live weight and water temperature (1-20 days, 2% at 15°C; 21-41 days, 1.8% at 13°C; 42-62 days, 1.7% at 12°C; 63-83 days, 1.5% at 11.5°C; 84-105 days, 1.5% at 11.2°C). However, feed was supplied in small quantities, assuring that fish ate to satiation (*ad libitum*) and no feed was wasted. Feed was added, when predetermined feed amount is not enough. The amount of feed given per day was recorded. Live weight was determined 6 times with 21 days intervals starting from the initiation of experiment to the end of experiment.

Blood (1-2 mL) was sampled from groups of fish (n = 12) by inserting a syringe into the caudal vessels. The blood samples were centrifuged (1500 g for 20 min) to obtain serum. Serum samples were stored -20°C until analysis of mineral content. Serum mineral contents

(Na^+, K^+, Cl^-) were measured in Lloyt Na/K/Cl analyser by ISE method. Dietary pH was measured as described by Tarakci. Dry matter (105°C, overnight), ash (550°C, overnight), crude protein (nitrogen×6.25, Gerhard Kjeldatherm, Königswinter, DE), ether extract (Velp Scientifica 148 Solvent Exractor Milan, IT), crude fiber (Ancom 220, Fiber Analyzer, Macedon, NY, USA) of diets were analysed by methods of AOAC (1990). Dietary $Na^++K^+-Cl^-$ or CAD level (meq kg^{-1}) were determined by Horst *et al.* (1997) and dietary Na/K ratio (mol/mol) was measured from the Na^+, K^+, Cl^- in diets which were analysed according by Kacar.

Calculations: In the study, the following predictions were used (Bascinar *et al.*, 2008; Cho, 1992). The weight gain (WG, g) calculated as: WG = last biomass-first biomass. The Specific Growth Rate (SGR% day^{-1}) was calculated as:

$$SGR = 100\times\left(\frac{(\ln w_2 - \ln w_1)}{\text{Feeding days}}\right)$$

The Daily Feed Intake (DFI%) was calculated as:

$$DFI = 100\times\left(\frac{\frac{\text{Feed consumed (g)}}{\text{Feeding days}}}{\text{Fish number}}\right)\left(\frac{\text{Initial biomass (g)} + \text{Final biomass (g)}}{2}\right)$$

The Feed Conversion Rate (FCR) was calculated as:

$$FCR = \frac{\text{Feed consumed}}{(\text{Increase in biomass + dead fish biomass})}$$

Thermal-unit Growth Coefficient (TGC) calculated as:

$$TGC = \frac{\text{Final body weight }(g^{1/3})\text{-Initial body weight }(g^{1/3})}{\sum \text{water temperature }(°C \times \text{Day})\times 100}$$

Protein Efficiency Rate (PER) calculated as:

$$PER = \frac{\text{Increase in biomass (g)}}{\text{Protein consumed (g)}}$$

Statistical analysis: Linear or non-linear regression was analysed using SPSS (2008) Statistical Package program. For curve fitting, treatment means were used. Stepwise Elimination Method was used to determine significant independent variables in Multiple Regression analyses.

RESULTS AND DISCUSSION

Growth: Weight gain, specific growth rate and thermal-unit growth coefficient increased linearly with increasing dietary CAD level and dietary Na/K ratio (Table 2 and 3).

Feed efficiency: Daily feed intakes ranged from 1.37-1.43 among groups and were not significantly influenced by dietary CAD-Na/K level (Table 2 and 3). Feed conversion rate decreased linearly with increasing dietary CAD level and dietary Na/K ratio. Protein efficiency rate increased linearly with increasing dietary CAD level and dietary Na/K ratio (Table 2 and 3).

Mineral contents in serum and mortality: Mineral contents of serum were not significantly influenced by dietary CAD level and dietary Na/K ratio (Table 2 and 3). Mortality ranged from 2-8% among groups and was insignificantly influenced by dietary CAD-Na/K level (Table 3).

Multiple stepwise regression: In multiple regression model including two independent variables, when dietary CAD level and Na/K ratio were taken into consideration, there was a multicollinearity problem in multiple regression analysis. However, after stepwise elimination method was applied for multiple regression analysis, dietary Na/K ratio as independent variable in simple linear regression was considered. In simple linear regression, all the regressions of dietary Na/K ratio on WG, SGR, TGC, FCR and PER were significant (Table 4, $p<0.05$). For example, 81% of variation in WG was explained by variation in dietary Na/K ratio and WG would be expected to be an increase of 0.926 with increasing dietary Na/K ratio. 86.6% variation in FCR was explained by variation in dietary Na/K ratio and FCR would be expected to be an decrease of 0.930 with increasing dietary Na/K ratio.

The results of the present study with rainbow trout indicated that the diets regulated in respect of both CAD levels and Na/K ratios improved the growth and feed efficiency. Dietary CAD-Na/K level of between 560-2.15 and 981-3.53 produced the best growth performance in rainbow trout. The studies about the effects of dietary CAD level and dietary Na/K ratio on growth performance in fish species are few in number. The most studied fish species on this topic is African catfish. Different dietary regulations caused to different response in African catfish in respect of growth performance and feed efficiency.When only dietary CAD was increased within the range -100 to 700 meq kg^{-1} (Dersjant-Li *et al.*, 1999), feed intake and growth increased linearly with an increase

Table 2: Growth performance, feed efficiency and serum mineral composition in experimental groups (Mean±Standard error)

	Dietary measured CAD* level (meq kg^{-1})				
	101	352	560	703	981
	Dietary Na/K (mol mol^{-1})				
Parameters	0.4	1.32	2.15	2.63	3.53
Growth					
WG (g)	138.50±3.5	142.50±6.5	161.50±0.5	167.50±6.5	166.50±3.5
SGR (%)	0.88±0.03	0.89±0.040	1.02±0.010	1.066±0.04	1.074±0.02
TGC	0.14±0.004	0.14±0.006	0.16±0.001	0.17±0.006	0.17±0.003
Feed efficiency					
DFI%	1.40±0.01	1.38±0.03	1.37±0.02	1.37±0.003	1.43±0.02
FCR	1.67±0.04	1.57±0.12	1.37±0.03	1.39±0.03	1.36±0.04
PER	1.28±0.03	1.34±0.10	1.50±0.03	1.52±0.04	1.55±0.05
Minerals in serum*mmol L^{-1}±SE					
Na^+	144.26±9.70	144.8±2.94	143.08±4.48	146.36±10.44	146.67±6.77
K^+	15.69±3.57	14.21±2.67	16.25±1.59	22.08±0.97	19.71±0.33
Cl^-	121.92±3.26	124.1±0.24	122.05±1.47	127.24±6.11	128.99±6.73
CAD meq L^{-1}	184.65±13.61	173.74±2.44	184.86±6.83	203.79±16.34	185.81±0.94

WG: Weight Gain, SGR: Specific Growth Rate, TGC: Thermal-unit Growth Coefficient, DFI: Daily Feed Intake, FCR: Feed Conversion Rate, PER: Protein Efficiency Rate, *The initial values for serum minerals were 138.71±0.86, 12.97±0.65 and 116.16±0.67 mmol L^{-1} for Na, K and Cl, respectively and 176.16±5.4 meq L^{-1} for CAD

Table 3: Regression equations and coefficients (R^2) of dietary CAD level and dietary Na/K ratio on growth performance, feed efficiency, serum mineral composition and mortality

Effect of dietary CAD level	Effect of dietary Na/K ratio	Main effect*
Growth		
WG = 135.10051 + 0.0374481 CAD. R^2: 0.8307	WG = 134.02208 + 10.60714 Na/K, R^2:0.8574	L
SGR = 0.8465542 + 0.0002586 CAD, R^2: 0.8637	SGR = 0.8397845 + 0.0729148 Na/K, R^2: 0.8832	L
TGC = 0.1343624 + 0.0000389 CAD, R^2: 0.8547	TGC = 0.1333105 + 0.0109845 Na/K, R^2: 0.8766	L
Feed efficiency		
DFI = 1.3775984 + 0.0000223 CAD, R^2:0.097	DFI = 1.3786842 + 0.0054672 Na/K, R^2:0.074	NS
FCR = 1.6774986 - 0.0003809 CAD, R^2: 0.8356	FCR = 1.6888492 - 0.1080908 Na/K, R^2: 0.8655	L
PER = 1.2560822 + 0.0003348 CAD, R^2: 0.8888	PER = 1.2468976 + 0.0946003 Na/K, R^2: 0.9129	L
Minerals in serum and mortality		
CAD_{Blood} = 179.74093 + 0.0126568 CAD, R^2: 0.153	CAD_{Blood} = 179.20837 + 3.6688088 Na/K, R^2: 0.166	NS
Na^+ = 143.49527 + 0.0028527 CAD, R^2: 0.410	Na^+= 143.49273 + 0.7683294 Na/K, R^2: 0.383	NS
K^+ = 13.128564 + 0.007897 CAD, R^2: 0.607	K^+= 12.940105 + 2.2173955 Na/K, R^2: 0.616	NS
Cl= 120.55574 + 0.0079769 CAD, R^2: 0.718	Cl^-= 120.48257 + 2.1814226 Na/K, R^2: 0.690	NS
Mortality = 2.072086 + 0.0054281 CAD, R^2: 0.413	Mortality = 2.0096567 + 1.4906996 Na/K, R^2: 0.401	NS

WG: Weight Gain, SGR: Specific Growth Rate, TGC: Thermal-unit Growth Coefficient, DFI: Daily Feed Intake, FCR: Feed Conversion Rate, PER: Protein Efficiency Rate, *L: Linear effect (p<0.05), NS: Non Significant (p>0.05)

Table 4: Results of the multiple stepwise regression analysis*

Linear regression model (stepwise)	R^2	Adjusted R^2	p-value
WG = 134.022+ 0.926 Na/K	0.857	0.810	0.024
SGR = 0.840+0.940 Na/K	0.883	0.844	0.018
TGC = 0.133+0.936 Na/K	0.877	0.836	0.019
FCR = 1.689-0.930 Na/K	0.930	0.866	0.022
PER = 1.247+0.955 Na/K	0.913	0.884	0.040

*Dependent variables, WG: Weight Gain, SGR: Specific Growth Rate, TGC: Thermal-unit Growth Coefficient, FCR: Feed Conversion Rate, PER: Protein Efficiency Rate

in dietary CAD and feed conversion rate was not significantly influenced. When only dietary Na/K ratio was increased (from 0.2-2.5 mol/mol) at optimal dietary CAD level (554-657 meq kg^{-1}), growth increased quadratically, feed conversion rate decreased quadratically and feed intake was not significantly influenced (Dersjant-Li *et al*., 2001). In the present study, growth increased linearly, feed conversion rate decreased linearly with increasing dietary CAD-Na/K level and daily feed intake was not significantly influenced. In contrast to this and the previous reports, Wilson *et al.* (1985) found no significant growth effects in rainbow trout for dietary CAD ranging from -90 to 638 mEq kg^{-1}. These results may be attributed to the feeding levels (2-2.5% of body weight), lacking of no regulation on dietary Na/K ratio and/or using the pure aminoacid (casein, 50%) as a protein source in test diets. Likewise, Poston and Rumsey (1983) noted that as the amount of crystalline amino acids increased in amino acid test diet, the dietary pH and CAD of the test diet decreased and the effects of CAD could not observed explicitly. It thought that Wilson *et al.* (1985) could not obtain the effects of optimal CAD in their study because of using excess crystalline aminoacid in the diets and/or lacking of no regulation on dietary Na/K ratio. In rainbow trout. Chiu *et al.* (1984, 1987, 1988) investigated the relation between dietary CAD and amino acid metabolism. Apart from CAD also other factors differed between experiments (Chiu *et al.*, 1988). These different results deserve further research.

Dietary Na/K ratio is actually new concept for fish diets or other animal's diets. Sodium is higher in extracellular fluids and potassium is higher in intracellular fluids in vertebrates. It is important for acid-base regulation, osmotic balance, the energy cost for maintaining Na and K gradients between intra-extracellular fluids, homeostasis, Na-K ATPase and Na-K pump activity in organism (Dersjant-Li *et al.*, 2001). These effects are indirectly affected growth performance, feed intake and general health of the animals. For example, when excess potassium was used, growth was depressed in chickens (Johnson and Karunajeewa, 1985) and young pigs (Golz and Crenshaw, 1984). Dersjant-Li *et al.* (2001) reported that the decreasing Na/K ratio in the diet resulted in excess K which consequently depressed growth in African catfish. Shearer (1988) reported that an adequate K of 0.6-1.2% in diet improved growth performance and insufficient K caused mortality and depressed growth in juvenile Chinook salmon. In the present experiment, growth was depressed in the low dietary CAD-Na/K levels of 101-0.4 and 352-1.32.

We think that this result cannot be attributed to excess dietary K. Because dietary K concentration was almost equal in all treatment groups (Table 1). Additionally, dietary K concentrations (0.95-1.01%) were in optimum limits reported for African catfish 0.9-1.3% (Dersjant-Li *et al.*, 2001) and juvenile Chinook salmon 0.6-1.2% (Shearer, 1988). According to results of the multiple stepwise regression analysis, low growth performance could be attributed to low dietary Na/K ratio in the experiment and dietary CAD level seems relatively less important in growth performance in this study. However optimum dietary CAD level has been determined for many animals such as cows, growing steers, lambs, dairy calves, swine, horse and poultry. Below the optimal dietary CAD level, growth performance decreased because of the increased energy requirement for maintenance of homeostasis (Dersjant-Li *et al.*, 1999; Patience *et al.*, 1987; Haydon and West, 1990). According to the result of the present study, dietary CAD level can be >560 meq/kg/dry matter in rainbow trout diet. This dietary CAD level is <700 meq kg^{-1} of African catfish (Dersjant-Li *et al.*, 1999) and higher than dietary CAD levels of other animals such as -128 meq kg^{-1} in anionic diet of cows for preventing milk fever, (Block, 1994), 277 meq kg^{-1} in swine diet (Patience *et al.*, 1987), 370 meq kg^{-1} in dairy calves diet (Jackson *et al.*, 1992), 500 meq kg^{-1} in lambs diet (Fauchon *et al.*, 1995), minimum 150 meq kg^{-1} in laying hens diet (Harms *et al.*, 1996), 180-300 meq kg^{-1} in broiler diet (Martinez *et al.*, 1998), 250-300 meq kg^{-1} in horse diet (Baker *et al.*, 1993). The results indicate that fish can tolerate higher dietary CAD levels than terrestrial animals (Dersjant-Li *et al.*, 1999). The hardness of water used in the study was very high (370 mg L^{-1} $CaCO_3$).

Water hardness is very important for fish culture and is a commonly reported aspect of water quality. It is a measure of the quantity of divalent ions such as calcium, magnesium and/or iron in water. Calcium and magnesium are essential in the biological processes of fish. The presence of free (ionic) calcium in culture water helps reduce the loss of other salts (e.g., sodium and potassium) from fish body fluids. Environmental calcium is also required to re-absorb these lost salts (Wurts and Durborow, 1992). In this study, setting of fish diet in respect of CAD level and Na/K ratio caused to improve in growth and feed efficiency of rainbow trout in very hard water providing the advantages to fish in respect of ionoregulation and biological processes. High hardness of water didn't mask the effects of dietary CAD-Na/K regulation on growth performance of rainbow trout. There are need to further studies, for determining the effects of dietary CAD-Na/K regulation on growth performance of rainbow trout in water having different hardness values.

Daily feed intake was similar in all treatment groups (p>0.05). However, weight gain increased by 11.7-17.3% in the dietary CAD-Na/K level of between 560-2.15 and 981-3.53 groups compared to the low dietary CAD-Na/K levels of 101-0.4 and 352-1.32.

Feed conversion rate decreased by 12.9-22.8% in the dietary CAD-Na/K level of between 560-2.15 and 981-3.53 groups compared to low dietary CAD-Na/K levels of 101-0.4 and 352-1.32. These differences in weight gain and feed conversion rate in treatment groups probably associated with differences in energy costs. Because of the significant decrease in protein efficiency rate in low dietary CAD-Na/K level group, it can be expected that in control and low dietary CAD-Na/K level group, more energy is needed to maintain mineral balance and homeostasis. Dersjant-Li *et al.* (2001) also reported that feed efficiency improved and therefore more fat was deposited and the body nitrogen, fat and energy levels increased linearly with increasing dietary Na/K in African catfish. In earlier reports, when only CAD regulated in diet, feed intake and growth increased with an increase in dietary CAD in African catfish and many animals (e.g., in dairy cows, (West *et al*., 1991), growing steers, (Ross *et al*., 1994), young dairy calves, (Jackson *et al*., 1992), lambs, (Fauchon *et al*., 1995) and in swines, (Patience *et al*., 1987). In African catfish, when only dietary Na/K increased at optimal dietary CAD, growth increased, feed conversion decreased without changing in feed intake. All the results evaluated together, separate regulation of dietary CAD and dietary Na/K produced different response in target animals. Thus dietary CAD and dietary Na/K can be taken into consideration together in preparing of diet.

With a changing dietary CAD-Na/K level, rainbow trout performed well with a wide range of Na levels in the diet and maintained a constant Na, K, Cl and CAD level in the serum. Dersjant-Li *et al*. (2001) reported that plasma K levels increased quadratically but plasma Na, Cl and CAD concentration were not changed with increasing dietary Na/K ratio in African catfish. Dersjant-Li *et al*. (2001) reported that significant lower plasma K concentration in the fish receiving low dietary Na/K ratio of 0.2 was associated with the low dry matter content of the body. When only CAD regulated in diet for the same fish species, plasma K concentration was not changed but plasma Cl concentration increased quadratically, plasma Na and CAD levels decreased quadratically with increasing dietary CAD level (Dersjant-Li *et al*., 1999). In rainbow trout, Wilson *et al*. (1985) reported that plasma chloride was not changed with changes of dietary Na and Cl levels. However, plasma Na concentration was high and plasma K concentration was low in a high-Na/low-Cl diet compared to a high-Cl/low-Na diet. Chiu *et al*. (1984) reported that increasing sodium and potassium levels of the diet in rainbow trout increased potassium concentration but did not affect sodium concentration in the blood. Consequently, different regulations in fish diet affected differently the plasma Na, K, Cl concentrations of fish.

Mortality rate was variable and ranged from 2-8% among groups. No clear effect of dietary CAD-Na/K level on mortality rate was observed (Table 3). Dersjant-Li *et al*. (2001) reported that mortality and disease rate was high in the fish receiving low dietary Na/K ratio of 0.2. For determining the effect of very low dietary Na/K ratio and/or CAD level on mortality rate in rainbow trout, there is need to further research.

The results of the present study imply that Na sources such as $NaHCO_3$ or Na_2CO_3 could be used to increase both dietary CAD level and dietary Na/K ratio in rainbow trout. Increasing both dietary CAD levels and dietary Na/K ratio has positive effects on growth performance of rainbow trout.

CONCLUSION

It was concluded that weight gain, specific growth rate, thermal-unit growth coefficient and protein efficiency rate increased linearly, feed conversion rate decreased linearly and daily fseed intake was not influenced with increasing dietary CAD level and dietary Na/K ratio between 101-0.4 and 981-3.53 (meq kg^{-1} mol/mol). The best growth was taken with the dietary CAD level and dietary Na/K ratio between 560-2.15 and 981-3.53 (meq kg^{-1} mol/mol) for rainbow trout.

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