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The Effects of Virginiamycin Added Feeding on the Growth Performance and Feed Utilisation of the Tilapia (*O. niloticus* L.,) Fry

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Abstract: In this study effects of feeding of tilapia (*O. niloticus*) fries with basal diet prepared on the base of fish meal and soybean oil cake in form of including 34.27% crude protein and 3000 kcal kg^{-1} digestible energy and treatment diets added in the rate of 20, 40, 60, 80 and 100 mg kg^{-1} virginiamycin to this feed on the growth, Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Productive Protein Value (PPV) and Survival Rate (SR) were determined during 90 days. Virginiamycin addition has resulted to increase in gaining live weight and length in all groups. Significant results have been obtained, especially, in 80 and 100 mg kg^{-1} VM rates in accordance with both control and other treatment groups ($p>0.05$). While progress of feed conversion ratio, protein efficiency ratio and productive protein value has been observed by adding 80 and 100 mg kg^{-1} VM to the basal diet again, better results were obtained from control group in adding lower ratio of virginiamycin. However, the differences between these values were not statistically significant ($p>0.05$). Survival rates have been 100% for control, VM20, VM40, VM60, VM80 and VM100 groups.

Key words: *Oreochromis niloticus*, virginiamycin, growth, feed conversion ratio, protein efficiency ratio, productive protein value

INTRODUCTION

Some factors such as obtaining faster fish growth, shortening breeding period and decreasing expended feed amount render possible to the more profitable breeding. For this reason, several feed additives are used in preparing mixed feed. The basic goals of using feed additives are as follows; to improve nutritional value by increasing feed quality or charm, to improve its durability or storage ability, to take the feed into easier receivable condition, to make easy its preparation or technology. In the frame of growth promoter derigueur feed additives both increase anabolic activity and provide less feed entry by helping to utilization of animals from feed[1]. The most common used group for this aim is antibiotics. Growth promoter effects of antibiotics are described as in order to increase growth rate and capacity, giving of antibiotics under the minimum inhibition concentration (MIC) for many patojen in long terms and low concentrations to the cultured animals for the purpose of consumption by oral[2]. Antibiotics used for accelerating growth in animals are declared as arcenilic acid, avilamycin, avoparcin, zinc bacitracin, dimetrydazol, erythromycin, efrotamycin, flavomycin, carbadox, lasalocid, lincomycin, monencin, olaquindox, oleandomycin, penicillin, roxarson, salinomycin, streptomycin, spiramycin, tetracycline, tylosin and virginiamycin[3,4]. Even though growth speeder and feed utilization improving effects of these matters aren't exactly known, it is declared that antibiotics make positive effects by reducing or preventing bacterial and protozoal activity can lead to diseases in intestinal channel progressing in secret condition in the form of not lead to clinic symptom, by suppressing bacterial propagation which gas or toxins included ammonia and nitroamins that can retard to growth speed of animals can be formed and secrete, by warning the development of bacteria which can synthesis to known or unknown nutrition elements, by decreasing proliferation of bacteria which can associate animal feed, by increasing to synthesis of vitamins and other growth factors, by providing development of bacteria group which streamlined digestion and by increasing absorption ability of intestines because of decreasing or removing of harmful bacteria-protozoa group[5,6,3]. Normal animals compared with animals taken antibiotic there are significant differences in organs related to intestine. For example, regeneration speed of small intestine mucosa can be more than normal animals. While ammonia production by bacteria in intestine destroys intestine mucosa cells and increases disease attack possibility, ammonia levels significantly decreases in animals fed with antibiotics, then this case results in a feed evaluation increase indirectly[3].

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Ergül (1988) mentioned that antibiotics as growth promoter increase daily live weight gain especially in flourishing animals, improve utilization of feed and increase resistance of animals against infections[7]. Thus totally 110 tones antibiotics per year are used as feed additive in fish farms and as coccidiostatic in fowls in farm animal production for stimulation of growth[8]. Virginiamycin is an antibiotic that forms joining of M and S factors in the rate of 4:1 and that have got synergic effect of these two factors. Numerous research were done concerning to usage in aquaculture too heading fish of virginiamycin used extensively in stock farmer and poultry nutrition. However there isn't exact oneness between these researches. Because of effects mentioned above, both in other farm animals and in aquaculture nutrition researches were done with virginiamycin added feeds besides several antibiotics, quite different results were obtain in respect of species, size, diet composition, virginiamycin content and environment properties[5,9-16].

In this study it was aimed to determine effects of feeding with 0, 20, 40, 60, 80, 100 mg kg^{-1} virginiamycin included feeds of tilapia (*Oreochromis niloticus*) fries newly beginning to feeding on growth, feed conversion and survival rate, to detect which rates of them can be used for diet in fry period.

MATERIALS AND METHODS

The study was conducted in Ankara University of Agriculture Faculty of Aquaculture and Fisheries Department Research Unit and Laboratory. Fish in experimental groups were fed with feeds added VM for 3 months and they were held in 12 glass aquariums (80x40x40 cm) and the water level was 32 cm tilapia (*Oreochromis niloticus*) fry were obtained from the hatchery brood stock of the Aquaculture and Fisheries Department Research Unit. The fry were fed with live feed for a while and then starter feed was given. Totally 300 fry were used which the initial mean total length and weight of them were 0.82±0.01 cm and 0.005±0.0001 g respectively. Basal diet was formulated to contain 34.27% crude protein and 3000 kcal kg^{-1} digestible energy based on mainly fish meal and soybean meal according to nutrient requirements of tilapia which stated by NRC [17] and Castaldo[18]. The composition of basal diet was given in Table 1 and 2. Firstly virginiamycin was dissolved in methanol and sprayed on to the treatment diets as described by Ahmad and Matty[5] and Ikai[19] during the pelleting of feeds at 20, 40, 60, 80 and 100 mg kg^{-1} levels of virginiamycin (Stafac 40 containing 40.000 mg kg^{-1} virginiamycin).

Table 1:The composition of the basal diet

Ingredients	(%)
Fish meal	23.36
Soybean meal	35.00
Maize	14.86
Wheat bran	10.00
Wheat	7.13
Vegetable oil	4.55
Lime stone	1.50
Bentonit	1.00
NaCl	1.00
Vitamin premix*	1.00
Mineral premix **	0.10
Cr_2O_3	0.50

Table 2: Analyzed nutrient composition of the basal diet

Nutrient composition	(%)
Dry matter	92.64
Moisture	7.36
Crude protein	34.27
Crude fat	7.22
Crude fiber	4.85
Crude ash	9.37
Nitrogen free extracts	36.93
Digestible energy (Kcal kg^{-1}) *	3000

*Calculated

* Rovimix 107 (Vit A: 4000000 IU/kg, Vit D3: 600000 mg kg^{-1}, Vit E: 40000 mg kg^{-1}, Vit K3: 2400 mg kg^{-1}, Vit B1: 4000 mg kg^{-1}, Vit B2: 6000 mg kg^{-1}, Niacin: 40000 mg kg^{-1}, Cal-D-Pantothenate: 10000 mg kg^{-1}, Vit B6: 4000 mg kg^{-1}, Vit B12: 10 mg kg^{-1}, Folic acid: 1600 mg kg^{-1}, D-Biotin: 100 mg kg^{-1}, Vit C: 40000 mg kg^{-1}, Inositol: 60000 mg kg^{-1})

** Remineral B (Manganez: 90000 mg kg^{-1}, Iron: 65000 mg kg^{-1}, Zinc: 80000 mg kg^{-1}, Copper: 12500 mg kg^{-1}, Cobalt: 400 mg kg^{-1}, Iodine: 1800 mg kg^{-1}, Selenium: 150 mg kg^{-1}).

In the feeding experiment, 12 aquariums (6 groups - 2 replicates) were used and 25 fish were stocked in each aquarium[20,21]. The water temperature was fixed as 26±2°C by the termostated heaters to provide optimum temperature for tilapia fry. The fish were fed ad libitum[22,23] . The analysis of nutrient composition of the test diets were based on AOAC methods[24]. Moisture was determined by oven-drying at 105°C for 24 h, crude protein (Nx6.25) by the Kjeldahl method and crude ash by combustion in a muffle furnace at 550°C for 16 h. Total lipid concentration was determined by extraction with chloroform-methanol.

Feed consumption and as depending on this feed conversion ratio was calculated according to De Silva and Anderson[25]. protein efficiency ratio was calculated according to El Sayed[23] and productive protein value was calculated according to Utne[26] by being weigh out to feed rest again in each measurement period. Live weight and total length of fish as individual was measured per 15 days during experiment; live weight and growth as length was interpreted by calculating to absolute, relative and specific growth. Calculating of growth parameters was done according to El Sayed[23] and De Silva *et al.*,[27].

Growth was computed in terms of relative weight and length gain (RWG-RLG), Specific Growth Rate (SGR), Condition Factor (CF) as follows.

- Relative weight or length gain (RWG - RLG) (%) = (final - initial /initial)x100
- Specific growth rate (SGR) (% day^{-1}) = [(ln final – ln initial)/days]x100
- Condition factor (CF) = (BW/TL^3)x100; where BW and TL are body weight (g) and total length (cm) respectively.

Feed utilization was calculated in terms of feed conversion ratio (FCR) as follows.

FCR = WtA/(Bf-Bi); where Bf and Bi are the final and initial biomass of the aquarium, respectively and WtA is feed provided. Since all fish were weighed individually initial and final biomass was calculated based on mean body weight and the number of fish in each aquarium.

Protein Efficiency Ratio (PER) = (weight gain/protein intake) and

Productive Protein Value (PPV) = ($B-B_0/I$); where B = final body nitrogen, B_0 = initial body nitrogen and I = nitrogen intake. All data were subjected to variance analysis (ANOVA) and means were separated by Duncan Multiply Range Test[28]. For this purpose Minitab for Windows 10.5 and MSTAT-C programs were used. The treatment effects were considered to be significant at $p<0.05$.

RESULTS

Growth, Condition Factor (CF), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Productive Protein Value (PPV) at the end of the experiment of tilapia (*Oreochromis niloticus*) fry fed with basal diet including 34.27% crude protein and 3000 kcal kg^{-1} digestible energy prepared on the basis of fish meal and soybean oil cake and treatment diet added in the rate of 20, 40, 60, 80, 100 mg kg^{-1} virginiamycin to this basal diet throughout experiment were given in Table 3.

Final mean live weight and live weight gain and final mean total length and length gain values were found as 3.04±0.15, 3.035±0.07, 5.55±0.10 and 4.73±0.02 respectively in control group while the same findings showed change in the interval of 3.35±0.13 (VM60) – 4.04±0.25 (VM80), 3.345±0.13 (VM60) – 4.035±0.25 (VM80), 5.81±0.08 (VM60) – 6.06±0.12 (VM80) and 4.99±0.05 (VM60) – 5.24±0.16 (VM80) respectively. Beside numerical increases were observed in virginiamycin added groups in comparison with control group on account of final mean live weight and live weight gain, VM80 group obtained the higher mean live weight and live weight gain showed significantly difference from either control group or virginiamycin added other groups statistically ($p<0.05$). Virginiamycin added other treatment groups were similar to each other while it was found that they were different from control group ($p<0.05$). The highest value was found in VM80 group again in point of final mean total length

Table 3:Performances of tilapia (*O. niloticus*)fry fed with virginiamycin added feeds in different levels

	Groups					
Parameters	Control	VM20	VM40	VM60	VM80	VM100
Experiment periot (days)	90	90	90	90	90	90
Initial / Final fish number (N_1/N_2)	50/50	50/50	50/50	50/50	50/50	50/50
Initial weight (g)	0.005±0.0001	0.005±0.0001	0.005±0.0001	0.005±0.0001	0.005±0.0001	0.005±0.0001
Final weight (g)	3.04±0.15^{c}	3.55±0.14^{b}	3.39±0.13^{b}	3.35±0.13^{b}	4.04±0.25^{a}	3.52±0.13^{b}
Mean weight gain (g)	3.035±0.07^{c}	3.545±0.14^{b}	3.385±0.13^{b}	3.345±0.13^{b}	4.035±0.25^{a}	3.515±0.13^{b}
Differences from control (%)	-	+16.80	+11.53	+10.21	+32.95	+15.82
Initial total length (cm)	0.82±0.01	0.82±0.01	0.82±0.01	0.82±0.01	0.82±0.01	0.82±0.01
Final total length (cm)	5.55±0.10^{c}	5.86±0.09^{b}	5.82±0.07^{b}	5.81±0.08^{b}	6.06±0.12^{a}	5.92±0.07^{ab}
Mean length gain (cm)	4.73±0.02^{c}	5.04±0.04^{b}	5.00±0.04^{bc}	4.99±0.05^{bc}	5.24±0.16^{b}	5.10±0.08^{b}
Differences from control (%)	-	+6.55	+5.71	+5.50	+10.78	+7.82
Initial condition factor	1.06±0.07	1.06±0.07	1.06±0.07	1.06±0.07	1.06±0.07	1.06±0.07
Final condition factor	1.71±0.02^{a}	1.76±0.08^{a}	1.69±0.02^{a}	1.67±0.01^{a}	1.74±0.03^{a}	1.67±0.02^{a}
Specific growth rate as weight (% day^{-1})	7.12±0.07^{c}	7.30±0.01^{b}	7.25±0.08^{bc}	7.23±0.01^{bc}	7.44±0.04^{a}	7.29±0.04^{b}
Specific growth rate as length (% day^{-1})	2.12±0.01^{b}	2.19±0.01^{a}	2.18±0.01^{ab}	2.18±0.01^{ab}	2.22±0.04^{a}	2.20±0.02^{a}
Relative weight gain (%)	60700±396.00^{c}	70900±141.00^{b}	67700±495.00^{bc}	66900±990.00^{bc}	80700±311.10^{a}	0300±254.60^{b}
Relative length gain (%)	576.83±2.44^{b}	614.63±4.39^{a}	609.76±5.37^{ab}	608.54±6.10^{ab}	639.02±20.00^{a}	621.95±9.03^{a}
Feed conversion ratio	2.72±0.18^{a}	2.31±0.02^{a}	2.17±0.24^{ab}	1.95±0.07^{ab}	1.54±0.01^{c}	1.81±0.17^{b}
Differences from control (%)	-	-15.07	-20.22	-28.30	-43.38	-33.45
Protein efficiency ratio	1.08±0.07^{d}	1.26±0.01^{cd}	1.36±0.15^{bcd}	1.50±0.05^{bc}	1.90±0.01^{a}	1.63±0.15^{ab}
Differences from control (%)	-	+17.75	+25.35	+39.49	+76.62	+50.28
Productive protein value (%)	18.15±1.16^{d}	19.86±1.35^{cd}	21.06±1.76^{bcd}	24.037±1.48^{bc}	30.54±1.94^{a}	25.65±1.89^{ab}
Differences from control (%)	-	+9.40	+16.01	+32.39	+68.27	+41.31

a-d; The values at the same row with different superscripts differ significantly ($p<0.05$).

and length gain. Total mean length of this group was similar to VM100; length gain was similar to all the groups except that control group ($p>0.05$).

Live weight and in length specific and relative growth values were found as 7.12±0.07, 2.12±0.01, 60700%±396.00, 576.83%±2.44 respectively in control group while the same findings showed change in the interval of 7.23±0.01 (VM60) – 7.44±0.04 (VM80), 2.18±0.01 (VM40 and VM60) – 2.24±0.04 (VM80), 66900%±990.00 (VM60) - 80700%±311.10 (VM80) and 608.54%±6.10 (VM60) - 639.02%±20.00 (VM80) respectively. The highest value was obtained from VM80 group in point of live weight and in length specific and relative growth; this group was significantly different from all the groups in weight while it was different from only control group in length ($p<0.05$).

Feed conversion ratio (FCR), protein efficiency ratio (PER) and productive protein value (PPV) were found as 2.72±0.18, 1.08±0.07, 18.15±1.16 respectively in control group while the same findings showed change in the interval of 1.54±0.01 (VM80) – 2.31±0.02 (VM20), 1.26±0.01 (VM20) – 1.90±0.01 (VM80), 19.86±1.35 (VM20) – 30.54±1.94 (VM80) respectively. The highest value was obtained from VM80 group in point of feed conversion ratio (FCR), protein efficiency ratio (PER) and productive protein value (PPV); improvement was obtained in quite high ratios such as 43.38%, 76.62%, 68.27% respectively in comparison with control group. VM80 and VM100 groups showed significantly difference from control groups in point of these parameters statistically ($p<0.05$).

DISCUSSION

Antibiotics are matters that their importance starts ever increase in modern feed formulations. Although there are a lot of studies, stated and positive concluded, for poultry or other farm animals, there are comparatively few studies on potentially growth promoter effects of antibiotics started to using for fish. However there isn't exact oneness between obtained findings these researches. Virginiamycin formed joining of M and S factors in the rate of 4:1 and had got synergic effect of these two factors is a growth promoter used extensively in poultry; recently it has been used in several fish species and crustacean culture.

The best live weight gain (4.04±0.25 g) was seen in 80 mg kg^{-1} virginiamycin included group in fry that initial live weights being 0.005±0.0002 g at the end of the experiment; others as follows; virginiamycin included groups of 20 mg kg^{-1} (3.55±0.14 g), 100 mg kg^{-1} (3.52±0.13), 40 mg kg^{-1} (3.39±0.13), 60 mg kg^{-1} (3.35±0.13 g) respectively and control group (3.04±0.15). The highest growth in weight obtained in 80 mg kg^{-1} virginiamycin included group was similar to obtained growth when feed included 38% crude protein in an experiment conducted with carp, while, it was different from obtained the highest live weight value in the group of 100 mg kg^{-1} when feed included 25% protein [29]. Live weight value in the group of 100 mg kg^{-1} being high from that of obtained in 80mg kg^{-1} in experiment conducted with feed included 25% protein didn't support estimation stated by Ahmad and Matty [5]. According to Ahmad and Matty[5] virginiamycin addition in the high protein level gave rise to increase in live weight while similar effect wasn't shown in the low protein level. 20 mg kg^{-1} group following to the best growth in weight after 80 mg kg^{-1} group was similar to Silva's[12] findings obtained from *Labeo rohita*. In this research conducted with tilapia it was found that virginiamycin addition to feed in growing concentration didn't increase to growth as directly proportional and value in the group given 100 mg kg^{-1} virginiamycin was the lover that that of 80 mg kg^{-1}. These findings can be interpreted as reducing of virginiamycin harmful bacterial and protozoan activity in digestive channel besides suppressing to beneficial bacteria situated in intestinal micro flora of increased virginiamycin amounts.

The highest feed conversion ratio belongs to control group with 2.72±0.18 whereas the lowest value belongs to 80 mg kg^{-1} virginiamycin included group with 1.54±0.01 in the experiment. Obtained feed conversion ratio is lower than that of carp[29], carp and tilapia by Viola and Arieli[9] and *Pangasius sutchi* by Pathmasothy[10]. These results showed that fish fed with feed included virginiamycin converts feed better in parallel Cravedi *et al.*,[5] stated that virginiamycin gave rise to a general improvement in feed conversion ratio for rainbow trout. The higher value in account of final protein efficiency ratio in parallel to feed conversion ratio was seen in 80 mg kg^{-1} virginiamycin given group. Other treatment groups added virginiamycin to their feed were showed significant differences from control group ($p<0.05$). This case shows that fish added virginiamycin to their feed can utilize better from protein. Kim *et al.,*[30] stated that growth promoter effect of virginiamycin in carp was week because stock density of fish was few (4-5 fish/m^2) and nutrient matters in natural environment reduced effects of virginiamycin on nutrition. In this research on the contrary of results obtained by Kim *et al.*,[30], being seen to growth promoter effect of virginiamycin can result from not utilizing of fish from feed in natural environment, taking of fish only pellet feed in controlled aquarium environment, stock density (200 number/m^3) and differences between fish species. Related to species differences, Viola and Arieli[9] stated that carp respond to growth promoters better than tilapia. Even completed diets in nutritional meaning this growth supporting are available under the several conditions. However this mechanism can't just be understood, so many theory are developed because of explaining this process of growth excitement. The most accepted theory is that antibiotics show an effect by changing to intestinal flora. This can be by reducing toxins produced pathogenic symptoms. Antibiotics can be effective for nutritive matters by reducing of organisms struggled with host or by helping to improve of bacteria being able to synthesized undefined nutritive matters.

The most important entry in aquaculture is feed. In this experiment feed conversion ratio was found as1.54 in fish fed with 80 mg kg^{-1} virginiamycin group, it was found as 2.72 in control group; this value was higher 43.38% than 80 mg kg^{-1} virginiamycin included group. So virginiamycin usage in the level of 80 mg kg^{-1} has shown that it was saved up 43.38% from feed. Supposing that a commercial fish feed is 100 TL kg^{-1}, a kilo of commercial virginiamycin is 1000 TL that 10 times expensive from feed. A firm will pay 1000 TL for 10 kg feed requirement. Again supposing that if the same firm buys 80 mg kg^{-1} virginiamycin included feed, required feed amount for taking fish to same weight will fall to 5.66 kg and its cost will be 566.2 TL. When a feed additive included 4% virginiamycin is bought for obtaining 80 mg kg^{-1} feed, adding 20 g from this to 10 kg feed will be enough. Cost of this to farmer will be only 20 TL. Thus farmer will reap 41.38% profit from feed again.

Because of using antibiotics and related to this risk of residue, a lot of international foundation workouts continuously in the frame of residue following program and they publish instructions with regard to obtained data. Whether there is deposit into tissues of fish fed with treatment feeds added virginiamycin in different values or not isn't determined within the available facilities. It is impossible that antibiotic residue is formed in tissues of fish fed with virginiamycin included feed up to reach market length in fry period during three month according to findings of Cravedi *et al.*,[13],[30,31]. Any finding related to deposition of metabolits or VM, M, S in the intestine, body and tissue of rainbow trout (*O. mykisss*) fed with 40 ppm VM, 35 ppm M and 12 ppm S included feeds throughout 15 days wasn't found by Cravedi *et al.*,[13]. Also it was stated that because extractable residues in liver appeared less than 20% of total radioactivity, virginiamycin didn't compose an important residue in tissue and 40 ppm might be used in aquaculture safely. Carillo *et al.*,[31] stated that virginiamycin residue wasn't found in tissue samples taken from big, white shrimps (*Penaeus vannamei*) fed with feed added 50, 80, 100, 200 ppm virginiamycin throughout 6 weeks even in the highest adding ratios. Similarly it wasn't found antibiotic as biological in tissues of mirror carp fed with feeds prepared by adding 80 ppm virginiamycin and using vegetable and animal protein sources[30].

In this experiment, most important improvements were obtained by adding virginiamycin in different values to feeds prepared with regard to nutrient requirement in terms of live weight, total length, feed conversion and protein efficiency ratios in tilapia (*Oreochromis niloticus*) fry. Both live weight and length gainings of virginiamycin added all groups were so much than control group. At the end of the experiment the best values were obtained from adding 80 mg kg^{-1} virginiamycin to feed in terms of live weight gain, protein efficiency ratio and feed conversion ratio. It was obtained to 32.95% increase in live weight 43.38% saving from feed in comparison with control group by adding to feed 80 mg kg^{-1} virginiamycin; it was found that the higher ratio than this (100 mg kg^{-1}) didn't increase anymore growth performance and feed conversion. Consequently when live weight gain and feed utilization are considered as economical criteria it can be recommended adding 80 mg kg^{-1} virginiamycin to their feed in nutrition of tilapia fry.

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