Residual Effects of Dietary 17α-Methyltestosterone on Second-Year Growth, Body Composition, and Gonosomatic Indices of Blue Tilapia, *Oreochromis aureus* (Steindachner)

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청틸라피아의 2년째 성장, 체조성 및 생식소 중량 지수에 미치는 17α-Methyltestosterone의 잔류 효과

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ABSTRACT

Blue tilapia, *Oreochromis aureus* (Steindachner) swim-up fry were fed a ration containing 0, 1, 10, or 60 ppm 17α-methyltestosterone (MT) for 30 days (sex reversal period). Fish that had been fed 0 ppm MT-treated feed during the sex reversal period were subsequently fed rations containing either 0 ppm MT, 10 ppm MT, or 60 ppm MT for the next 39 days (post sex reversal period) ; fish that had been fed 60 ppm MT-treated feed during the sex reversal period were subsequently fed rations containing either 0 ppm MT, 10 ppm MT, or 60 ppm MT during the post sex reversal period. One group was fed 1 ppm MT-treated feed, while another group was fed 10 ppm MT-treated feed during both periods. The following growing season (10 months later), the residual effects of MT on weight gain, body composition, and gonosomatic indices (GSI) were evaluated. MT had no residual effect on growth of either sex. Groups that ate MT-treated feed were significantly ($P < 0.05$) larger than those that received no MT during the sex reversal period, because females in the latter group lowered average weight gain. MT had a positive residual effect on body fat content in males, but had a negative effect on body moisture content. MT had a positive residual effect on body moisture content in females. MT had a significant negative effect on male and female GSI at the end of the post sex reversal period. MT consumption during the sex reversal period had a significant negative residual effect on male GSI. MT fed

after sexual development (post sex reversal period) did not have a residual effect on male or female GSI.

INTRODUCTION

Concentrations of exogenous hormones in tissues of common carp, *Cyprinus carpio* (Lone and Matty 1981), coho salmon, *Oncorhynchus kisutch* (Fagerlund and Dye 1979), rainbow trout, *O. mykiss, Oreochromis mossambicus* (Johnstone et al. 1983), and *O. aureus* (Goudie et al. 1986a, 1986b) decrease rapidly after the hormones were removed from the diets. Less than 0.5% of the original concentrations remained after three weeks.

Despite the fact that exogenous hormones are retained for only a short time, 17α-mehyltestosterone (MT) has been shown to affect growth of tilapia a year after removal from the diet. Muhaya (1985) found that sex-reversed *O. niloticus* with MT grew faster than non-treated males 13 to 14 months after the hormone was withdrawn from the diet, which suggested that the hormone had residual anabolic effects.

MT has been used to produce all-male populations in tilapia (Hunter and Donaldson 1983). Consequently, it is important to quantify the residual effects of various dietary MT concentrations and durations of feeding on both males and females.

The objectives of this study were to evaluate the residual effects of several dietary levels of MT and durations of feeding during the first three months of life on second-year growth, body composition, and gonosomatic indices of *O. aureus* (Steindachner).

MATERIALS AND METHODS

Fry Production and Sex Reversal Period

Fry and fingerlings used for this experiment were introduced from previous experiment (Jo et al. 1995).

Post Sex Reversal Period

On August 14, 1986, fry were stocked in 3.05 m diameter circular plastic pools. The group that had been fed 0 ppm Mt-treated feed during the sex reversal period was divided into three sub-groups ; for the next 39 days, the three sub-groups were fed either 0, 10, or 60 ppm MT-treated feed. These groups were designated 0−0, 0−10, and 0−60, respectively. The group that had been fed 60 ppm MT-treated feed during the sex reversal period was divided into three sub-groups ; for the next 39 days, the three sub-groups were fed either 0, 10, or 60 ppm MT-treated feed. These groups were designated 60−0, 60−10, and 60−60, respectively. The groups that had been fed 1 ppm or 10 ppm MT-

treated feed during the sex reversal period were fed 1 or 10 ppm MT-treated feed, respectively, for the next 39 days. They were designated 1−1 and 10−10, respectively. Each treatment was randomly assigned to six pools. There were 25 fish in each replicate.

Fish were fed a commercial floating catfish fingerling feed (36% protein). Hormone treated feed was prepared and stored as described previously (Jo et al. 1995). Fish were fed *ad libitum* twice daily.

On September 23, 1986, each fish was manually sexed and weighted to the nearest 0.1 g. Gonads were removed from eight randomly selected fish from each replicate at the end of the post sex reversal period and were weighted to the nearest milligram. The remaining fish from each treatment were combined for over-wintering.

Over-Wintering

On October 10, 1986, eighty randomly selected fish from each treatment were stocked in individual 1,200 ℓ tanks for over-wintering. Tanks were located in a heated building and were aerated continuously. Every three days, one-third of the water was replaced with heated pond water. Water temperature ranged from 16 to 22℃. Fish were fed a commercial floating catfish feed at 0.5% of their body weight daily.

Second-year Growth Period

On May 29, 1987, ten fish from the 0−0, 0−10, 0−60, 1−1, 10−10, 60−0, 60−10, and 60−60 treatments were randomly chosen and stocked in each of four replicate 2 m^3 hapas (mesh size : 2 × 1.5 m) that were suspended in a 0.1 ha earthen pond to determine the residual effects of the MT treatments. The 0−0, 0−10, 0−60, and 1−1 MT treatments had both sexes, so males and females were separated to prevent growth depression due to reproductive activities. Males and females within each of these treatments were stocked into two replicate hapas.

Fish were fed a commercial catfish floating feed (36% protein) *ad libitum* twice daily. Three randomly chosen fish from each replicate were sampled at harvest (September 27) for proximate analyses. Whole fish were immediately frozen and were later ground to determine percentage body protein, percentage body fat, and percentage body moisture. Percentage nitrogen was determined by the Kjeldahl procedure (AOAC 1950) ; percentage nitrogen was multiplied by 6.25 to calculate percentage protein. Percentage fat was determined by the Gerber method (AOAC 1984). Percentage moisture was determined as described by AOAC (1975).

Gonads were removed from five randomly selected fish from each replicate at harvest (September 27) and were weighed to the nearest milligram. Gonosomatic indices (GSI) were determined by using the following formula : GSI=(gonad weight/body weight) × 100.

Statistical Analysis

Sex ratios were tested for goodness of fit by chi-square. Regression analysis was used to determine the relationship between concentration of MT in the diet on both proximate analysis and GSI. Differences in weights were assessed by analysis of variance. Differences among group means were assessed by Duncan's new multiple range test and Student's *t-test* (Steel and Torrie 1980).

RRSULTS AND DISCUSSION

Sex Ratio

Percentages of males and females for each hormone treatment are listed in Table 1. *O. aureus* has the WZ sex-determining system (Guerrero 1975), so males and females were assumed to be produced in equal numbers. Sex ratio in the control group was not statistically different from a 1 : 1 sex ratio ($\chi^2=2.05$; df=1 ; P > 0.1). The 0−10 and 0−60 treatments did not alter sex ratio ($\chi^2=1.80$, df=1 ; P > 0.1, and $\chi^2=0.35$, df=1, P > 0.5, respectively).

Table 1. Percentages of males and females at various concentrations and durations of 17α-methyltestosterone (MT) in the diet of blue tilapia, *Oreochromis aureus*

Treatments*	Females	Males
0−0	44	56
0−18	44	56
0−60	48	52
1−1	41	59
10−10	1	99
60−0	0	100
60−10	0	100
60−60	0	100

* The first number is the concentration (ppm) of MT during the sex reversal period ; the second number is the concentration (ppm) of MT during the post sex reversal period.

Sex ratio of the 1−1 treated group was significantly different from a 1 : 1 ratio ($\chi^2=4.25$; df=1 ; P < 0.025). These results are in contrast to those from a previous study where the sex ratio of a 1−1 treated group was not different from a 1 : 1 ratio (Jo 1988).

The 10−10 treatment produced a population which was nearly 100% male ; only two females were not sex reversed. The 60−0, 60−10, and 60−60 treatments produced all-male populations.

Residual Effects on Growth

Net weight gains of males, females, and males and females combined (overall), are listed in Table 2. Except for the 1−1 group, all groups treated with MT during the sex reversal period grew significantly faster than untreated fish (overall net weight gains), regardless of the hormone concentration during the post sex reversal period. Fish fed a ration containing 60 ppm during the sex reversal period gained significantly ($P < 0.05$) more weight than groups fed a ration containing 0, 1, or 10 ppm. Fish fed a ration containing 10 ppm during the sex reversal period were significantly heavier than those fed a ration containing 0 or 1 ppm (overall net weight gains). There was no difference in wieght gain between the groups that received rations containing 0 or 1 ppm MT during the sex reversal period. Groups fed 60 ppm MT-treated feed gained 25% to 29% more (overall net weight gain) than the control group, while the group fed 10 ppm MT-treated feed gained 16% more than the controls. These results indicate that 10 and 60 ppm MT-treated feed produced a positive residual effect on overall weight gain.

Table 2. Residual effects of various concentrations and durations of dietary 17α-methyltestosterone (MT) on average net weight gains (g) of males, females, and males and females combined (overall) blue tilapia, *Oreochromis aureus* at the end of the second-year grow-out period. Weights followed by same letter are not statistically different ($P < 0.05$)

Treatment*	Net weight gain		
	Overall	Male	Female
0−0	222.2c	285.4ab	158.9a
0−10	218.9c	260.8ab	177.0a
0−60	222.4c	266.0ab	178.8a
1−1	221.7c	271.7ab	171.7a
10−10	257.2b	257.2b	−
60−0	284.0a	284.0ab	−
60−10	286.9a	286.9a	−
60−60	278.1a	278.1ab	−

* The first number is the concentration (ppm) of MT during the sex reversal period ; the second number is the concentration (ppm) of MT during the post sex reversal period.

However, the residual effect was not expressed in both sexes. Weight gain of MT-treated males was equal to or lower than that of the control males (Table 2). On the other hand, observed mean weight gains of MT-treated females in the 1−1, 0−10, and 0−60 treatments were 8.1%, 11.4%, and 12.5% greater, respectively, than that of the control females. However, these differences were not statistically significant. Males gained significantly more than the females in the 0−0, 0−10, 0−60, and 1−1 groups. The relatively low weight gains of females depressed overall average weight gains of the 0−0, 0−10,

0−60, and 1−1 groups.

Results of this experiment indicated that MT produced no residual effect on second-year growth of sex-reversed *O. aureus* a year after the hormone was withdrawn from the diet. These results differ from those found by Muhaya (1985) for *O. niloticus*. In that study, sex-reversed males gained more than non-treated males in their second year of growth. The differences may be due to sepecies-specific differences.

Normal male tilapia grow faster than females (Tave 1988). Results of this experiment confirmed that males grew faster than females, both with and without the influence of MT.

Residual Effect on Body Composition

Residual effects of MT on proximate analysis of male and female *O. aureus* are listed in Table 3. The residual effects of MT during both sex reversal and post sex reversal periods on body composition of males are illustrated in Fig. 1, and the residual effect of MT during the post sex reversal period on proximate analysis of females is illustrated in Fig. 2.

Table 3. Residual effects of various concentrations and durations of dietary 17α-methyltestosterone (MT) on mean percentage body protein, mean percentage body fat, and mean percentage body moisture in male and female blue tilapia, *Oreochromis aureus* at the end of the second-year grew-out period

Treatment	Male			Female		
	Protein	Fat	Moisture	Protein	Fat	Moisture
0−0	16.6	5.4	73.4	16.9	5.9	71.8
0−10	16.3	4.2	75.0	16.4	4.4	73.7
0−60	16.3	6.1	73.2	16.7	5.5	72.9
1−1	16.3	6.1	73.5	16.8	5.4	72.6
10−10	15.7	4.2	75.6	−	−	−
60−0	16.1	5.9	74.0	−	−	−
60−10	16.0	5.3	73.9	−	−	−
60−60	16.5	6.8	72.8	−	−	−

* The first number is the concentration (ppm) of MT during the sex reversal period ; the second number is the concentration (ppm) of MT during the post sex reversal period.

There was a significant ($P < 0.05$) inverse relationship between concentration of dietary MT in both the sex reversal and post sex reversal periods and moisture content of male *O. aureus*. On the other hand, there was a significant positive relationship between post sex reversal dietary MT levels and moisture content in females.

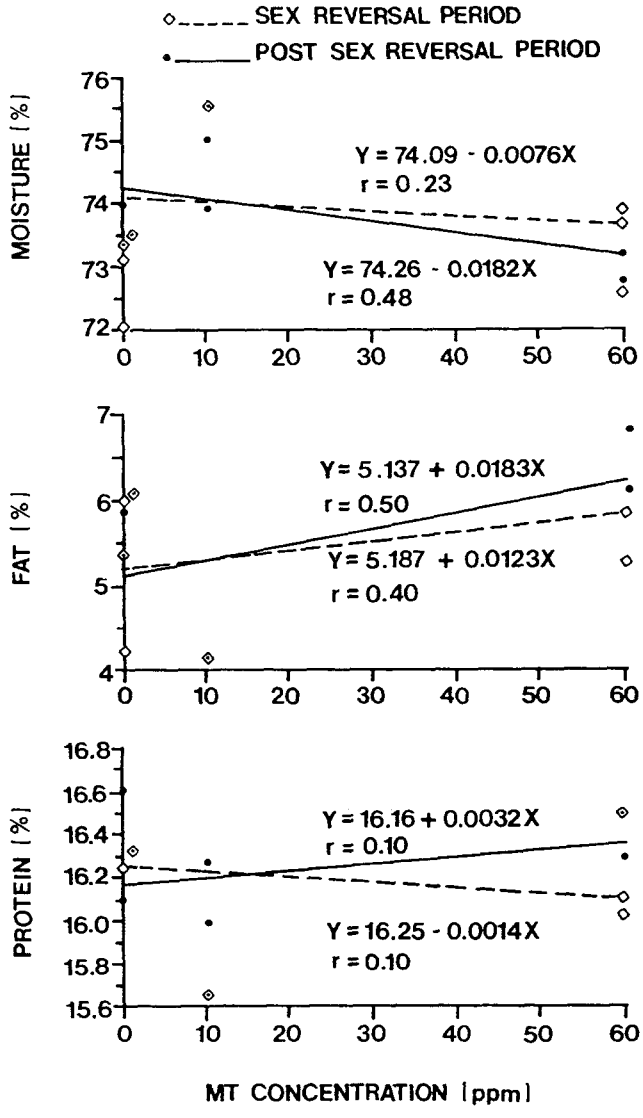

Fig. 1. Residual effects of various concentrations and durations of dietary 17α-methyltestosterone (MT) during the sex reversal and post sex reversal periods on body compositions of male blue tilapia, *Oreochromis aureus* at the end of second-year growth.

There was a significant positive relationship between dietary MT concentrations in both sex reversal and post sex reversal periods and percentage body fat in males. In females, fat levels unaffected by dietary MT during the post sex reversal period.

Percentage body protein in both males and females at the end of the second year were not altered by dietary MT.

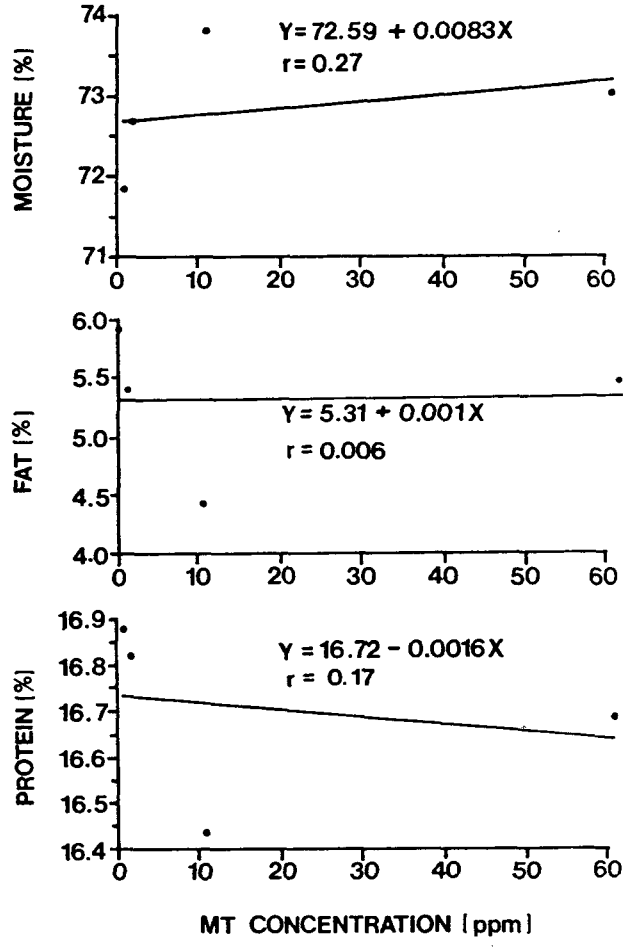

Fig. 2. Residual effects of various concentrations and durations of dietary 17α-methyltestosterone (MT) during the post sex reversal periods on body compositions of female blue tilapia, *Oreochromis aureus* at the end of second-year growth.

Gonosomatic Indices

Effects of dietary MT on gonosomatic indices (GSI) of males are listed in Table 4. There was a significant inverse relationship between MT concentrations in the feed during the sex reversal period and male GSI at the end of the post sex reversal period (Fig. 3). Consumption of MT-treated feed during the post sex reversal period also had a significant adverse effect on male GSI at the end of the post sex reversal period.

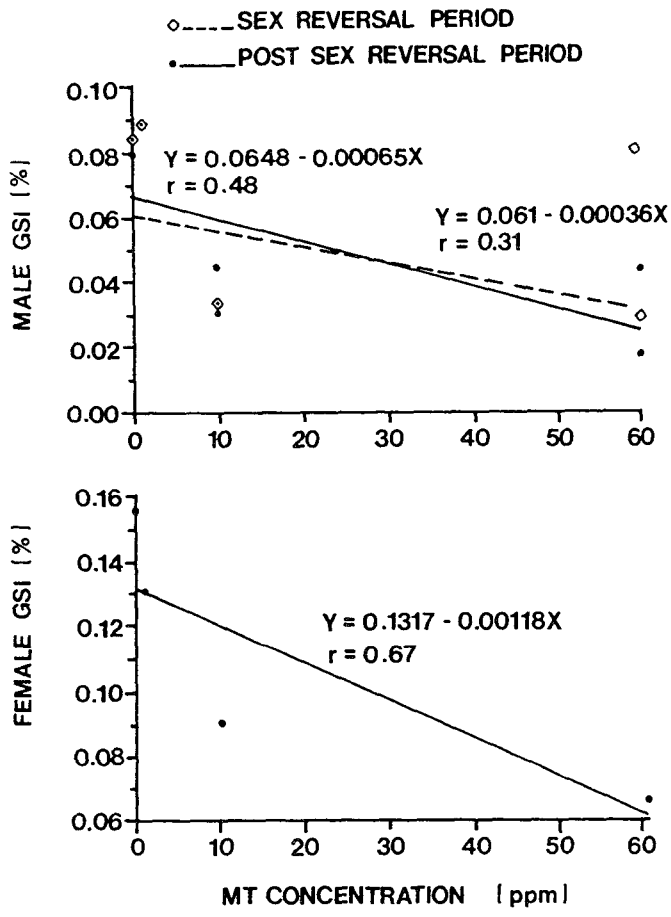

Fig. 3. Effects of various concentrations and durations of dietary 17α-methyltestosterone (MT) on gonosomatic indices (GSI) of male and female blue tilapia, *Oreochromis aureus* at the end of the post sex reversal period.

Effects of dietary MT on female GSI at the end of the post sex reversal period are listed in Table 4. There was a significant inverse relationship between dietary MT concentrations during the post sex reversal period and female GSI at the end of the post sex reversal period (Fig. 3). Other species exhibited similar responses to exogenous androgens. Ashby (1957) reported that 60 ppm testosterone inhibited development of germinal tissue of brown trout, *Salmo trutta*. McBride and Fagerlund (1973), Fagerlund and McBride (1975), and Billard et al. (1982) reported that 1 ppm MT-treated feed caused degeneration of testes, inhibited gametogenesis, and prevented vitellogenesis in the ovaries in coho salmon. Eckstein and Spira (1965) reported that 50 to 1000 ppm testosterone and methyltestosterone in the water delayed gonadal differentiation of *O. aureus*.

Residual effects of dietary MT on male GSI are listed in Table 4. There was a significant inverse relationship between dietary MT concentration during the sex reversal period and male GSI at the end of the second year (Fig. 4). MT consumption during the post sex reversal period had no effect on male GSI at the end of the second year.

Residual effects of dietary MT on female GSI are listed in Table 4. No significant residual effect was observed between dietary MT and female GSI (Fig. 4).

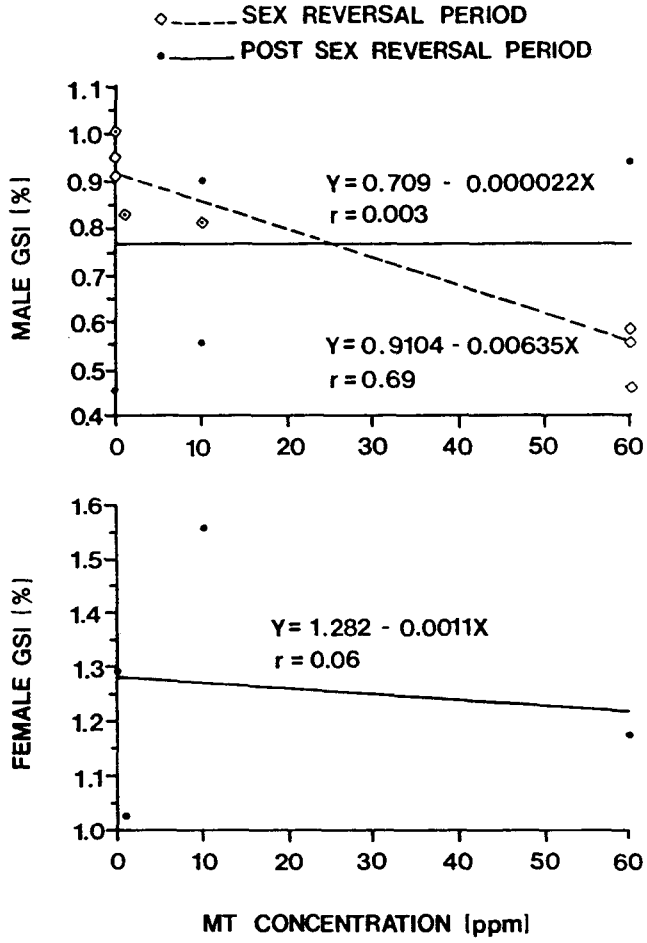

Fig. 4. Residual effects of 17α-methyltestosterone (MT) in the diet on gonosomatic indices (GSI) of male and female blue tilapia, *Oreochromis aureus* at the end of the second year.

The effect of dietary MT on gonadal development were reduced over time. These data suggest that dietary MT has less effect on gonadal growth after sexual differentiation has occurred.

Table 4. Effects of various concentrations and durations of dietary 17α-methyltestosterone (MT) on mean gonosomatic indices (GSI) of male and female blue tilapia, *Oreochromis aureus* at the end of the post sex reversal and at the end of the second-year growth period (residual effects)

Treatment	Post sex reversal period		Second-year growth period	
	Male	Female	Male	Female
0−0	0.085	0.156	1.019	1.293
0−10	0.045	0.090	0.901	1.558
0−60	0.044	0.066	0.940	1.174
1−1	0.091	0.131	0.837	1.029
10−10	0.036	−	0.809	−
60−0	0.080	−	0.455	−
60−10	0.032	−	0.556	−
60−60	0.018	−	0.584	−

* The first number is the concentration (ppm) of MT during the sex reversal period ; the second number is the concentration (ppm) of MT during the post sex reversal period.

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요 약

청틸라피아(*Oreochromis aureus*)의 자어에 0, 1, 10, 60 ppm의 17α-methyltestosterone (MT) 을 30 일간 성전환기 사료에 섞어 먹인후 다음 39 일간(성전환 후기) 0 ppm 을 먹인 그룹은 다시 세그룹으로 나누어 0 ppm MT (0−0), 10 ppm MT (0−10), 및 60 ppm MT (0−60)을 먹였고, 60 ppm 을 먹인 그룹은 다시 3 그룹으로 나누어 0 ppm MT (60−0), 10 ppm MT (60−10), 및 60 ppm MT (60−60)을 먹였으며, 초기 30 일간 1 ppm과 10 ppm MT를 먹인 것은 계속해서 각각 1 ppm과 10 ppm을 먹여서 월동시킨 다음 10개월 후인 이듬해 9월 말경에 체조성과 생식소 중량 지수에 미치는 영향을 조사하였다.

암수 모두에서 2 년째의 성장에 대한 MT의 잔류 효과는 없었다. MT를 먹인 그룹의 성장은 전혀 먹이지 않은 대조군보다 통계적으로 유의성있게 좋았는데($P < 0.05$) 그 이유는 대조군에 있는 암컷의 성장 지연으로 인한 전체 평균 중량의 감소 때문이었다. 사료 중의 MT 함량이 증가하는데 따라 수컷의 조지방 함량은 증가하여 순상관 관계의 잔류 효과를 나타내었으나 체 수분 함량은 오히려 MT가 증가함에 따라 감소하는 역상관 관계의 결과가 나타났다. 암컷에서는 사료 중 MT가 증가함에 따라 체 수분 함량이 감소하는 역상관 관계의 잔류 효과를 보였다. 성전환 후기의 암수 모두의 생식소 중량 지수에

역상관 관계를 나타내었고, 성전환기에 사료에 섞어 섭취된 MT는 2 년째의 수컷의 생식소 중량 지수에 잔류 효과를 나타내어 MT 농도가 증가하는데 따라 생식소 중량 지수가 감소하는 역상관 관계를 나타내었다. 그러나 생식소 발달이 완료된 성전환 후기에 먹인 MT는 암수 모두에서 생식소 중량 지수에 잔류 영향을 보이지 않았다.

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