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Research Article

Ractopamine supplementation in diets for Nile tilapia (*Oreochromis niloticus*) at grow-out phase: effect on body composition and fatty acid profile

Thiago Dias Trombeta[1], Eduardo Uribe Tapia[2], Bruno Olivetti de Mattos[3] Raquel Tatiane Pereira[4], Bruno Dallago[1], Renato Silva Leal[4] Galileu Crovatto Veras[5] & Jurij Wacyk[6]

[1]Faculty of Agronomy and Veterinary Medicine, University of Brasília, Brasília, Distrito Federal, Brazil
[2]Department of Aquaculture, Catholic University of the North, Coquimbo, Chile
[3]Center of Agricultural Sciences Environmental and Biological, Federal University of Recôncavo Bahia Cruz das Almas, Bahia, Brazil
[4]Department of Animal Sciences, Federal University of Lavras, Lavras, Minas Gerais, Brazil
[5]Department of Animal Science, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[6]Department of Animal Production, University of Chile, Santiago, Chile
Corresponding author: Thiago Dias Trombeta (thiago.trombeta@unb.br)

ABSTRACT. Ractopamine is a drug used as an additive in animal production to improve growth, body composition, and production efficiency, although its use in fish is still poorly known. This study aimed to evaluate ractopamine hydrochloride (RAC) effects at increasing levels (0, 10, 20, and 40 mg kg^{-1}) in Nile tilapia (*Oreochromis niloticus*) diets during 40 days by considering growth performance, body composition, and muscle fiber morphometry. One hundred and twelve fish (mean initial weight 518.63 ± 5.49 g) were housed in 16 circular tanks (450 L), following a completed randomized design with four treatments and four replicates. Body composition was significantly affected ($P < 0.05$), and the content of lipids in fish fillets dropped 26.7% (20 mg kg^{-1} RAC) and 26.1% (40 mg kg^{-1} RAC), as well as the amount of unsaturated fatty acids C14:0 and C18:0 in fish fillets ($P < 0.05$) by treatment with 10 mg kg^{-1} of RAC. Results demonstrate that fish fed 20 mg kg^{-1} RAC for 40 days changed Nile tilapia fillet chemical composition, decreasing lipid levels, altering the fatty acid profile, and increasing protein levels. RAC did not affect any productive performance parameters evaluated for muscle fiber morphometry.

Keywords: *Oreochromis niloticus*; β-adrenergic; lipolysis; muscle fiber; protein deposition; lipid oxidation

INTRODUCTION

One of the main production costs in aquaculture is the diet (Brown 2012), which is affected by constant increases in feedstuff prices. The industry is constantly searching for new ingredients and strategies to reduce economic and environmental costs (Naylor et al. 2009, Ajiboye et al. 2012).

One of these strategies is to use feed additives to improve weight gain, feed efficiency, and increase muscle mass and carcass yield (Mersmann 1998, Burnett et al. 2016). Cimaterol, clenbuterol, L-644, zilpaterol, and ractopamine (RAC) are commonly employed in the pork, bovine, ovine, and turkey industries (Moody et al. 2000, Mersmann 2002, Beucher et al. 2015). Despite the benefits mentioned

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above, each additive has different effects on productive performance depending on factors such as the species of the animal, period of use, and dose RAC is a widely used additive in the pork industry (Mersmann 1998, Apple et al. 2007, Kutzler et al. 2011, Almeida et al. 2012). Its mechanism of action is associated with the animal adrenal system stimulation, which, via a complex signaling network, increases the lipolysis rate by reducing fat and increasing protein deposition in the carcass (Mills et al. 2002, Almeida et al. 2013, Ferreira et al. 2013).

Despite the regularization of the use of RAC as an additive in swine feed in more than 20 countries, especially the USA and Brazil, for aquatic organisms, there still needs to be regulation of the use of RAC as a food additive. However, some studies have shown that using RAC in food improves productive performance and body composition. They promote an increase in muscle protein deposition and a decrease in fat accumulation in some fish species such as *Ictalurus punctatus* (Rafinesque) (Mustin & Lovell 1993, 1995), *Oncorhynchus mykiss* (Walbaum) (Moccia et al. 1998, Vandenberg & Moccia 1998, Jalali et al. 2010). Few reports are available using Nile tilapia (*Oreochromis niloticus*) (Mundim et al. 2016, Tovo-Neto et al. 2017).

In the fish industry, Nile tilapia is one of the most raised species worldwide. It is present in all continents, with an approximate production of 4.4 million tons and a growth rate of 9% compared to 2015 (FAO 2022). In order to increase its competitiveness, the Nile tilapia industry is improving not only its productivity but also the nutritional quality of its products and fillet yields via different strategies (MacFadyen et al. 2012, FAO 2016, Valladão et al. 2016, Roriz et al. 2017) such as genetic and nutritional improvement programs. Therefore, to improve performance and body composition, the present study aimed to evaluate the effects of including ractopamine hydrochloride in Nile tilapia diets on the grow-out phase for 40 days.

MATERIALS AND METHODS

This research was conducted at the Fish Farming Center Granja do Ipê (Federal District, Brazil) (15°54'20.88"S, 47°59'19.76"W). One hundred and twelve adult Nile tilapia (518.63 ± 5.49, mean of weight ± standard error mean, SEM), kindly provided by a commercial fish farm, were distributed into 16 450-L tanks (7 fish per tank). Each individual was identified by using PIT tags.

The basal experimental diet (Table 1) was prepared based on Nile tilapia nutritional requirements at the grow-out phase, according to Furuya et al. (2010). Each experimental diet was supplemented with an equal number of vitamins and minerals. Treatments were composed of four levels of RAC and four replicates according to a completely randomized design. The graded RAC (Ractosuin®, Ouro Fino Animal Health, Ribeirão Preto, São Paulo, Brazil) concentrations (0, 10, 20, and 40 mg kg^{-1}) were added in powder form to the basal diet. Ingredients were thoroughly mixed and moistened with 40% water before being pelleted in a professional extruder (INBRA LABOR PQ-30, INBRAMAQ, Brazil) to form 4 mm-long pellets.

Fish were reared in standard recirculating systems (15 m^3 of total volume) and mechanical and bio-filters with a water flow rate of 2.5 L min^{-1} per tank. Water quality parameters were monitored daily and kept within the values described as appropriate for this species (Diana et al. 2017). The average water temperature was 25.9 ± 0.8ºC, dissolved oxygen 6.4 ± 0.6 mg L^{-1}, pH 7.1 ± 0.5, ammonia and nitrite <0.05 mg L^{-1} and nitrate <1000 mg L^{-1}.

Fish were fed to apparent satiety twice daily for 40 days and were kept under a natural photoperiod of about 12:12 h light:dark. The solid residue in each tank was removed by siphoning tanks after all feeding behavior had ceased.

At the end of the feeding trial, all fish were euthanized by a lethal dose of 2 g L^{-1} benzocaine (ethyl 4-aminobenzoate 99%, Sigma Aldrich Co, USA) (Ross & Ross 2008). They were individually counted and weighed to determine: weight gain [IWG (g) = final weight (g) - initial weight (g)]; survival rate [SR (%) = final fish number × 100 / initial fish number]; feed conversion ratio [FCR = feed intake (g) / weight gain (g)], the specific growth rate [SGR (% d^{-1}) = ln final weight - ln initial weight × 100 / days] and protein efficiency ratio [PER = weight gain (g) / protein intake (g)].

In addition, from the euthanized fish, two fish per experimental tank (n = 8 per treatment) were randomly sampled to determine the dressing percentage [DP (%) = carcass (with head and viscera removed) weight (g) × 100 / body weight (g)], the hepatosomatic index [HSI = liver weight (g) × 100 / body weight (g)] and the liposomatic index [LSI = viscera weight (g) × 100 / body weight (g)]. A sample of fillet and white muscle from the dorsal musculature of these fish was also collected to assess chemical and fatty acid composition and fiber diameter analysis.

The proximate composition of diets and fillet was determined by standard methods according to the Association of Official Analytical Chemists (AOAC 2012) as follow: moisture by drying for 24 h at 110°C

Table 1. Formulation and proximate composition of the basal diet. [a]Assurance levels per kilogram of vitamins and minerals (mg kg^{-1} diet): vit. A (min) 1000000 UI, vit. D3 (min) 250000 UI, vit. E (min) 12500 UI, vit. K3 (min) 1250 mg, vit. B1 (min) 1875 mg, vit. B2 (min) 1875 mg, vit. B6 (min) 1250 mg, vit. B12 (min) 2500 mcg, vit. C (min) 12.5 g, pantotenic acid (min) 5000 mg, niacine (min) 10.0 g, folic acid (min) 625 mg, biotine (min) 62.5 mg, coline (min) 50 g, copper (min) 625 mg, iron (min) 6250 mg, manganese (min) 1875 mg, cobalt (min) 12.5 mg, iodo (min) 62.5 mg, zinc (min) 6250 mg, selenium (min) 12.5 mg, Inositol (min) 12.5 g. [b]Butylated hydroxytoluene (BHT).

Ingredient (g kg^{-1})		Proximate composition (% dry matter)	
Poultry by-product meal	150.00	Dry matter (g kg^{-1})	925.2
Soybean meal	272.10	Crude protein (g kg^{-1})	349.8
Fish meal	33.30	Crude lipid (g kg^{-1})	65.1
Rice meal	300.00	Crude ash (g kg^{-1})	67.0
Meat and bone meal	47.90	Gross energy (kcal kg^{-1})	3318
Corn meal	181.50		
Soy oil	1.80		
Premix (vitamins and minerals)[a]	4.00		
Limestone (calcite)	1.10		
Dicalcium phosphate	2.50		
Antioxidant (BHT)[b]	0.30		
Antifungal (calcium propanoate)	0.50		
Salt	5.00		
Total	1000.00		

to constant weight; crude protein by the Kjeldahl method (N×6.25%); crude fat by diethyl ether extraction; ash by heating at 450°C for 24 h.

A homogenized fish fillet was also freeze-dried to assess fatty acid analysis (model Liotop L101, São Carlos, Brazil). The total lipid content was first extracted by homogenization in chloroform/methanol (v:v, 2:1) according to Folch et al. (1957), and then methylation to produce fatty acid esters (FAMEs). The esters that resulted from the esterification step were subjected to a gas chromatography analysis (GC 2010, Shimadzu, Kyoto, Japan) in a flame ionization detector (FID) using a capillary column (100 m × 0.25 mm × 0.2 µm). The identification and quantification of fatty acids were made by comparing the Supelco 37 retention times pattern of the FAME mixture components (CRM47885 - CAS 75-09-2) with all the samples. All the chemical analyses performed from the fish fillets were carried out in triplicate.

The white muscle fiber diameter was analyzed according to Almeida et al. (2008). The muscle samples were collected from the dorsal region, immersed in isopentane, cooled in liquid nitrogen (-195°C), and stored at -80°C until processing. Transversal sections (10 µm) were obtained by a cryostat at -23°C (CM1850 Leica, Germany), dehydrated in ethanol, diaphanized in xylene, and stained with hematoxylin and eosin (Bancroft & Gamble 2007). Transversal sections were generated to measure the fiber diameter (Dubowitz & Brooke 1973). The fiber cross-section diameter (µm) was determined by measuring 300 muscle fibers from each fish per treatment based on the smallest diameter method according to Dubowitz & Brooke (1973). Histological images were obtained under compound microscopy (CX31 Olympus, Japan) with a digital camera (Altra SC30, Olympus, Japan) attached to a computerized imaging analysis system (AxioVision, Carl Zeiss, Germany). Lastly, the white muscle fibers measurements were grouped into three diameter classes ≤30, 30-100, and >100 µm (Valente et al. 1999). The muscle fiber frequency was expressed as a percentage (%) of the number of fibers from each diameter class concerning the total number of recorded fibers.

Data analyses were performed by checking the normality using Shapiro-Wilk's test. The percentage values were normalized by transforming into arcsine (√) to achieve homogeneity of variance. All the results were expressed as the mean ± SEM and evaluated by a one-way ANOVA, followed by Duncan's *post-hoc* test using SPSS (version 20.0). All the statistical analyses set the significance level at $P < 0.05$.

RESULTS

All the performance parameters (IWG, SR, FCR, CR, SGR, PER, DP, HSI, and LSI) were not affected ($P > 0.05$) by the experimental diets (Table 2).

Experimental treatments significantly changed the proximal composition of fillets, except for ash content (Table 3). Both the fish fed 20, and 40 mg kg^{-1} diets changed ($P < 0.05$) values for total fat and crude protein contents. Fish fed 20 mg kg^{-1} diets showed a lower 26.7% total fat content, while the fish fed the 40 mg kg^{-1} diets dropped 26.1%, compared to the 0 mg kg^{-1} diets. Interestingly, for crude protein, fish 20 and 40 mg kg^{-1} diets showed a higher 6.6 and 6.4% increase ($P < 0.05$) compared to the 0 mg kg^{-1} diets (Table 3).

Treatments also significantly changed the fatty acid (FA) profile of Nile tilapia fillets (Table 4). An increase in C14:0 (miristic acid) and a decrease in C18:0 (stearic acid) was observed for the fish fed the 10 mg kg^{-1} diet compared to the other treatments ($P < 0.05$).

No significant effects ($P > 0.05$) in the diameter of white muscle fiber were observed in the fish fed the RAC supplementation levels compared to those fed the control diet (Table 5).

DISCUSSION

No significant differences were found for productive performance in the values for the performance parameters obtained. Which has often been reported by studies conducted with *O. niloticus* (Tovo-Neto et al. 2017); *Piaractus mesopotamicus* (Bicudo et al. 2012, Drumonnd et al. 2018); and *O. mykiss* (Moccia et al. 1998, Vandenberg & Moccia 1998). However, some studies have reported improved growth in RAC-supplemented fish. Mustin & Lovell (1993) observed a significant increase of up to 17% in weight gain in *I. punctatus* fed 20 and 100 mg kg^{-1} RAC for 35 days. The same was observed by Haji-Abadi et al. (2010), who noticed improvements in weight gain, SGR, and PER in *O. mykiss* fed 10 mg kg^{-1} RAC for 84 days. In the studies by Mustin & Lovell (1993) and Jalali et al. (2010), the best growth was observed in fish-fed RAC at higher doses or for longer exposure times than those in the present study. In this study, the absence of significant difference in performance and yield variables, in addition to being probably associated with short RAC supply in the diet, is also directly related to the average weight of the tilapia at the beginning of the experimental period. Adult specimens were used and were close to the slaughter weight to conduct the research. Tilapias in this life stage show smaller and slower growth, which certainly interfered with detecting differences in productive performance variables.

The main effects observed on the RAC-supplemented fish are associated mainly with lower total fat content (Mustin & Lovell 1993, Vandenberg & Moccia 1998, Haji-Abadi et al. 2010). In this study, a significant decrease of approximately 15% fat was observed in the fillets of the Nile tilapia fed 20 and 40 mg kg^{-1} RAC compared to the control treatment. These results are like those reported by Mustin & Lovell (1993) for catfish, who described a significant 14% decrease in fat in the catfish fed 20 and 100 mg kg^{-1} RAC fillets. The same was stated by Vandenberg & Moccia (1998) in *O. mykiss* fed 10 mg kg^{-1} RAC for 56 days, who found a significant 7% decrease in total fat in fish carcasses compared to the control treatment. One of the mechanisms of body fat reduction in animals attributed to RAC is the ability to inhibit lipogenesis by reducing insulin sensitivity in adipocytes (Mills et al. 2002). In addition, the reduction in fish fat is associated with increased lipolysis due to the β-adrenergic agonist effect on adipose tissue with the activation of β-receptors. The binding of RAC with β-adrenergic receptors on adipocyte membranes promotes a series of cascading reactions from the membrane to the interior of the cell, resulting in the action of phosphorylation and activation of hormone-sensitive lipases, catalyzing the degradation of triglycerides (Mersmann 1995), promoting the release of fatty acids and glycerol. The activation of hormone-sensitive lipases leaves mainly unsaturated fatty acids available for oxidation and energy production (Mersmann 2002), which normally results in a change in the lipid profile of animals.

Unlike other studies, the inclusion of RAC in the diet did not increase PUFA deposition in the tilapia fillet of the present study. Studies that tested the inclusion of RAC in the diet associated with L-carnitine demonstrated an increase in PUFA in *O. mykiss* fillet (Jalali et al. 2010) and the carcass of *Labeo calbasu* (Singh et al. 2021). The increase in PUFA in fish is of interest to human health since fatty acids such as linoleic, α-linolenic, arachidonic, eicosapentaenoic, and docosahexaenoic acids are directly related to the prevention of cardiovascular diseases. In the present study, the failure to detect some of these PUFAs is probably due to the low inclusion of the diet fishmeal composition used to feed tilapia since the composition of body fatty acids reflects the diet. On the other hand, the inclusion of 40 mg kg^{-1} of RAC in the tilapia diet promoted an increase in MUFA. Among the benefits of these fatty acids on human health is an increase in insulin sensitivity and consequent reduction in blood

Table 2. Growth performance of Nile tilapia fed diets containing levels of ractopamine hydrochloride. Results were obtained from a one-way ANOVA and Duncan's test. IWG: individual weight gain; SR: survival rate; FCR: feed conversion ratio; SGR: specific growth rate; PER: protein efficiency ratio; DP: dressing percentage; HIS: hepatosomatic index; LSI: liposomatic index.

Parameter	Ractopamine level (mg kg^{-1})				Value F	*P*-value
	0	10	20	40		
Initial weight (g)	519.81 ± 10.43	507.69 ± 10.51	535.00 ± 13.15	512.22 ± 9.52	1.18	0.32
Final weight (g)	617.94 ± 15.16	558.20 ± 24.44	604.00 ± 22.99	604.37 ± 23.04	1.50	0.22
IWG (g)	73.13 ± 11.32	71.82 ± 11.59	81.58 ± 12.05	103.20 ± 10.58	1.15	0.34
SR (%)	93.75 ± 6.25	76.25 ± 17.72	85.00 ± 9.57	90.00 ± 5.77	0.48	0.70
FCR	1.68 ± 0.34	2.77 ± 0.88	1.81 ± 0.36	1.51 ± 0.15	1.07	0.40
SGR (% d^{-1})	0.32 ± 0.05	0.32 ± 0.04	0.35 ± 0.05	0.44 ± 0.04	1.09	0.36
PER	1.87 ± 0.27	1.59 ± 0.67	1.74 ± 0.28	1.92 ± 0.18	0.12	0.95
DP (%)	92.33 ± 0.49	91.15 ± 0.29	91.27 ± 0.32	91.29 ± 0.46	1.77	0.18
HIS (%)	2.05 ± 0.14	2.33 ± 0.12	2.29 ± 0.15	2.18 ± 0.24	0.51	0.68
LSI (%)	7.67 ± 0.49	8.84 ± 0.29	8.72 ± 0.32	8.71 ± 0.46	1.77	0.78

Table 3. Fillet proximal composition of Nile tilapia fed diets containing levels of ractopamine hydrochloride. Results were obtained from a one-way ANOVA and Duncan's test. Different superscript letters in a same row indicate significant differences ($P < 0.05$).

Parameter	Ractopamine level (mg kg^{-1})				Value F	*P*-value
	0	10	20	40		
Moisture (%)	670.2 ± 02.1^{a}	672.3 ± 2.4^{a}	691.0 ± 3.0^{b}	685.5 ± 3.7^{b}	11.41	< 0.05
Protein (%)	800.5 ± 10.7^{a}	819.7 ± 5.6^{a}	853.5 ± 2.8^{b}	852.3 ± 12.4^{b}	8.55	< 0.05
Fat (%)	197.9 ± 10.8^{b}	178.7 ± 5.5^{b}	145.1 ± 2.9^{a}	146.2 ± 12.4^{a}	8.44	< 0.05
Ash (%)	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	2.29	0.10

Table 4. Fillet fatty acid profile of Nile tilapia fed diets containing levels of ractopamine hydrochloride. SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Results were obtained from a one-way ANOVA and Duncan's test. Data expressed as the area percentage of fatty acid methyl esters (FAMEs). Different superscript letters in a same row indicate significant differences ($P < 0.05$).

Fatty acid (mg kg^{-1})	Ractopamine level (mg kg^{-1})				Value F	*P*-value
	0	10	20	40		
C13:0 (tridecylic)	11.26 ± 2.25^{ab}	14.63 ± 2.71^{b}	7.45 ± 0.34^{a}	8.01 ± 0.76^{a}	3.77	0.02
C14:0 (myristic acid)	2.39 ± 0.13^{a}	3.09 ± 0.44^{b}	2.18 ± 0.10^{a}	2.26 ± 0.12^{a}	3.78	0.02
C16:0 (palmitic acid)	24.75 ± 0.81	20.74 ± 2.66	25.91 ± 0.77	25.44 ± 0.43	2.84	0.06
C16:1(palmitoleic acid)	4.88 ± 0.33	5.70 ± 0.33	4.61 ± 0.19	4.82 ± 0.17	2.96	0.05
C18:0 (stearic acid)	6.49 ± 0.38^{b}	4.76 ± 0.82^{a}	6.41 ± 0.12^{b}	6.17 ± 0.13^{b}	3.35	0.03
C18:1 (oleic acid)	33.30 ± 2.11^{ab}	29.22 ± 2.51^{a}	33.76 ± 1.21^{ab}	36.82 ± 0.87^{b}	3.26	0.04
C18:2 (linoleic acid)	10.76 ± 0.44	19.55 ± 4.82	14.15 ± 0.89	12.55 ± 0.57	2.53	0.08
C18:3 (linolenic acid)	1.57 ± 0.33	1.62 ± 0.52	2.98 ± 1.74	1.33 ± 0.29	0.42	0.74
∑ SAFA	4.89 ± 1.98	42.33 ± 4.10	41.96 ± 0.75	41.89 ± 0.56	0.42	0.74
∑ MUFA	38.17 ± 2.41^{ab}	33.30 ± 2.69^{a}	38.38 ± 1.37^{ab}	41.65 ± 0.94^{b}	3.23	0.04
∑ PUFA	11.66 ± 0.38	20.25 ± 4.97	16.01 ± 1.51	13.05 ± 0.78	2.22	0.11

glucose, decreased risk of metabolic syndrome, and lowering blood pressure (Santos et al. 2013). An increase in MUFA in body composition was also demonstrated when *L. calbasu* fingerlings were fed a diet containing 1 g kg^{-1} of L-canitine and 10 mg kg^{-1} of RAC (Singh et al. 2021).

In the present study, despite the absence of a significant difference in the muscle fibers morphometric

Table 5. Muscle fiber diameter of Nile tilapia fed diets containing levels of ractopamine hydrochloride. Results were obtained from a one-way ANOVA and Duncan's test.

Muscle fiber diameter class (%)	Ractopamine level (mg kg^{-1})				Value F	*P*-value
	0	10	20	40		
<30 µm	2.08 ± 0.57	3.30 ± 0.99	4.12 ± 1.12	1.95 ± 0.67	1.11	0.36
>30 and ≤100 µm	40.16 ± 7.54	40.56 ± 11.31	42.13 ± 2.91	40.97 ± 7.05	0.02	0.99
>100 µm	57.76 ± 3.96	56.14 ± 4.24	53.75 ± 2.60	57.08 ± 2.30	1.75	0.18

analysis, there was an increase in protein levels in the fillet. As expected, the protein levels increase was accompanied by a moisture increase since 3 to 4 g of water is a deposition for every 1 g of protein deposited. The increased muscle protein levels due to using RAC may be related to increased protein synthesis or decreased muscle protein degradation (See et al. 2004, Marcolla et al. 2017). By binding to β-receptors in the plasma membrane of muscle cells, RAC potentiates the synthesis of skeletal muscle proteins due to the hypertrophy of white and intermediate muscle fibers (Marcolla et al. 2017). This increase in skeletal muscle results from increased myofibril gene expression due to RAC feeding (Gunawan et al. 2020). Salem et al. (2006) observed that *O. mykiss* fed with diets containing RAC showed that changes in RNA production resulted in the increased expression of genes MHC-f and β-actin, which suggests anabolic effects for higher protein depositions on muscle myofibrils. Supplementation with RAC and L-carnitine also promoted muscle growth in *L. calbasu* (Singh et al. 2021).

In fish, a better understanding of the interactions of β-adrenergic compounds in energy metabolism is still necessary, especially when considering specimens' different physiological characteristics, culture, environmental conditions, and the amount and location of adrenergic beta receptors.

CONCLUSION

This research has shown that the inclusion of 20 mg kg^{-1} RAC in Nile tilapia diets significantly reduces fat, increases the protein content in tilapia fillet composition, and can potentially be used as an additive by the fish food manufacturing industry.

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