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ORIGINAL ARTICLE

Range on vitamin and mineral contents and proximate composition of commercial tilapia feeds in Brazil

Variações nos conteúdos de vitaminas e minerais e composição proximal de rações comerciais para tilápia no Brasil

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ABSTRACT: The number of feed mills manufacturing aquafeeds has increased considerably, and as a consequence so has the range of quality commercial fish feeds. A better knowledge of the actual composition of feeds available in the Brazilian market is key to meet the main challenges currently come across by fish farmers: hampered growth rate and increased incidence of diseases of stocked fish. Mineral and vitamin contents, proximal composition, *in vitro* pepsin digestibility and peroxide values were thus determined in 15 commercial tilapia *Oreochromis* spp. feeds (32% protein contents, grower phase) marketed in Brazil from 2017 to 2020 with the aid of atomic spectrometry and HPLC techniques. A broad range of mineral and vitamin contents were registered among the samples and also in comparison to recommended values in specialized literature. Particularly, all diets registered excessive amounts of trace mineral elements as Fe that registered up to 13 times the recommended requirement and also Cu (up to 43.35 mg/kg) and Mn (up to 114.28 mg/kg). The vitamin A (mean value registered 2538.55 IU/kg) were lower than the recommended requirement in 13 out of the 15 sampled commercial feeds. The highest peroxide values of feeds were associated with low pepsin digestibility. The results were discussed in the context of available information on the dietary mineral and vitamin requirements for tilapia *Oreochromis* spp.

RESUMO: *O número de fábricas de rações, bem como a diversidade na qualidade das rações comerciais para peixes, vem aumentando consideravelmente. O melhor conhecimento da real composição de rações comumente comercializados no Brasil é fundamental para o entendimento dos principais desafios enfrentados pelos piscicultores nos estoques confinados: crescimento deficiente e aumento da incidência de doenças. O teor de minerais e vitaminas e a composição proximal, digestibilidade em pepsina in vitro e índice de peróxidos foram determinados em 15 alimentos comerciais para tilápias Oreochromis spp. (32% proteína, fase de crescimento) comumente comercializados no Brasil entre 2017 e 2020, análises conduzidas por espectrometria atômica e HPLC. Uma ampla variação dos teores minerais e vitamínicos foi registrada entre as amostras e também em comparação aos valores recomendados na literatura especializada. Particularmente, todas as dietas registraram quantidades excessivas de elementos traço como Fe - que registrou até 13 vezes a exigência recomendada, e também Cu (até 43,35mg/kg) e Mn (até 114,28 mg/kg). A vitamina A (valor médio registrado 2.538.55 UI/kg) foi inferior à exigência recomendada em 13 das 15 rações comerciais amostradas. Os maiores valores de peróxido foram geralmente registrados em rações comerciais com baixa digestibilidade. Os resultados foram discutidos no contexto das informações disponíveis sobre as necessidades dietéticas de minerais e vitaminas para tilápia.*

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1 Introduction

Farmed Tilapia *Oreochromis* spp. accounts for nearly 60% of the total aquaculture production in Brazil (IBGE, 2018). The farming and husbandry of tilapias has expanded from traditional, extensive pond culture to intensive cage farming in private or public Brazilian reservoirs, sprouting the demand for high quality feeds aiming at better feeding efficiency, fish growth, and health. Nowadays, tilapia production is based mainly in commercial aquafeed accounting for 60 to 70% of the total cost (Barroso *et al.*, 2019).

There are approximately 100 companies processing and marketing a gross total of 1.38 million tons of aquafeeds in Brazil, an average yearly growth rate of 6.2 % (SINDIRAÇÕES, 2021). While fish feed companies have been increasing, the diversity in quality and prices of commercial fish feeds are criticized by fish farmers. Impaired growth and poor feed conversion, body deformities and metabolic disorders related to low dietary vitamin and mineral contents are problems experienced by fish farmers aiming at intensification of farming operations using commercial fish feeds (Hardy, 2001; Kubitza & Cyrino, 1999). Although vitamin and mineral contents usually deemed as the easiest problems to circumvent in the formulation and processing of aquafeeds, vitamin and mineral deficiencies are the main problems related to the feeding and nutrition of intensively farmed fish (Hardy, 2001).

Economically productive fish farming depends upon an adequate supply of less expensive and readily available feed ingredients with high nutritional quality. Although the advancement on the knowledge of the nutritional requirements of cultured fish has been noticeable, data on commercial fish feed in Brazil are scarce. Additionally, there has been no attempt hitherto to evaluate mineral and vitamin compositions of feeds produced by commercial industries and to examine their suitability using criteria for nutritional requirements which have been reported for tilapia. The present paper aimed to investigate the chemical composition, and mineral and vitamin contents of 15 commercial diets routinely used in the farming and husbandry of tilapias (32% crude protein, growth phase) in Brazil.

2 Materials and Methods

Sampling procedures

Five hundred and forty samples of fifteen, commercial feed brands, 36 label-disclosed formulations (32 % crude protein contents), were randomly taken at commercial fish farming operations from 2017 to 2020 (Table 1). All sampled feeds contained at least three of the following protein sources: corn gluten meal (5), soybean meal (9), fish meal (15), meat and bone meal (9), wheat bran (11), and hydrolyzed feather meal (4). The commercial feeds were all recommended for the farming of tilapia, growth phases.

Proximate analysis

Chemical analysis of the samples were carried out according to AOAC methods (AOAC, 2012) in triplicate. Samples were dried to a constant weight at 105°C for 24 hr to determine the dry matter content. Crude protein was determined by the Kjeldahl method (N x 6,25) after acid digestion. Crude fat analysis was carried by Folch's method. Crude fiber was determined with the aid of acid-base hydrolysis and ash content was determined by incineration in muffle furnace (550°C; 12 hr). To access the quality of the commercial diets, digestible crude protein was determined by pepsin digestibility *in vitro* method (AOAC, 2012), using 0.2% of pepsin (Sigma-Aldrich, USA). The peroxide value was determined according to the official AOCS Peroxide Value method Cd 8– 53 (AOCS, 2003).

Mineral contents

Analysis for determination of mineral contents of samples were carried out by atomic spectrometry, graphite furnace atomization. Powdered samples (1.0 g; triplicate) were digested with 6.0 mL of concentrated hydrogen peroxide and diluted to 10.0 mL with double deionized water (milli-Q Millipore). The concentrations of zinc (Zn), copper (Cu), calcium (Ca), sodium (Na), cobalt (Co), iron (Fe), manganese (Mn), and selenium (Se) were determined following manufacturer specifications. For determination of total phosphorus (P), samples assayed by the phosphovanado-molybdate method (AOAC, 2012), standard calibration technique using aqueous standards performed for comparison.

Vitamin contents

Determinations of vitamin contents of samples were carried out by High-Performance Liquid Chromatography – HPLC (Thermo Scientific, HPLC System, USA) following methods described by Dionex Corporation Annual Report (2010). For extraction of water-soluble vitamins, 0.100 g of powdered samples (triplicate) were added to 100-mL volumetric flasks and stirred in 80 mL of deionized water. After 15 minutes of ultrasonic extraction, water was added to the mark. For extraction of fat-soluble vitamins, 0.125 g of powdered samples (triplicate) were added to 10-mL volumetric flasks and mixed to 8,0 mL of dichloromethane (CH_3OH-CH_2Cl_2), 1:1, v/v. After 15 min of ultrasonic extraction, 1:1, v/v dichloromethane were added to the mark. The HPLC was fitted with a 5µm C^{18} reverse phase column and used for the simultaneous identification of water-soluble [thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), biotin (B_7), folic acid (B_9), cyanocobalamin (B_{12}), ascorbic acid (C) and choline], and fat-soluble [retinol (A), cholecalciferol (D_3), dl-α-tocopherol (E)] vitamins using varying wavelengths; hydrochloride salt was used for the analysis of vitamins B_1 and B_6. Analytical standards were prepared with a range from 0.01 – 10 mg L^{-1} for vitamins A and D_3; the calibration range for vitamin E was 1.0 - 1000 mg L^{-1}. All samples were prepared with a 1:10 methanol dilution solvent and percolated through a 0.2-μm filter (Millex-GN, Sigma-Aldrich, USA).

Statistical analysis

Results were reported as mean ± standard deviation and analysis of data were carried out by one-way ANOVA. Values on peroxide and pepsin digestibility were tested by Pearson's correlation method (α=0.05).

3 Results and Discussion

TheThe variability on the chemical composition of the commercial aquafeeds ranged within acceptable limits for dry matter, ash and crude fiber but lipid contents lied below labeled values for all sampled feeds (Table 2). Although crude protein contents varied very little among the diets (31.49 – 33.84 %), values on pepsin digestibility varied to a great extent (44.94 – 82.01 %). Protein contents is the main parameter used by fish farmers to assess the quality of commercial feed. However, crude protein contents are rarely a true reflection of the actual protein and amino acid profile of any given diet (Mariotti, 2008).

Diets formulated with protein sources of low biological value do have high contents of non-protein nitrogen compounds that causes an imbalance in the amino acid's ratio and an increase in metabolic excretion that negatively affects water quality (Klein *et al.*,2014). Furthermore, the imbalance of functional amino acids impairs fish growth, metabolism and homeostasis (Andersen, 2016).

Table 1. Centesimal composition of commercial feeds (CF) according to manufacturer's label.

Tabela 1. Composição centesimal das rações comerciais de acordo com o rótulo do fabricante.

Commercial feed	DM (% min)	CP (% min)	EE (% min)	Ash (% max)	CF (% max)
CF 1, CF 2, CF 3	88.0	32	6.0	12.0	5.5
CF 4, CF 5, CF 6	88.0	32	7.5	12.0	4.5
CF 7	89.5	32	8.0	10.5	4.0
CF 8	86.0	32	6.0	14.0	7.0
CF 9	89.0	32	7.5	11.0	4.0
CF 10	88.0	32	6.0	12.0	5.0
CF 11	85.0	32	7.0	15.0	5.0
CF 12, CF 13, CF 14	88.0	32	6.5	12.0	5.0
CF 15	90.0	32	6.0	10.0	5.0

Dry matter (DM), Crude Protein (CP), Ether Extract (EE), Crude Fiber (CF).
Matéria seca (DM), proteína bruta (CP), extrato etéreo (EE) e fibra bruta (CF).

The values of digestible protein and lipid oxidation of the feed ingredients are important to develop well-balanced and sustainable diets (Pond *et al.*, 2005), and should be monitored to determine the quality of fish feed. Feeds samples # CF 1, CF 3, CF 9, CF 10, CF 12, CF 13, and CF 14 presented peroxide values exceeding 20 meq kg^{-1}. Although tilapia seems to be tolerant to the effects of oxidized dietary lipid (Kubiriza *et al.*, 2017), a general rule is that peroxide values exceeding 20 meq kg-1 fat, contribute to off flavour and include toxic compounds frequently-associated with rancidity (Connel, 1995). Moreover, diets samples # CF 3, CF 9 and CF 10, which presented the highest peroxide values (45.68; 44.83; 58.56, respectively), also registered the lowest in vitro digestibility (44.94; 60.83; and 57.93, respectively). Given that a moderate correlation of peroxide values and pepsin digestibility were detected (Table 2), it is fair to infer that lipid oxidation may have contributed to reduced protein digestibility, as reported by Anderson et al. (1995). Furthermore, the presence of oxidized oil in fish diets has been shown to induce gastrointestinal and systemic oxidative distress in Nile tilapia (Kubiriza *et al.*, 2017) and other fishes such as Atlantic salmon (Sutton *et al.*, 2006) and African catfish (Baker & Davies, 1997).

Because of its high protein contents and essential, ideal amino acids profile, digestibility and palatability, fish meal used to be the choice dietary protein source for aquafeeds' formulations and processing, but it was gradually replaced by an assortment of plant and animal by-products as a flexible, sustainable solution whilst minimizing the final cost of the diets (Hardy, 2001). Soybean meal, corn gluten, and wheat bran are the main plant protein sources currently used in tilapia diets. Increasing plant protein contents usually yields parallel reduction of dietary protein digestibility (NRC, 2011). In addition, essential amino acids, such as lysine and methionine, are generally deficient in plant protein sources and corresponding plant protein-based diets (NRC, 2011). Rendered animal protein ingredients, such as meat and bone meal, blood meal, and feather meal are routinely used in formulation and processing of tilapia feeds, but their nutritional quality may vary to a great extent. Although the replacement of a large proportion of dietary fish meal with alternative sources can be accomplished without deleterious effects on growth performance and health of tilapia, the quality and potential interactions among ingredients should be considered and the additivity of individual digestibility should be determined and informed to potential buyers through the product label (Yones & Metwalli, 2015).

Mineral contents of sampled diets varied widely as compared to the species requirements and recommendations (Furuya *et al.*, 2010; NRC, 2011); actually, only Na contents matched reported tilapia's nutritional requirements (Table 3). Both macro (Ca, P) and microminerals (Zn, Cu, Co, Fe, Mn and Se) contents were considerably higher than tabled recommendations in all sampled feeds. There is actually a tendency to overdose minerals in commercial feeds to ensure a generous safety factor (Nguyen *et al.*, 2019). However, the overdosing of minerals is a concern to natural populations of aquatic animals, and the excess of certain metallic elements in animal tissue can pose potential adverse impacts on human health (Fernandes *et al.*, 2008). For instance, the highest registered Fe content (sample # CF 3) was 13 times higher than the level recommended by NRC (2011). Fish gain Fe for bodily functions predominantly from the diet and its bodily contents must be carefully regulated to provide sufficient

Fe for biological functions, whilst avoiding Fe overload that can lead to oxidative stress and cumulative toxic effects such as reduced growth, histopathological alterations on hepatocytes and increased mortality (Guo *et al.*, 2017).

No information is available regarding the chemical form or biological availability of the mineral elements used in tilapia feeds. For instance, ANFAL (2000) recommends supplementing commercial diets for omnivorous fish with zinc oxide at 30-150 mg kg^{-1}. However, Sá *et al*. (2004) reports that zinc oxide has lower bioavailability than zinc sulfate and recommend the use of 79.51 mg kg^{-1} of zinc sulfate in plant ingredients-based diets for Nile tilapia. Also, plant protein sources present high amounts of phytate-bonded phosphorus which may represent the higher amount of P detected in commercial feeds as total P contents. However, the phytate-bonded phosphorus is poorly available to fish (Furuya *et al.*, 2010). The excretion of excess dietary P taint fish farm effluents and compromise the water quality causing high eutrophication rates, production of toxic compounds to fish and may affect Zn, Fe, and Mg availability (Furuya *et al.*, 2010). The degradation of aquatic system also produces a series of economic impacts, such as the increase in water treatment costs, damage to economic activities, such as aquaculture itself and damage to human health as a result of waterborne diseases (Tundisi, 2003).

Contents of both dietary minerals and water-soluble vitamin of sampled commercial feeds were above the required levels, with the exception of vitamins Bs, Bs, ascorbic acid, and choline all present in adequate amounts in most feed samples (Table 4). Little evidence has been registered for hypervitaminosis associated to dietary water-soluble vitamins as these compounds are quickly metabolized and readily excreted when the intake as fed exceeds body storage capacity (Halver, 2003).

The fat-soluble vitamins contents varied rather broadly (Table 5). Vitamin D contents were at least 10 times higher than recommended in samples # CF 1, CF 3, CF 5, CF 8, CF 12, and CF 13, while vitamin E contents ranged between 52,6 to 148,78 UI kg^{-1}, that is, a much narrow variation range. No reports on adverse effects of vitamin D overload in tilapia could be located. As a rule, when dietary intake exceeds metabolic needs, animals store fat-soluble vitamins either in cell compartments, mainly hepatocytes, or by accumulation in the body lipid depots, (NRC, 2011). Although fish can accumulate enough fat-soluble vitamins in their tissues to fall into toxic conditions, it is unlikely to occur as a result of the intake of the sampled diets for fish are very tolerant to high levels of dietary vitamin D_3 (Lock *et al.*, 2010). Actually, Atlantic salmon (Graaf et al., 2002) showed no signs of hypervitaminosis D when fed diets containing exceedingly high levels of vitamin D3. Surprisingly, the vitamin A contents were lower than the recommended supplementation of requirement for tilapia in 13 out of the 15 sampled commercial feeds. Vitamin A is a key nutrient for fish and plays important roles in many physiological processes including growth regulation, vision, reproduction, embryogenesis and cell differentiation, thus deserving special attention in the formulation and processing of aquafeeds (Halver, 2003). For instance, Campeche *et al.* (2009) registered distinct deficiency signs of vitamin A in Nile tilapia fed 0 to 1.200 IU vitamin A kg^{-1} diet and moderate deficiency signs in Nile tilapia fed with 1.800 to 3.600 IU vitamin A kg^{-1} diet.

Table 2. Analyzed centesimal composition of the commercial feeds (CF) (dry matter basis).

Tabela 2. Análise de composição centesimal das rações comerciais (com base na matéria seca).

	DM %	CP %	EE%	Ash %	CFb %	PD %	PV meq/kg[1]
CF 1	91.79±1,38	31.84±1.06	4.52±0.91	10.38±2.12	5.43±2.05	64.72±14.56	23.76±17.85
CF 2	91.55±1,01	31.56±1.05	5.16±0.73	9.92±1.09	4.04±1.13	78.17±4.01	14.76±7.23
CF 3	92.32±0.52	32.42±0.96	5.12±0.68	11.31±2.04	4.95±1.06	44.94±4.59	37.96±17.08
CF 4	91.52±1.21	32.48±1.13	4.71±1.70	9.52±2.32	4.28±1.20	70.27±8.10	41.92±19.46
CF 5	93.00±0.63	31.49±1.05	5.04±0.91	9.34±1.77	2.77±1.59	78.11±11.88	5.53±16.24
CF 6	91.89±0.85	33.84±0.68	4.37±0.41	10.15±1.91	3.63±0.71	79.89±0.78	11.94±0.46
CF 7	92.06±0.84	31.97±0.66	4.99±0.82	10.19±1.49	4.45±1.63	79.56±0.77	14.19±4.75
CF 8	93.49±1.72	31.78±0.93	5.53±0.54	14.58±3.33	5.07±2.43	78.04±8.34	5.45±4.84
CF 9	88.75±2.01	31.57±1.33	4.85±1.25	9.50±2.12	2.36±0.98	57.39±13.03	44.83±18.32
CF 10	88.62±2.06	32.90±1.13	3.87±2.13	7.54±2.18	3.64±1.57	55.94±5.71	58.56±13.34
CF 11	90.72±0.34	33.26±0.26	5.23±2.76	10.44±1.78	4.16±0.01	82.01±0.05	5.83±0.61
CF 12	93.47±2.09	32.54±1.54	4.50±0.78	11.25±1.99	6.36±1.86	69.71±11.89	22.20±15.84
CF 13	90.83±0.47	33.70±2.52	4.38±0.43	9.41±2.15	6.39±0.26	78.00±8.41	35.92±3.13
CF 14	91.96±1.27	33.84±0.42	4.23±0.18	10.31±2.02	5.68±1.22	75.56±1.22	35.79±6.13
CF 15	90.55±0.90	33.23±1.89	4.49±0.59	9.48±1.88	4.19±0.96	81.31±0.50	11.23±5.21
Mean	91.39	32.55	4.73	10.23	4.76	73.72	24.66
Std.dev	1.39	1.11	0.45	1.45	1.77	8.93	16.75
Parameter						**Correlation**	**Significance**
Peroxide value	Pepsin digestibility					-0.5368	0.0046

Dry matter (DM), Crude Protein (CP), Ether Extract (EE), Crude Fiber (CFb), Pepsin digestibility (PD), Peroxide value (PV)

[1] PV of oil in the commercial diets; PV up to 10 meq/kg were considered with low/no-onset of oxidation.

Mean (n = 36/Commercial feed) ± standard error.

Matéria seca (DM), Proteína Bruta (CP), Extrato Etéreo (EE), Fibra Bruta (CFb), Digestibilidade da Pepsina (PD), Valor de Peróxido (PV).

[1] PV do óleo presente nas dietas comerciais; PV até 10 meq/kg foram considerados com baixo/sem início de oxidação.

Média (n = 36/ração comercial) ± erro padrão.

Table 3. Mineral content of some commercial feeds (CF) for Nile tilapia with 32% of crude protein, available in Brazil.

Tabela 3. Teor mineral de algumas rações comerciais para tilápia-do-Nilo com 32% de proteína bruta, disponíveis no Brasil.

Minerals (symbol)	Zn mg/kg	Cu mg/kg	Ca g/kg	Co mg/kg	Fe mg/kg	Mn mg/kg	Na g/kg	Se mg/kg	P g/kg
Reference range[1]	20 - 79.51	4.00	1.5 – 4.3	ND	60 - 85	4 - 7.0	1.5 – 4.30	0.25	0.45- 5.0
CF 1	133.53±30.91	30.38±12.08	26.36±6.17	1.13±0.64	736.76±313.73	79.37±14.79	3.47±2.60	1.78±2.08	16.26±8.05
CF 2	345.32±40.97	38.10±21.60	26.88±2.94	1.03±1.107	440.53±70.54	114.28±15.74	5.39±6.24	1.39±0.64	16.04±2.31
CF 3	141.85±4.22	20.05±4.01	13.87±0.91	0.71±0.18	1181.70±201.42	84.85±13.6	1.65±0.28	2.03±0.40	17.60±4.31
CF 4	246.95±12.18	17.21±6.34	16.50±2.98	0.32±0.29	613.91±307.72	68.00±5.97	2.31±0.33	0.96±0.75	16.86±0.98
CF 5	562.28±110.99	33.72±18.31	28.07±2.59	0.24±0.30	359.19±155.02	46.25±9.94	3.73±0.84	1.42±0.29	16.16±3.92
CF 6	70.71±56.27	15.29±5.28	25.98±1.75	0.54±0.03	499.84±138.99	55.57±7.41	6.44±0.94	1.60±0.11	15.42±1.16
CF 7	231.76±138.20	31.12±17.08	25.70±5.56	0.53±1.08	637.82±266.79	108.49±27.23	3.74±4.18	3.51±1.06	17.11±8.70
CF 8	138.40±43.52	15.92±4.77	36.40±8.25	2.39±0.70	444.10±146.01	79.33±20.04	4.38±0.93	1.58±0.90	28.62±15.71
CF 9	135.83±15.40	19.89±2.94	22.81±1.90	0.31±0.04	465.33±101.76	70.67±13.78	4.02±0.92	1.05±0.38	18.88±2.34
CF 10	92.14±21.78	19.43±2.71	17.90±3.74	0.2±0.26	454.86±107.71	51.29±11.36	3.53±0.39	1.03±0.52	50.68±8.56
CF 11	139.20±16.97	16.00±12.98	29.84±1.38	0.0	607.60±140.71	75.40±23.41	5.17±1.15	0.0	17.04±12.32
CF 12	175.01±36.26	15.79±6.86	28.47±6.15	1.81±1.65	357.12±307.38	61.42±11.14	3.50±2.60	1.31±±0.78	13.71±8.00
CF 13	199.33±28.99	43.35±7.32	23.01±4.18	0.62±0.05	397.00±38.18	111.00±21.21	3.41±0.60	0.77±0.42	20.60±8.53
CF 14	176.25±13.54	39.96±2.88	24.99±1.39	0.58±0.58	437.75±108.12	108.00±12.73	3.60±0.15	0.76±0.16	20.00±13.27
CF 15	96.29±22.34	24.79±8.24	25.38±3.70	0.36±0.48	680.29±208.49	56.14±23.23	5.05±0.83	2.3±0.93	12.46±8.53
Mean	192.32	25.40	24.81	0.72	554.25	78.00	3.96	1.43	19.83
Std.dev	123.75	9.85	5.58	0.64	209.98	23.03	1.20	0.81	9.30

ND – not determined.

[1] Min and Max levels in Nile tilapia (*Oreochromis* spp.) requirements according to Furuya et al. (2010) and NRC (2011).

Mean (n = 36/Commercial feed) ± standard error.

ND – não determinado.

[1] Níveis de Min e Max nas exigências de tilápia-do-Nilo (*Oreochromis* spp.) de acordo com Furuya et al. (2010) e NRC (2011).

Média (n = 36/ração comercial) ± erro padrão.

Table 4. Water-soluble vitamins content of some commercial feeds (CF) for Nile tilapia with 32% of crude protein, available in Brazil.

Tabela 4. Teor de vitaminas hidrossolúveis de algumas rações comerciais para tilápia-do-Nilo com 32% de proteína bruta, disponíveis no Brasil.

Water-soluble vitamins (symbol)	B1 mg/kg	B2 mg/kg	B3 mg/kg	B5 mg/kg	B6 mg/kg	B7 mg/kg	B9 mg/kg	B12 µg/kg	C mg/kg	Choline mg/kg
Reference range[1]	0.50	5 - 6.00	5 – 26.00	10.00	3 – 16.6	0.06 - 0.60	0. 82 - 1.00	ND	50 - 600	800 - 1000
CF 1	10.49±3.89	19.35±13.74	53.81±45.83	159.58±106.70	13.62±12.07	0.26±0.10	8.87±6.06	54.6±7.23	432.57±241.13	3359.5±941.76
CF 2	7.58±4.04	11.25±5.97	111.7±88.92	297.95±256.95	13.96±9.47	0.31±0.09	11.83±10.46	49.14±3.56	529.42±349.79	867.72±447.31
CF 3	8.31±1.70	14.94±6.58	79.49±47.99	177.22 ±42.38	8.31±2.56	0.31±0.05	2.04±0.47	51.38±8.61	400.42±276.01	554.68±399.59
CF 4	8.96±2.09	16.89±2.81	29.29±43.82	107.87 ±44.54	7.27±3.16	0.38±0.04	1.38±0.63	54.1±5.38	848.92 ±231.46	7372.5±1047.73
CF 5	6.78 ±1.72	14.75±3.01	190.57±±53.06	511.47±204.00	13.62±7.94	0.29±0.07	7.21±2.76	44.14±5.36	370.71±288.42	841.09±310.75
CF 6	4.43±2.58	34.76±6.27	44.44±65.53	182.63±171.80	13.47±3.56	0.57±0.14	13.03±6.34	52.21±16.27	783.22 ±104.61	919.33±175.75
CF 7	4.08±3.38	13.37±11.77	109.11±87.12	359.05±251.62	13.28±10.46	0.35±0.11	4.95±4.14	17.96±10.95	476.81±304.33	940.18±257.55
CF 8	11.65±2.19	9.7±2.10	20.15±12.09	112.92±109.74	14.29±15.62	0.46±0.20	17.52±10.63	19.22±2.25	1361.7±963.91	542.53±78.27
CF 9	41.45±29.70	12.75±3.64	38.9±36.52	94.11±48.38	11.0±1.91	0.75±0.18	5.28±1.96	43.61±10.73	720.2 ±54.88	661.59±157.25
CF 10	6.09±1.34	6.7±2.71	44.9±37.28	64.34±65.02	8.81±1.21	0.41±0.14	3.85±2.26	34.63±13.21	208.04±32.53	581.83±191.64
CF 11	9.19 ±4.28	15.17±6.12	2.86±2.09	57.68±50.09	31.53 ±14.47	0.0	0.0	0.0	279.11±27.46	464.41±368.46
CF 12	8.77 ±3.93	11.63±13.90	22.99±6.33	122.21±168.44	13.95±12.13	0.21±0.10	17.66±6.59	23.51±5.78	949.78 ±243.31	1044.7±1048.62
CF 13	19.65±6.04	17.85±1.57	112.61±23.56	35.31 ±9.07	17.53±4.09	0.71±0.38	7.79±0.54	19.1±2.59	320.58±20.25	952.13±340.30
CF 14	18.76±4.12	16.94±1.93	106.04±43.68	35.25 ±3.42	17.05±1.22	0.65±0.11	5.57±1.34	19.53±2.14	364.36±86.78	784.12±340.30
CF 15	6.16 ±4.27	10.09±3.58	28.37±5.60	117.01±103.29	14.61±8.65	0.39±0.13	4.71±2.41	40.33±11.68	134.96±26.88	1016.6±313.81
Mean	11.49	15.08	66.25	162.31	14.15	0.40	7.45	34.90	545,39	1393,53
Std dev	9.44	6.43	50.79	132.61	5.63	0.20	5.44	17.09	328,94	1791,66

ND – not determined.

[1] Min and Max levels in Nile tilapia (*Oreochromis* spp.) requirements according to Furuya *et al.* (2010) and NRC (2011).

Mean (n = 36/Commercial feed) ± standard error.

ND – não determinado.

[1] Níveis de Min e Max nas exigências de tilápia-do-Nilo (*Oreochromis* spp.) de acordo com Furuya *et al.* (2010) e NRC (2011).

Média (n = 36/ração comercial) ± erro padrão.

Table 5. Fat-soluble vitamins content of some commercial feeds (CF) for Nile tilapia with 32% of crude protein, available in Brazil.

Tabela 5. Teor de vitaminas lipossolúveis de algumas rações comerciais para tilápia-do-Nilo com 32% de proteína bruta, disponíveis no Brasil.

Fat-soluble vitamins (symbol)	A IU/kg	D3 IU/kg	E IU/kg
Requirement[1]	3586 – 6967	374	74.5 – 111.11
CF 1	2036.5±1111.60	3984.9±3213.53	52.6±30.37
CF 2	1441.3±1054.16	3179.8±3867.15	94.4±60.11
CF 3	1463.0±480.70	4868.9±5626.13	148.78±87.95
CF 4	3624.7±4811.80	735.6±1809.78	117.67±44.52
CF 5	2063.6±2030.38	4350.9±4985.95	100.27±23.54
CF 6	1067.5±1015.04	817.8±532.22	136.79±33.56
CF 7	10675.08±5211.40	1129.5±1999.89	119.06±54.58
CF 8	1968.6±3264.43	5562.9±2871.81	137.02±46.01
CF 9	1361.1±1006.84	3228.3±1344.94	160.19±48.50
CF 10	623.5±987.44	2119.0±792.12	61.53±67.64
CF 11	1884.7±610.95	677.4±249.99	56.09±29.66
CF 12	5752.6±1143.23	3740.6±6011.15	95.3±9.50
CF 13	1297.1±1127.12	4018.6±2008.31	119.02±37.61
CF 14	1847.9±923.09	2883.0±1545.27	142.88±28.44
CF 15	971.0±366.03	796.1±1080.14	55.08±42.99
Mean	2538.55	2739.55	106.45
Std dev.	2577.33	1749.66	36.55

[1] Min and Max levels in Nile tilapia (*Oreochromis* spp.) requirements according to Furuya *et al.* (2010) and NRC (2011).

Mean (n = 36/Commercial feed) ± standard error.

[1] Níveis de Min e Max nas exigências de tilápia-do-Nilo (*Oreochromis* spp.) de acordo com Furuya *et al.* (2010) e NRC (2011).

Média (n = 36/ração comercial) ± erro padrão.

4 Conclusion

In conclusion, this study showed that mineral and vitamin contents of randomly sampled commercial fish feeds available in the Brazilian market still vary to a great extent, but no reason can be pinpointed. However, it is safe to consider that such variations can derive from ever increasing replacement of fishmeal by surrogate, plant protein sources such as corn gluten meal, soybean meal, and meat and bone meal, spoilage of feed ingredients and processed feeds by inadequate storage conditions, varying feed formulation and processing capabilities within processing plants and contamination of feed ingredients at their origin, transport or storage. Findings that most elements were found in concentrations above the recommended, required levels, reinforces the need to improve quality control of the manufacturing process, nutrient analysis and biological assays of finished products included.

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