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Microwave irradiation and pelleting method affected feed chemical composition and growth performance and feed utilization of sex-reversed Nile tilapia, *Oreochromis niloticus* (L.)

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Abstract

Microwave processing and pelleting methods were assessed to improve aquafeed quality for sex-reversed Nile tilapia. The 2 × 3 experimental feeds were prepared with and without microwave irradiation of ingredients prior to pelleting with either a meat mincer, extruder or steam conditioner followed by a meat mincer. Five feedstuff ingredients, including fish meal, meat and bone meal, soybean meal, broken rice and rice bran, were irradiated separately at optimal times, according to the third-order polynomial regression analysis between *in vitro* digestibility and microwave irradiation times ($r = 0.681$–0.942, $P < 0.001$, $n = 42$). The prepared feeds were studied for chemical compositions and responses in fish growth performance and feed utilization efficiency. The fish (1.57 ± 0.01 g initial weight) were fed *ad libitum* for 4 months. All proximate chemical compositions of experimental feeds, except protein, were influenced by microwave processing, pelleting methods or their interaction ($P < 0.05$). There were no significant effects from either parameter on water quality during the fish trial. Fish fed steamed microwave-irradiated feed, mechanically pelleted with a meat mincer, showed the highest growth performance (weight gain 18.91 ± 0.73 g and specific growth rate $2.15 \pm 0.26\%$ day^{-1}) and feed utilization efficiency (feed conversion ratio 1.31 ± 0.05 g feed g gain^{-1} and protein efficiency ratio 2.27 ± 0.08 g gain g protein^{-1}) compared with the other dietary groups. These findings indicate that microwave irradiation of feed ingredients prior to steam conditioning followed by mincing can improve aquafeed production quality.

Keywords: feed utilization, growth, microwave, Nile tilapia, pelleting

Introduction

The Nile tilapia (*Oreochromis niloticus*) is one of the most economically important fish in the world. Global production of farmed tilapia exceeded 1 500 000 metric tons in 2002 (Fessehaye 2006) and is increasing annually, with an estimated value of US$ 1.8 billion, about equal to that of salmon and trout (De Silva, Subasinghe, Bartley & Lowther 2004). Nile tilapia grows and reproduces in a wide range of environmental conditions and tolerates stress induced by handling (Getinet & Amrit 2007). In order to increase the income of the fish industry, many researchers have focused on accelerating the fish growth rate.

Aquafeed processing is an important factor to increase feed utilization and growth rate and decrease discharge of waste and nutrients into the aquatic environment. Feed utilization of fish depends on the nutrient composition of the feed component and the ability of fish to digest and absorb the nutrients (Riche, Trottier, Ku & Garling 2001). Therefore, various formulations have been developed for rearing Nile tilapia (Amirkolaie, Verreth & Schrama 2006; Tran-Duy, Smit, Van Dam & Schrama 2008; Abdel-Tawwab, Ahmad, Khattab & Shalaby 2010). Nevertheless, limited information is available on the sex-reversed fish. Recently, development of suitable feed for commercial production of sex-reversed Nile tilapia using *in vitro* digestibility technique has been reported (Engkagul, Kovitvadhi, Kovitvadhi, Siruntawineti, Choowongkomon, Unajak, Aeidnoie, Preprame, Trenet, Sunthornchot, Meeswad & Rungruangsak-Torrissen 2010). Based on these findings, the main appropriate feed ingredients consisted of fish meal, meat and bone meal, soybean meal, broken rice and rice bran.

Feed quality plays a key role in digestibility of fish (Thongprajukaew, Yawang, Dudae, Bilanglod, Dumrongrittamatt, Tantikitti & Kovitvadhi 2013). Therefore, improvement of feed ingredient quality for aquafeed production is of interest to the aquaculture sector (Sunde, Eiane, Rustad, Jensen, Opstvedt, Nygard, Venturini & Rungruangsak-Torrissen 2004; Kumar, Sahu, Pal, Choudhury & Mukherjee 2006). Microwave processing has achieved a remarkable acceptance by the food industry and chemical engineers (Oliveira & Franca 2002). Generation of heat throughout the materials leads to faster heating rates and shorter processing times when compared with conventional heating (Arocas, Sanz, Hernando & Fiszman 2011). In feed production, microwaving can improve feedstuff qualities by altering some physicochemical properties to enhance enzymatic hydrolysis (Palav & Seetharaman 2007; Thongprajukaew *et al.* 2013).

Pelleting techniques are also an important part of the process, since it may improve the nutritional and physical quality of feed ingredients (Behnke 1996; Booth, Allan & Warner-Smith 2000). A comparative study on pelleting machines showed that extruded feed improved growth performance of shrimp when compared with feed produced by a meat mincer (Gokulakrishnan & Bandyopadhyay 1995). Moreover, a combination with steam conditioning has also been reported to improve the physical quality of pelleted aquafeed (Hilton, Cho & Slinger 1981; Booth *et al.* 2000). The aim of this study was to select a suitable method for improving feed utilization efficiency in sex-reversed Nile tilapia. The findings obtained could provide an improved method for the development of aquafeed production.

Materials and methods

Preliminary study for selecting suitable microwave irradiation time

Microwave irradiation of feedstuffs

All feedstuffs were obtained from Feed Specialties Co., Ltd., Pathum Thani Province, Thailand. A 300-g sample of each feedstuff was placed in a plastic box (23 cm diameter $\times$ 10.5 cm height), mixed with an appropriate amount of distilled water (1:2 *w*/*v* for fish meal, meat and bone meal and broken rice, and 1:3 *w*/*v* for soybean meal and rice bran), covered and then cooked in a 700W microwave oven (EM-700T; Sanyo, Osaka, Japan) under agitation for 0, 5, 10, 15, 20, 25 and 30 min. The irradiated feedstuffs were dried at 60°C for 48 h, ground and sieved before studying *in vitro* digestibility.

Extraction of digestive enzymes for in vitro digestibility

Three-month-old sex-reversed Nile tilapia (67.28 $\pm$ 2.78 g body weight and 15.47 $\pm$ 0.20 cm total length) were randomly collected from a private farm in Nakhon Pathom Province, Thailand. The fish were killed by chilling in ice for 30 min and the small intestines were then carefully dissected. Digestive enzymes were extracted according to Rungruangsak-Torrissen (2007). The samples were homogenized in 50-mM Tris-HCl buffer (pH 8) containing 200-mM NaCl (1:2 *w*/*v*) using a micro-homogenizer (THP-220; OMNI International, Kennesaw, GA, USA). The homogenate was centrifuged at 15 000 *g* for 30 min at 4°C. The supernatant was collected and then kept at −80°C until used for *in vitro* digestibility study.

In vitro digestibility of irradiated feedstuffs

The *in vitro* digestibility reaction of raw and microwave-irradiated feedstuffs was performed according to the method as described by Thongprajukaew, Kovitvadhi, Kovitvadhi, Somsueb and Rungruangsak-Torrissen (2011). The fish meal and meat and bone meal were determined for protein digestibility, whereas broken rice and rice bran were determined for carbohydrate. For soybean meal, exceeding 500 g kg^{-1} contains carbohydrates in forms of both nitrogen-free extract (NFE) and fibre. In order to improve the quality, carbohydrate digestibility is very important but it has not yet been examined, whereas the increase in protein digestibility by microwave pretreatment has been reported previously (Sadeghi, Nikkhah & Shawrang 2005; Thongprajukaew, Kovitvadhi & Chandang 2015). The reaction mixtures contained 5 mg of each dried feedstuff, 10 mL of 50 mM phosphate buffer (pH 8.2), 50 μL of 0.5% chloramphenicol (Sigma-Aldrich, Saint Louis, MO, USA) and 125 μL of dialysed crude enzyme extract; these were incubated at 25°C for 24 h. Each feedstuff was run in six replications. For control, the crude enzyme extracts were replaced by extraction buffer in order to reduce the values due to liberated either amino acids or sugars from feedstuffs. The *in vitro* digestibility of protein was determined by measuring the increase of liberated reactive amino groups of cleaved peptides. The reaction contained 200 μL of digested solution, 2 mL of 50 mM phosphate buffer and 1 mL of 0.1% trinitrobenzene sulphonic acid (Sigma-Aldrich, Saint Louis, MO, USA). These mixtures were incubated in the dark at 60°C for 1 h and then 1 mL of 1 M HCl added. The absorbance was measured at 420 nm and compared with *DL*-alanine (Sigma-Aldrich) standard curve**.** The digestibility of carbohydrate was analysed by mixing 1 mL of digested solution with 500 μL of 1% dinitrosalicylic acid (Sigma-Aldrich), heating in boiling water for 5 min, cooling to room temperature, measuring absorbance at 540 nm and comparing with maltose (Sigma-Aldrich) standard curve.

Preparation of experimental feeds

The ingredients of experimental feeds for sex-reversed Nile tilapia are shown in Table 1. All main ingredients were separately irradiated for the appropriate time, based on *in vitro* digestibility studies of protein and carbohydrate from a preliminary study (Table 2). All ingredients were finely ground and sieved (0.5 mm) and weighed according to the formulation before mixing. The solid ingredients were mixed, and then fish oil, premix and water were added until a dough-like consistency was obtained. The glutinous mixture was passed through a meat mincer (3–4 mm die diameter). For extrusion, the dry ingredients were premixed in a vertical mixer and ground in a Dinnissen hammer mill. Subsequently, the ingredients were mixed in a horizontal mixer (30 kg) for 15–20 min. Extrusion was using a twin-screw extruder with barrel length of 425 mm and a length/diameter ratio of 4.9:1. The ingredients were extruded as described, producing extrudates having a diameter ranging from 3 to 4 mm. Steam conditioning was performed at 100°C for 30 min followed by meat mincer pelleting, as described above. All prepared feeds were then dried at 60°C for 12 h in a hot-air oven, cut, sieved and stored in black polyethylene bags at 4°C, until used in feeding. These six prepared feeds were similar in pellet size (3–4 mm).

Chemical analyses

One hundred grams of each feedstuff and experimental feed were randomly sampled from three points, pooled to obtain homogeneity, ground and then sieved. Chemical compositions, including crude protein, lipid, fibre and ash, were analysed according to standard methods of the AOAC (2005). NFE was calculated as follows: 1000 – (crude protein + crude lipid + crude fibre + ash).

Table 1 Ingredients of experimental feed for rearing sex-reversed Nile tilapia

Ingredient*	Inclusion (g kg^{-1})
Fish meal (58% protein)	245
Meat and bone meal (50% protein)	180
Soybean meal (42% protein)	60
Broken rice	350
Rice bran	150
Fish oil	10
Premix†	5

*Feedstuffs and their inclusion levels were used based on *in vitro* digestibility screening and *in vivo* growth trials of sex-reversed Nile tilapia, reported by Engkagul *et al.* (2010).

†Premix: 1 kg feed contained 1 130 000 IU vitamin A, 1 043 170 IU vitamin D_3, 30 000 IU vitamin E, 3.25 g vitamin K_3, 12 g vitamin B_1, 5 g vitamin B_2, 30 g vitamin B_6, 12 g vitamin B_{12}, 30 g vitamin C, 10 g niacin, 27 g pantothenic acid, 30 mg selenium and 30 g calcium.

Table 2 Regression analyses between microwave irradiation time (X) and *in vitro* digestibility (Y). These equations were used for predicting suitable microwave irradiation times (min) for feedstuffs

Feedstuff	Regression equation	r	Irradiation time (min)
Fish meal*	$Y = 8.0569 + 0.0743X + 0.0026X^2 - 0.0003X^3$	0.681 ($P < 0.001$, $n = 42$)	12
Meat and bone meal*	$Y = 4.8673 - 0.1684X + 0.0375X^2 - 0.0018X^3$	0.784 ($P < 0.001$, $n = 42$)	11
Soybean meal†	$Y = 44.834 + 5.641X - 0.146X^2 - 0.009X^3$	0.818 ($P < 0.001$, $n = 42$)	10
Broken rice†	$Y = 766.426 + 16.592X - 0.9X^2 + 0.014X^3$	0.839 ($P < 0.001$, $n = 42$)	13
Rice bran†	$Y = 570.733 + 11.634X + 1.219X^2 - 0.11X^3$	0.942 ($P < 0.001$, $n = 42$)	10

*Determination of *in vitro* protein digestibility.
†Determination of *in vitro* carbohydrate digestibility.

All chemical compositions were expressed in g kg^{-1} dry matter. Gross energy (GE) was determined using a ballistic bomb calorimeter (Cal 2 k, Digital Data Systems, Randburg, South Africa).

Growth trial of sex-reversed Nile tilapia

One-month-old sex-reversed Nile tilapia was obtained from the National Aquaculture Genetics Research Institute, Department of Fisheries, Pathum Thani Province, Thailand. Fish were acclimatized for 2 weeks in an indoor culture system and were fed to satiation with a commercial feed (280 g kg^{-1} protein). Subsequently, fish (1.57 ± 0.01 g initial weight and 4.58 ± 0.01 cm initial length) were randomly distributed into 18 aquaria (40 cm width × 76 cm length × 48 cm height) at a density of 15 fish per aquarium. The static water system was continuously supplied by air pumps during experiment. The trial was conducted in six dietary groups in triplicate for 4 months under 12-h light/12-h dark cycle. Fish were fed *ad libitum* twice daily at 09.00 and 16.00 h. Food scraps were siphoned off after feeding for 1 h, dried until reaching a constant weight and used to estimate feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER). Dead fish were removed and survival was recorded daily. Growth performance and feed utilization parameters were recorded every 10 days during the trial.

Water quality management

The water was partially replaced (30%) daily. Water qualities of the cultured system were analysed every 10 days. The parameters measured were temperature (Hg thermometer), conductivity (conductivity metre), pH (pH metre), dissolved oxygen (azide modification), free carbon dioxide (titration), total alkalinity (phenolphthalein and methyl orange indicators), total hardness (EDTA titration) and total ammonia nitrogen (phenate method), according to standard method of the APHA, AWWA and WPCF (1998). The water quality parameters during the experimentation period were temperature 25.05 ± 0.12°C, conductivity 163.78 ± 3.62 μS cm^{-1}, pH 7.68 ± 0.04, dissolved oxygen 6.95 ± 0.16 mg L^{-1}, free carbon dioxide 6.65 ± 0.16 mg L^{-1}, total alkalinity 82.24 ± 2.43 mg L^{-1}, total hardness 136.05 ± 9.12 mg L^{-1} and total ammonia nitrogen 0.02 ± 0.01 mg L^{-1}.

Growth performance calculations

Growth performance and feed utilization parameters of the individual fish were calculated using the following formulae:

Specific growth rate (SGR, % day^{-1}) $= 100\,[(\ln W_t - \ln W_0)/(t - t_0)]$,

where W_t = mean weight (g) at day t and W_0 = mean weight (g) at day t_0.

Weight gain (WG, g) = Final body weight (g) − initial body weight (g)

Average daily growth (ADG, g day^{-1}) = Net weight gain (g)/rearing period (day)

Condition factor (CF, g cm^{-3}) $= 100 \times$ [Live body weight (g)/total body length (cm)^3]

Feed intake (FI, g day^{-1}) = Individual feed intake (g)/rearing period (day)

Feed conversion ratio (FCR, g feed g gain^{-1}) = Dry feed fed (g)/wet weight gain (g)

Protein efficiency ratio (PER, g gain g protein^{-1}) = Wet weight gain (g)/protein intake (g)

Statistical analysis

The preliminary study was performed in a completely randomized design (CRD). Six replications of the *in vitro* digestibility were subjected to regression analysis in order to predict the suitable microwave irradiation time. The relationship between both parameters was expressed by the equation $Y = b_0 + b_1X + b_2X^2 + b_3X^3$, where Y is the *in vitro* digestibility values (mmol *DL*-alanine g^{-1} or μmol maltose g^{-1} for protein or carbohydrate digestibility, respectively), X is the irradiation time (min), and b_0, b_1, b_2 and b_3 are the parameters. The model was fitted and their performance was evaluated on the basis of R^2. Optimal time for maximizing the *in vitro* digestibility value was estimated using third-order polynomial regression analysis. The proximate chemical compositions of the treated and untreated feedstuffs were compared by Student's *t*-test. For the growth trial, the aquarium was considered as the experimental unit. Data from three replicate observations were expressed as mean ± SEM. The percentage was verified for normality after arcsine transformation. All data were analysed by two-way ANOVA for the effect of microwave processing (with and without microwave irradiation) and the effect of pelleting methods (meat mincer, extruder or steam conditioner followed by a meat mincer). All data were analysed using SPSS version 17 (SPSS Inc., Chicago, IL, USA).

Results

In vitro digestibility of microwave-irradiated feedstuffs

Protein digestibility of fish meal increased after 5–15 min irradiation (Fig. 1a), while protein digestibility of meat and bone meal increased after 10-min irradiation (Fig. 1b). Irradiation for longer than 20 min (fish meal) and 25 min (meat and bone meal) negatively affected protein digestibility. The equations from third-order polynomial regression analysis ($r = 0.681–0.784$, $P < 0.001$, $n = 42$) suggested that 12 and 11 min were appropriate for improving protein quality in fish meal and meat and bone meal, respectively (Table 2).

A significant increase in carbohydrate digestibility was observed in the irradiated soybean meal (Fig. 2a), broken rice (Fig. 2b) and rice bran (Fig. 2c) when compared with untreated ingredients ($P < 0.05$). The digestibility values were highest after 10-min irradiation for soybean meal and rice bran, and 5–25 min for broken rice, and then decreased with increasing irradiation duration. Third-order polynomial regression analysis ($r = 0.818–0.942$, $P < 0.001$, $n = 42$) of irradiation times and carbohydrate digestibility predicted that 10, 13 and 10 min, respectively, were optimal for improving carbohydrate quality in the three raw materials (Table 2).

Proximate chemical compositions of microwave-treated and untreated feedstuffs

A comparison of the chemical compositions of microwave-treated and untreated feedstuffs is shown in Table 3. Fish meal lipid and fibre contents were significantly increased due to microwave irradiation, while the other constituents did not fluctuate. Protein and lipid from meat and bone meal decreased after irradiation. Different trends were observed for contents of ash and NFE of the same feedstuff. For soybean meal, the only change was a decrease in lipid in the treated sample. Dramatically decreased lipid content was observed in irradiated broken rice, relative to native. Rice bran protein was significantly increased after irradiation, while fibre was decreased. Each chemical composition varied depending on type of feedstuff.

Proximate chemical compositions of irradiated feeds

Chemical compositions of experimental feeds are shown in Table 4. All chemical compositions, except protein and NFE, were affected by microwave processing ($P < 0.001$). Ash and GE contents were higher in microwave-irradiated feeds than in nonirradiated feeds and vice versa for lipid and fibre. The processing method directly affected crude lipid ($P < 0.001$), ash ($P < 0.05$), NFE ($P < 0.001$) and GE ($P < 0.04$) but not protein and fibre ($P > 0.05$). The interactions between microwave processing and pelleting methods affected lipid ($P < 0.05$), ash ($P < 0.004$) and GE ($P < 0.02$) contents but not protein, fibre and NFE contents.

Growth trial of sex-reversed Nile tilapia

Survival rate, growth performance and feed utilization of sex-reversed Nile tilapia fed different dietary

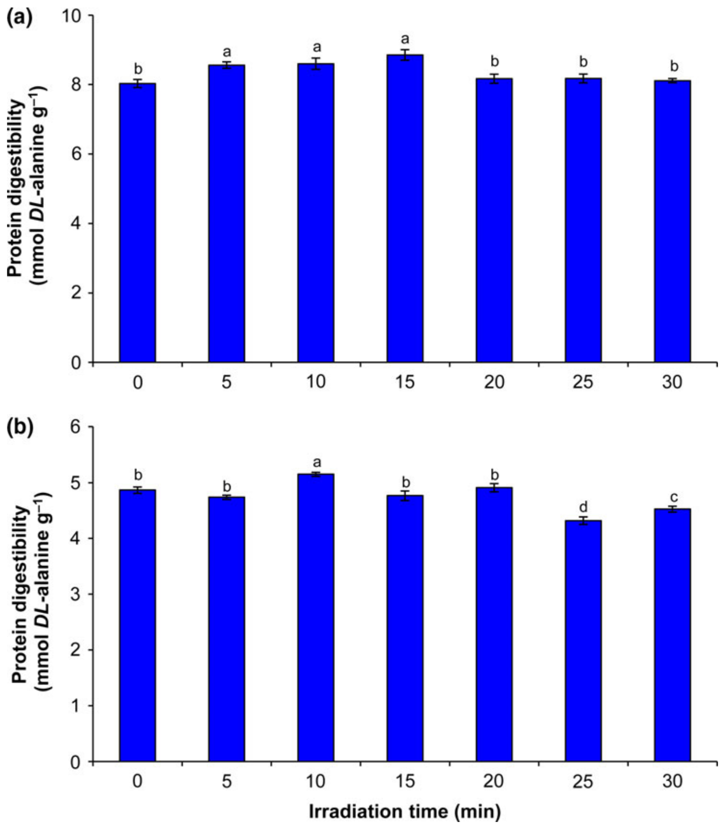

Figure 1 *In vitro* protein digestibility (mmol *DL*-alanine g^{-1}) of microwave-irradiated fish meal (a) and meat and bone meal (b) using digestive enzyme extracts from sex-reversed Nile tilapia (n = 6). Data with different superscripts are significantly different (P < 0.05).

treatments are shown in Table 5. Survival was not influenced by microwave processing, pelleting methods or their interaction. All growth performance parameters and feed utilization (except CF and FI) were influenced by microwave processing (P < 0.001). Fish fed steamed microwave-irradiated feeds, mechanically pelleted with a meat mincer, had the highest growth performance and PER, but lower FCR, than fish fed other feeds. Microwave processing affected all growth performances (P < 0.001), except CF and FI. Similar findings were also observed due to the pelleting methods, excluding a significant effect on FI. Interaction of both parameters had no effect on survival or final weight.

Discussion

Optimal time for improving *in vitro* digestibility of feedstuffs

The highest protein digestibility of fish meal and meat and bone meal was observed after microwave irradiation for 11 and 12 min, respectively. Similar findings in protein improvement have been reported in microwave-irradiated moth bean (Negi, Boora & Khetarpaul 2001), Bengal gram, green gram, horse gram (Khatoon & Prakash 2006), canola seed (Sadeghi & Shawrang 2006; Ebrahimi, Nikkhah & Sadeghi 2010) and cottonseed meal (Sadeghi & Shawrang 2007). This could occur due to the unfolding of protein structure and its denaturation as a result of microwave pretreatment (Negi *et al.* 2001). This change could lead to the reorganization of hydrophobic amino acids on the surface, which generally played as the active sites of pepsin and trypsin (Ebrahimi *et al.* 2010), for promoting proteolytic digestion along the gastrointestinal tract of animals.

Carbohydrate digestibility of soybean meal, broken rice and rice bran was highest after microwave irradiation for 10, 13 and 10 min, respectively. Water solubility, starch gelatinization, crystallinity, amylose content, starch diameter and starch degradation have been reported to play important roles in the enzymatic digestion of carbohydrates (Zhao, Xiong, Qiu & Xu 2007; Chung & Liu 2010). Improvement of carbohydrate qual-

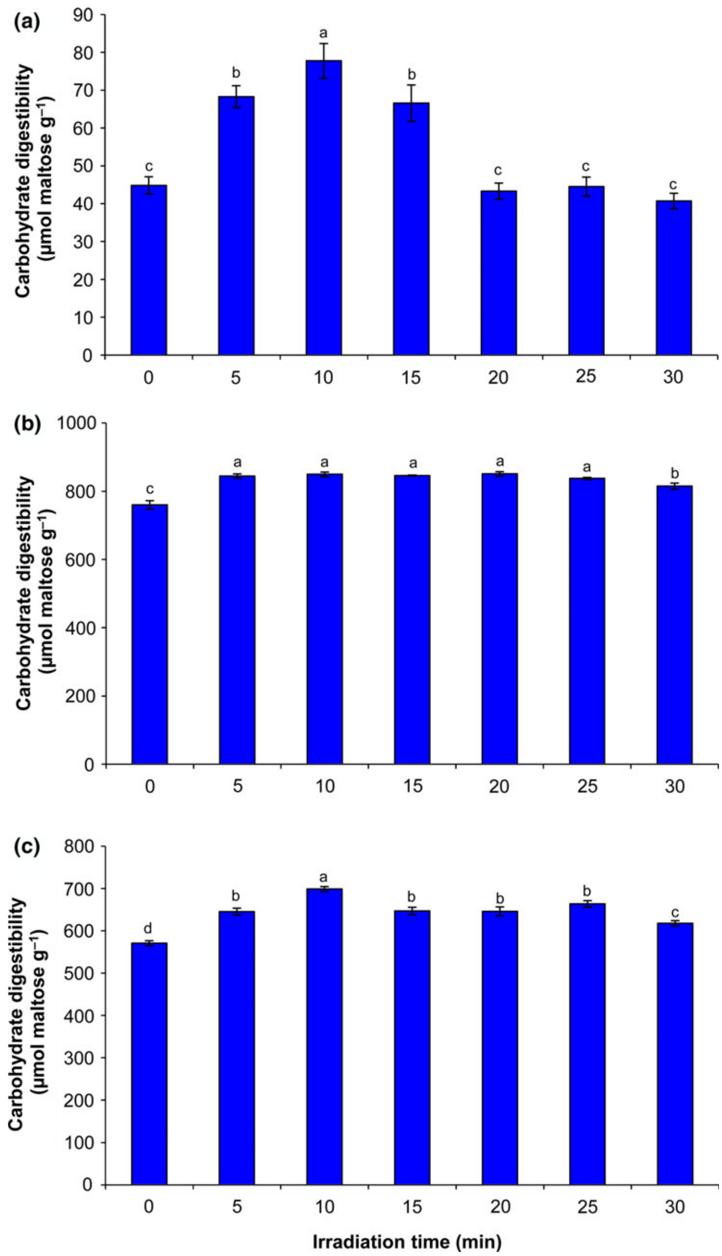

Figure 2 *In vitro* carbohydrate digestibility (μmol maltose g^{-1}) of microwave-irradiated soybean meal (a), broken rice (b) and rice bran (c) using digestive enzyme extracts from sex-reversed Nile tilapia ($n = 6$). Data with different superscripts are significantly different ($P < 0.05$).

ity, based on *in vitro* determination of digestibility, in different raw materials after microwave irradiation has been reported in various foodstuffs (Negi *et al.* 2001; Khatoon & Prakash 2006) as well as feedstuffs such as rice straw (Ma, Liu, Chen, Wua & Yu 2009), a feedstuff mixture (Thongprajukaew *et al.* 2011) and palm kernel meal (Thongprajukaew *et al.* 2013). However, longer irradiation time could cause a significant reduction in digestibility of carbohydrate and protein by retarding the physicochemical properties, such as the pasting property of starch (Palav & Seetharaman 2007), and aggregation of starch granules (Anderson & Guraya 2006) and protein (Sadeghi & Shawrang 2007). The optimal times for microwave irradiation of feedstuffs in the present study differed from some previous reports. This is likely due to differences in various factors that affected microwave pretreatments, namely irradiation time, irradiation power and substrate concentration (Ma *et al.* 2009), as well as the source of tested enzymes used for *in vitro* digestibility screening.

Table 3 The proximate chemical compositions of untreated and microwave-treated feedstuffs. Microwave pretreatment of each feedstuff was conducted according to the optimal times, as described in Table 2. Data were calculated from duplicate analyses and expressed in g kg^{-1} dry matter

Chemical composition	Fish meal		Meat and bone meal		Soybean meal		Broken rice		Rice bran	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
Crude protein	586.0 ± 1.6	586.6 ± 2.8	522.5 ± 0.1*	513.8 ± 0.7*	535.3 ± 0.3	532.9 ± 1.0	76.3 ± 1.9	76.0 ± 0.4	147.2 ± 1.3*	152.2 ± 1.5*
Crude lipid	129.4 ± 0.1*	142.3 ± 1.7*	131.6 ± 1.3*	118.0 ± 0.1*	26.7 ± 1.0*	22.5 ± 1.5*	12.8 ± 1.2*	5.0 ± 1.1*	193.4 ± 5.8	190.4 ± 2.6
Crude fibre	1.8 ± 0.2*	3.8 ± 0.2*	11.3 ± 0.1	12.7 ± 1.7	36.0 ± 0.1	34.7 ± 2.0	nd	nd	55.3 ± 0.9*	44.7 ± 2.0*
Ash	243.0 ± 6.7	235.0 ± 1.7	283.8 ± 0.4*	292.1 ± 0.4*	70.9 ± 2.4	71.2 ± 1.8	5.0 ± 0.5	5.7 ± 0.2	98.7 ± 0.8	95.9 ± 0.8
NFE	39.8 ± 7.1	32.3 ± 3.8	50.8 ± 1.3*	63.4 ± 1.8*	331.1 ± 2.6	338.7 ± 3.2	905.9 ± 2.2*	913.3 ± 1.2*	505.4 ± 5.1	516.8 ± 7.7

nd, not detected; NFE, nitrogen-free extract. Significant differences between treated and untreated feedstuffs are indicated by * ($P < 0.05$).

However, all of these studies demonstrated that microwave irradiation is an efficient method for improving the enzymatic digestibility and quality of feedstuffs for sex-reversed Nile tilapia.

Proximate compositions of microwave-treated and untreated feedstuffs and experimental feeds

Microwave pretreatment can cause significant changes in some proximate compositions, depending on the type of feedstuff. Changes in protein content that occur due to C–N bond scissions in the backbone of polypeptide chains, or physical changes like unfolding, can increase the availability of nitrogen atoms (Molins 2001; Fernandes, Barreira, Antonio, Rafalski, Oliveira, Martins & Ferreira 2015). Moreover, irradiation can modify the molecular properties of protein, as well as other N-containing compounds, by forming covalent cross-linkages or by conversion to higher molecular weight aggregates (Sadeghi & Shawrang 2007). On the other hand, the decrease in crude protein of microwave-irradiated feedstuff is probably due to the denaturation of protein (Sakla, Ghali, El Farra & Rizk 1988) or the partial loss of amino acids after pretreatment. Such effects would change the protein content, as detected by the Kjeldahl method.

Lipid content also changed, similar to protein. The loss of this constituent during cooking could occur from the oxidation of unsaturated fatty acids, which depends on time and temperature of processing (Stewart, Raghavan, Orsat & Golden 2003; Malheiro, Oliveira, Vilas-Boas, Falcão, Bento & Pereira 2009). On the other hand, increased crude lipid content in microwave-irradiated feedstuffs indicated the formation of stable saturated fatty acids from unsaturated fatty acids (Chumwaengwapee, Soontornchai & Thongprajukaew 2013).

Crude fibre content increased in microwave-irradiated fish meal. Bressani (1993) proposed that this increase could be due to the formation of protein-fibre complexes after pretreatment. On the other hand, crude fibre in microwave-irradiated rice bran decreased. These findings suggested a reduction in the main cell wall constituents (cellulose, hemicellulose and lignin), which caused an increase in NFE after pretreatment (Thongprajukaew *et al.* 2013). A significant increase in crude ash content of microwave-irradiated meat and bone meal was postulated to be due to

Table 4 Chemical compositions of experimental feeds prepared using different microwave processing and pelleting methods. Data were calculated from duplicate analyses and expressed in g kg^{-1} dry matter

	Microwave			Without microwave			*P*-values of the factors		
Chemical composition	Meat mincer	Extruder	Steam and meat mincer	Meat mincer	Extruder	Steam and meat mincer	Wave	Method	W × M
Crude protein	339.8 ± 1.4	335.9 ± 3.3	335.3 ± 1.6	334.0 ± 3.9	336.7 ± 1.3	334.7 ± 1.3	0.273	0.610	0.279
Crude lipid	68.9 ± 1.8	52.5 ± 3.2	75.4 ± 0.1	86.4 ± 1.4	61.5 ± 1.0	89.5 ± 2.0	**<0.001**	**<0.001**	**0.046**
Crude fibre	5.7 ± 0.5	5.7 ± 0.4	5.5 ± 0.1	8.6 ± 0.4	8.0 ± 0.1	8.2 ± 0.2	**<0.001**	0.381	0.377
Ash	143.1 ± 2.5	136.3 ± 1.3	140.3 ± 0.5	127.4 ± 0.5	128.7 ± 1.5	127.8 ± 1.6	**<0.001**	**0.040**	**0.003**
NFE	443.9 ± 2.7	468.9 ± 5.5	442.8 ± 1.1	443.6 ± 4.8	464.3 ± 3.8	440.7 ± 2.0	0.313	**<0.001**	0.712
GE (kcal kg^{-1})	4355 ± 41	4400 ± 61	4295 ± 20	4286 ± 18	4136 ± 1	4189 ± 24	**<0.001**	**0.039**	**0.013**

NFE, nitrogen-free extract; GE, gross energy. Significant values are indicated by bold type ($P < 0.05$).

microwave irradiation inducing a chelating reaction (Chumwaengwapee *et al.* 2013). Changes in these constituents directly affected NFE content.

For experimental feeds, microwave processing, pelleting methods or their interaction affected nearly all chemical compositions, except protein. The findings from the current study are in contrast to previous reports, which found no differences in the chemical compositions of microwave-irradiated feed for juvenile Siamese fighting fish, when compared with feeds produced from native or gamma-irradiated feedstuffs (Thongprajukaew *et al.* 2011), but not for the fine nutritional profiles (Thongprajukaew 2011). Some of the current findings from experimental feeds were not correlated with the summation of chemical compositions from all native feedstuffs. This might be due to the complicated interactions among constituents, which affected the proximate chemical compositions of feeds during processing.

Growth trial of sex-reversed Nile tilapia

Pearson correlation analysis indicated no association between the processing methods or feed chemical compositions and growth or feed utilization parameters ($P = 0.132$–0.974). Also, the microwave processing and pelleting methods did not affect either parameter during the first 60 days of all dietary treatments (data not presented). However, prolongation of the feeding trial was sufficient to cause significant changes in the growth performance of sex-reversed Nile tilapia. Similar findings were observed in Atlantic salmon (Sunde *et al.* 2004), trout and sea bream (Santigosa, Sánchez, Médale, Kaushik, Pérez-Sánchez & Gallardo 2008) and grass carp (He, Liang, Li, Sun & Shen 2013) after rearing for 90, 84 and 60 days, respectively. In addition, microwave processing, pelleting methods and their interaction did not affect water quality; all water quality parameters were within the standards for aquaculture.

The replacement of native feed ingredients with the microwave-irradiated form influenced the overall feed physicochemical properties of the feed, in relation to enhanced enzymatic hydrolysis (Thongprajukaew 2011) as well as fish growth performance (Thongprajukaew *et al.* 2011). These findings indicate that the nutritional quality can be manipulated by physicochemical alterations. Generation of heat throughout the materials by microwaving leads to faster heating rates and shorter processing times when compared with conventional heating, where heat is usually transferred from the outer surface to the internal materials (Arocas *et al.* 2011; Fan, Wang, Ma, Ma, Liu, Huang, Zhao, Zhang & Chen 2013). Moreover, microwave irradiation also has several other advantages, including greater energy efficiency, reduced startup and stopping time, space savings, higher levels of safety and automation, precise process control and improved nutritional quality of irradiated raw materials (Arocas *et al.* 2011; Xu, Zhou, Miao, Gao, Cai & Dong 2013).

The pelleting method could also improve the nutritional and physical qualities of feed ingredients as well as fish growth performance (Behnke 1996; Booth *et al.* 2000). The feed produced by microwaving and mechanical pelleting with a combination of steam conditioning and mincing provided superior growth performance and feed utilization of Nile tilapia. This is due to the heat

Table 5 Survival, growth performance and feed utilization of sex-reversed Nile tilapia when fed with pellets prepared using different microwave processing and pelleting methods. Data are for fish samples collected at the end of the experiment (120 days)

Parameter	Microwave			Without microwave			*P*-values of the factors		
	Meat mincer	Extruder	Steam and meat mincer	Meat mincer	Extruder	Steam and meat mincer	Wave	Method	W × M
Survival (%)	100	97.77 ± 3.85	97.77 ± 3.85	93.33 ± 6.66	93.33 ± 11.50	97.77 ± 3.85	0.221	0.822	0.638
Final body length (cm)	8.24 ± 0.01	8.49 ± 0.12	8.61 ± 0.07	7.95 ± 0.07	8.08 ± 0.05	8.30 ± 0.08	**<0.001**	**<0.001**	0.358
Final body weight (g)	15.61 ± 0.16	18.36 ± 0.57	20.46 ± 0.78	15.36 ± 0.33	15.55 ± 1.12	16.28 ± 1.10	**<0.001**	**<0.001**	**0.003**
CF (g cm^{-3})	2.79 ± 0.02	2.99 ± 0.04	3.20 ± 0.05	3.05 ± 0.15	2.95 ± 0.15	2.85 ± 0.11	0.281	0.124	**<0.001**
WG (g)	14.04 ± 0.20	16.80 ± 0.61	18.91 ± 0.73	13.81 ± 0.37	13.95 ± 1.05	14.68 ± 1.09	**<0.001**	**<0.001**	**0.002**
ADG (g day^{-1})	0.12 ± 0.01	0.14 ± 0.01	0.16 ± 0.01	0.11 ± 0.01	0.12 ± 0.02	0.12 ± 0.01	**<0.001**	**0.001**	**0.013**
SGR (% day^{-1})	1.91 ± 0.03	2.05 ± 0.05	2.15 ± 0.26	1.91 ± 0.05	1.89 ± 0.26	1.93 ± 0.06	**<0.001**	**0.001**	**0.002**
FI (g day^{-1})	0.22 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.20 ± 0.01	0.337	**0.010**	**0.001**
FCR (g feed g gain^{-1})	1.88 ± 0.32	1.45 ± 0.06	1.31 ± 0.05	1.80 ± 0.87	1.88 ± 0.16	1.63 ± 0.10	**<0.001**	**<0.001**	**0.001**
PER (g gain g protein^{-1})	1.57 ± 0.03	2.05 ± 0.08	2.27 ± 0.08	1.66 ± 0.08	1.58 ± 0.13	1.83 ± 0.12	**<0.001**	**<0.001**	**<0.001**

CF, condition factor; WG, weight gain; ADG, average daily gain; SGR, specific growth rate; FI, feed intake; FCR, feed conversion ratio; PER, protein efficiency ratio. Significant values are indicated by bold type ($P < 0.05$).

and moisture provided by the steam conditioning, which can improve the degree of gelatinization (Lundblad, Issa, Hancock, Behnke, McKinney, Alavi, Prestløkken, Fledderus & Sørensen 2011) and digestibility of starch (Robinson, Manning & Li 2004). Similarly, the feed produced by steam conditioning before pelleting also provided higher growth and feed utilization of Nile tilapia than the feed produced by extrusion only. This is because steam conditioning is generally conducted under low processing temperatures and requires low input of mechanical energy (Lundblad *et al.* 2011). These conditions result in less destruction of nutrients during steam pelleting, as compared with extrusion (Robinson *et al.* 2004). Similar findings on growth and feed utilization after feeding with pellets produced by steam conditioning have been reported in silver perch (Booth *et al.* 2000), rainbow trout (Hilton *et al.* 1981) and chicken (Lundblad *et al.* 2011).

Based on a same-species comparison, fish fed with steamed microwave-irradiated feed, mechanically pelleted with a meat mincer (SGR 2.15% day^{-1}), appeared to demonstrate superior growth performance compared with fish fed with feed containing 200 g kg^{-1} microwave-irradiated palm kernel meal (SGR 1.98% day^{-1}) (Thongprajukaew, Rodjaroen, Tantikitti & Kovitvadhi 2015) or feed containing 200 g kg^{-1} pre-extruded maize flour (SGR 2.08% day^{-1}) (Amirkolaie *et al.* 2006). The findings from the current study indicate that this is a promising alternative processing method for producing an effective feed for sex-reversed Nile tilapia and is easier and less expensive than production by an extruder. However, further study of the physical property of the experimental feeds is important as well in order to improve nutritional quality for aquafeed production.

Conclusions

Microwave irradiation of feedstuffs at optimal times could improve the digestibility of carbohydrate and protein. The microwave processing and pelleting methods as well as their interactions affected nearly all of the proximate chemical compositions. Sex-reversed fish fed steamed microwave-irradiated feed, mechanically pelleted with a meat mincer, had the highest growth performance and feed utilization efficiency when compared with the other dietary groups. Further improvement in the nutritional quality of aquafeed through microwave processing and pelleting methods should be investigated.

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