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Research Article

Dynamical Hybrid System for Optimizing and Controlling Efficacy of Plant-Based Protein in Aquafeeds

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In this paper, a mathematical model was used to evaluate a dynamical hybrid system for optimizing and controlling the efficacy of plant-based protein in aquafeeds. Fishmeal (FM), raw rapeseed meal (RM), and a fermented meal with yeast (RM-Yeast) and fungi (*Aspergillus* oryzae RM-Koji) were used as test ingredients for the determination of apparent digestibility coefficients (ADCs) of dry matter, crude protein, crude lipid, energy, and essential amino acids (EAA) for olive flounder (*Paralichthys olivaceus*, 7 ± 0.02 g) using diets containing 0.5% Cr_2O_3 as an inert indicator. Among all ingredients tested, FM had the maximum ADC of dry matter ($P < 0.05$), protein ($P < 0.05$), lipid ($P > 0.05$), and energy ($P > 0.05$). Fermented meals (RM-Yeast and RM-Koji) showed higher ADC ($P < 0.05$) of crude protein compared with RM, while there was no significance in ADCs of crude lipid and energy among different forms of rapeseed meal. Besides, ADC of crude lipid for RM-Yeast and RM-Koji, on the one hand, and ADC of gross energy for RM-Yeast, on the other hand, were not varied from that for FM ($P > 0.05$). Amino acid digestibility reflects protein digestibility in most cases. Interestingly, protease, lipase, and amylase activities were better expressed in RM-Koji, RM-Yeast, and FM over RM, respectively. The current results deliver important information on nutrients and energy bioavailability in raw and fermented RM, which can be implemented to accurately formulate applied feeds for olive flounder. Compared with other applicable systems, the complexity of the approach implemented has been considerably reduced.

1. Introduction

Because of the rising cost and demand for fishmeal on the international market, consideration has been given to increasing the use of plant origin protein sources in cultured fish feeds [1, 2]. For decades, soybean meal has become the most vastly used plant protein meal in aquaculture [3], and more protein sources, such as rapeseed meal, are being investigated as promising candidates for aquafeeds [4]. Indeed, rapeseed meal (RM) has become the second most

markedly traded protein source of plant origin after soybean due to booming global production/supply (40.51 million metric tonnes in 2017) [5]. Rapeseed meals have substantial potential as a fishmeal substitute in aquafeeds because of their protein contents (32% to 45%) and amino acid profile compared with soybean meal [6, 7]. However, owing to the prevalence of antinutritional factors (ANFs) and high fiber levels in rapeseed meals [8], its use has been imperfect, with the main adverse effects in fish being reduced palatability, feed intake, and digestibility [9].

To separate protein fractions from remaining ingredients, including ANFs, various approaches have been implemented, but each has its own set of downsides, such as protein loss and denaturation, expense, commercial viability, and environmental sustainability [10]. Microbial fermentation practice has been described as a less expensive and more efficient option. Indeed, during fermentation, microbial activities can partially degrade ANFs like cell walls and phytates, as well as breaking down large molecules, for example, starch and protein. into smaller molecules that are easier to digest and absorb [11–13]. So far, there is a widespread agreement that, after fermentation, nutrient bioavailability in rations for different animals amplifies [14–19]. Nonetheless, the performance of this substitution varies greatly depending on the fish species and experimental conditions. Changing dietary feedstuffs without affecting the animal's performance also necessitates knowledge of the feedstuff composition, especially the apparent digestibility coefficients of the nutrients.

Paralichthys olivaceus, also known as the olive flounder, is a commercially remarkable fish that is extensively farmed in Asia. ADCs of different protein sources for olive flounder have already been investigated in recent studies [20–24]. However, there is no accessible information on the digestibility of rapeseed products for this species. Therefore, this study sought to determine rapeseed meal digestibility and whether microbial fermentation of the meal could enhance nutrients digestibility and digestive enzymes in olive flounders.

2. Materials and Methods

2.1. Experimental Diets. The indicator approach (chromic oxide = Cr2O3 at 0.5%) was used to determine nutrient and energy digestibility (ADCs) in feedstuffs of test diets (TD) comprised of 70% basal or references diet (RD) (54.45% crude protein and 14.75% lipid) and 30% of each of the test ingredients (Table 1). Fishmeal (FM) was used as the key protein contributor in the basal diet. Raw rapeseed meal (RM) and fermented meal with yeast (RM-Yeast) and fungi (*Aspergillus* oryzae RM-Koji) were used as test ingredients for ADCs. Commercially available FM and RM were used, while RM-Yeast and RM-Koji were made as previously mentioned [4, 25]. Ingredients proximate analysis and amino acid compositions are displayed in Table 2.

2.2. Fish and Feeding Trial. The trial was done at Kagoshima University's Kamoike Marine Production Laboratory

TABLE 1: Ingredients (g/kg) of basal and test diets.

Ingredients	Basal diet	Test diets
Fishmeal	70	49
Rapeseed meal forms[1]	-	30
Wheat flour	4	2.8
Polack liver oil	5	3.5
Soybean lecithin	4	2.8
Vitamin mix[2]	3	3
Mineral mix[3]	3	3
Stay-C[4]	0.1	0.1
Activated gluten[5]	5	5
α-Cellulose	4.4	2.63
Attractant	1	1
Cr_2O_3	0.5	0.5

[1]Rapeseed meal forms: rapeseed raw meal (RM), fermented rapeseed meal with yeast (RM-Yeast), or fungi (RM-Koji).[2]A kilo of mixed vitamins contains biotin (0.01 mg), Ca pantothenate (0.27 mg), choline chloride (7.87 mg), folic acid (0.01 mg), inositol (3.85 mg), niacin (0.77 mg), ß-carotene (0.10 mg), vitamin B_1 (0.06 mg), vitamin B_2 (0.19 mg), vitamin B_6 (0.05 mg), vitamin B_{12} (0.0001 mg), vitamin D_3 (0.01 mg), vitamin E (0.38 mg), vitamin K_3 (0.05 mg), ρ-aminobenzoic acid (0.38 mg), and cellulose (1.92 mg).[3]A kilo of mineral mixture contains Al (OH)$_3$ (0.01 mg), Ca (IO$_3$)$_2$ (0.01 mg), Ca lactate (12.09 mg), CoSO$_4$ (0.04 mg), Fe citrate (1.1 mg), K$_2$HPO$_4$ (8.87 mg), MgSO$_4$ (5.07 mg), MnSO$_4$ (0.03 mg), Na$_2$HPO$_4$ (3.23 mg), and ZnSO$_4$ (0.13 mg).[4]L-Ascrobil-2-phosphate-magnesium.[5]A-glu SS (Glico Nutrition Co., Ltd. Osaka, Japan).

(Japan). Flounder juveniles (7 ± 0.02 g) were provided from a local private farm and adapted to test conditions for 10 days while fed the basal diet. Fishes ($n = 120$) were allocated in four experimental groups in triplicate (15 fish per tank) using 12 polycarbonates 100 L tanks supplied with a maximum of 80 L flow-through seawater, where tanks were prepared with an inlet, outlet, and constant aeration. During the entire trial, the natural light/dark regime was applied, while maintaining the water quality at the ideal limits in terms of temperature (20.2 ± 1.1°C), dissolved oxygen (DO = 6.90 ± 0.3 mg/L), and pH (7.9 ± 0.4). Hand-feeding was used to visible satiation twice a day (9 : 00 and 16 : 00 h), and the daily feed supply and leftover feed, which was recovered 40 minutes after each feeding to avoid contamination with feces, were reported. Feces collection started seven days after feeding to enable all previously ingested materials to be evacuated and lasted four weeks until adequate samples were collected for chemical analysis. Due to the difficulty of collecting flounders' feces, which are semiliquid and therefore dissolve quickly in water, samples were collected four times a day (10 h, 12 h, 14, and 16 h) by siphoning after the morning meal to limit the leaching of nutrients. Samples were immediately filtered through a filter paper (Whatman # 1), gathered per tank, and quickly kept for nutrient contents investigation at –20°C.

2.3. Digestive Enzyme Activity. Fish were depleted of food for 24 hours at the trial end, and their intestines were washed in pure cold water, cut into small pieces, and pooled for enzyme activity analysis. For the analysis, 2 g of each group sample was homogenized in 5 mL Tris-HCl buffer (50 mM, pH 8.0) and centrifuged at 10000 g and 4°C for 30 minutes. The supernatant was regarded as a crude enzyme solution and

TABLE 2: The chemical composition and amino acids' profile of tested feedstuffs.

Items	Feedstuffs			
	FM	RM	RM-Yeast	RM-Koji
Crude protein	65	40	46.84	46.78
Crude lipid	8.2	2.66	2.02	1.96
Ash	15.5	7.45	7.65	8.36
Fiber	0.35	11.40	10.41	10.88
Gross energy (kJ/g)[1]	ND	17.01	16.90	17.81
Phytic acid (g/kg)	ND	41.00	31.24	33.44
Glucosinolates (μmol/g)	ND	120.45	112.81	108.40
Amino acid profile (g/100 g of a dry ingredient)				
Arginine	4.62	2.14	2.45	2.81
Histidine	2.58	1.31	1.43	1.49
Isoleucine	2.00	1.26	1.34	1.38
Leucine	4.68	2.47	3.83	3.41
Lysine	4.07	1.78	1.75	1.65
Methionine	1.78	0.63	0.83	0.91
Phenylalanine	3.18	2.15	1.46	1.47
Threonine	2.74	1.68	2.00	1.93
Valine	2.37	1.61	1.56	1.77

[1]Calculated using multiplying combustion values (23.6, 39.5, and 17.2 kJ/g) in ratios of protein, lipid, and carbohydrate, respectively.

was used to detect the activity of intestine enzymes [26]. Protease activity was detected with the protocol of Sigma's Nonspecific Protease Activity Assay, utilizing casein as a substrate [27]. Released fatty acids by triglyceride enzymatic hydrolysis in a stabilized olive oil emulsion were quantified to inspect lipase activity [28]. Amylase activity was examined using iodine to reveal nonhydrolyzed starch [29].

2.4. Analytical Approaches and Calculation Formulas. The basic AOAC methods [30] were used to analyze the proximate composition of feed ingredients, diets, and feces. Moisture was checked by oven-drying to a stable weight at 110°C. Crude protein, crude lipids, crude fiber, and ash were assessed by methods of Kjeldahl, Soxhlet extraction, FiberCap procedure, and incineration for 4 h at 550°C in a muffle furnace, respectively. The concentration of Cr_2O_3 in diets and feces was detected using the method of Furukawa and Tsukahara [31]. Amino acids' profile was obtained with high-performance liquid chromatography (Shimadzu Co., Kyoto, Japan) [32]. ANFs in test ingredients were examined in terms of glucosinolates by their alkaline degradation and ferricyanide reaction [33] and phytic acid content with a colorimetric technique [34].

The following formulas were used to computerize the ADCs for the nutrients and energy:

$$ADC_{nutrient(\%)} = 100 - \left(\frac{\%Cr_2O_{3diet}}{\%Cr_2O_{3feces}} \times \frac{\%nutrient_{feces}}{\%nutrient_{diet}} \right),$$

$$ADC_{gross\,energy(\%)} = 100 - \left(\frac{\%Cr_2O_{3diet}}{\%Cr_2O_{3feces}} \times \frac{gross\,energy\,(kJ/g)_{feces}}{gross\,energy\,(kJ/g)_{diet}} \right), \quad (1)$$

$$ADC_{dry\,matter(\%)} = 100 - \left(100 \times \frac{\%Cr_2O_{3diet}}{\%Cr_2O_{3feces}} \right).$$

Test ingredients ADCs (ADC_I) were determined according to the digestibility of the RD and TD using the equation of Bureau et al. [35].

$$ADC_I = ADC_I + \left(\frac{0.7D_R}{0.3D_I} \times \frac{ADC_T}{A\,DC_R} \right), \quad (2)$$

where

ADC_T = ADC of the test diets

ADC_R = ADC of the reference or basal diet

D_R = % nutrient or gross energy (Kj/g) of the reference diet

D_I = % nutrient or gross energy (kJ/g) of the test ingredients

2.5. Statistical Analysis. Data were statistically computerized using one way-ANOVA (Package SuperANOVA version 1.11, Abacus Concepts, Berkeley, USA). Data were displayed as means values plus/minus the standard error of the mean

TABLE 3: Nutrients and gross energy contents (%) of the basal and tested diets.

Items	Basal diet	RM	RM-Yeast	RM-Koji
Dry matter	64.84 ± 0.25^{b}	51.38 ± 2.57^{a}	53.66 ± 2.87^{a}	53.15 ± 3.97^{a}
Crude protein	86.04 ± 0.36^{c}	73.16 ± 0.64^{a}	80.88 ± 1.58^{b}	79.79 ± 1.60^{b}
Crude lipid	85.64 ± 0.35^{b}	72.06 ± 2.38^{a}	81.20 ± 3.53^{ab}	80.49 ± 3.86^{ab}
Gross energy	81.07 ± 0.62^{b}	67.92 ± 3.10^{a}	73.97 ± 1.41^{ab}	65.62 ± 1.51^{a}

Values (mean $\pm$ SEM, $n = 3$) within a row with common superscripts are not different ($P > 0.05$).

($\bar{X} \pm S.\ E.\ M$). Duncan's multiple range test detected significant differences between means. Variations at $P < 0.05$ are significant statistical differences.

3. Results

Table 2 represents the chemical composition and amino acids' content of tested feedstuffs used in the experimental rations. No phytic acid or glucosinolate contents were detected in FM, and the highest contents were in RM, while RM-Yeast and RM-Koji showed remarkably lower contents. FM has the highest amino acid contents, while RM-Yeast and RM-Koji amino acid compositions were better compared with RM, except for phenylalanine.

ADCs values for FM, RM, RM-Yeast, and RM-Koji in the test diets for olive flounders are shown in Table 3. FM has the highest ADC ($P < 0.05$) for dry matter (64.84%), while the dry matter ADCs for the other ingredients were relatively lower but not meaningfully different from each other ($P > 0.05$). Similar trends were observed for ADC of crude lipid and energy with the exception that crude lipid coefficients were numerically closer to that of FM in the case of RM-Yeast and RM-Koji, while the energy coefficient was numerically closer to that of FM in the case of RM-Yeast. Only ADC of crude protein showed a clear pattern, in which FM recorded the highest coefficient among all treatments ($P < 0.05$), and RM-Yeast and RM-Koji together recorded also a greater coefficient ($P < 0.05$) than RM.

Apparent availability coefficients (EAA %) of amino acids in test ingredients for Japanese flounder are presented in Table 4. FM has superior amino acids EAA ($P < 0.05$), while RM recorded the lowest values. However, EAA recorded no noteworthy variances between FM and RM-Yeast for arginine, isoleucine, lysine, and valine. Moreover, there were no differences in the EAA between the different rapeseed forms for arginine and histidine. Also, RM-Yeast and RM-Koji showed the same availability of leucine and methionine.

Table 5 represents the enzyme activity in the intestine of flounder fish-fed test diets. Intestinal enzyme activities (protease, lipase, and amylase) in juveniles' flounders fed RM recorded minimal values, while the best activities were achieved with RM-Koji, followed by RM-Yeast and FM, respectively.

4. Discussion

The sustainability of aquaculture has become the endeavor of researchers' focus, especially providing ecofriendly solutions to overcome various production problems [36]. From a nutritional standpoint, dietary alteration is a notable way of amending animals' wellbeing and performance [37, 38]. The findings of this work provide a comprehensive assessment of the influence of processing plant meals on the bioactivity of nutrients from rapeseed products when given to olive flounders. The composition of the test ingredients had a substantial impact on the ADCs of different nutrients, for example dry matter, crude protein, and crude lipid, and energy. FM has the highest ADC ($P < 0.05$), while there are no important variances for crude lipid and energy when compared with RM-Yeast and crude lipid when compared with RM-Koji. However, no variances ($P > 0.05$) were recorded for dry matter, crude lipid, and energy between different rapeseed forms, while RM-Yeast and RM-Koji together recorded a significantly higher protein coefficient than RM ($P < 0.05$). These results indicate that, at this addition level, *Paralichthys olivaceus* cannot excellently utilize RM and derivate in diets when compared with FM. This is consistent with the results of Nagel et al. [39], suggesting rapeseed-derived poor palatability and deterioration of the diets for juvenile turbot. In this trial, FM had a substantially higher ADC ($P < 0.05$) of dry matter than all the test diets.

The amount of fecal material produced is determined by the dry matter digestibility of the feed and plant protein meals are always associated with low-digestible materials and lower dry matter digestibility than FM, due in part to their content in ANFs [40]. Table 2 showed the reduced fiber, phytic acid, and glucosinolates levels in RM-Yeast and RM-Koji. Nonstarch polysaccharides, fibers, glucosinolates, and phytic acids, which also make up a large part of RM, are the main plant ingredients that contribute the most to digestive disability and waste production. The nutritional quality of RM is primarily determined by the presence of glucosinolates and phytic acids, which have been shown to have an unfavorable influence on feed taste, intake, and absorption [41, 42]. There were some main variations in the digestibility of amino acids, but EAA digestibility reflects protein digestibility in general. Despite that, once again FM recorded numerically the highest EAA for all amino acids, while RM recorded the lowest. Protein digestion in fish can be hampered by high phytic acid levels combined with high fiber levels [8, 43, 44]. The effect of fiber on nutrient digestibility is thought to interfere with nutrient mobility along the gastrointestinal tract, resulting in limited nutrient absorption ability. Riche et al. [45] showed that phytates form complexes with proteins reducing the availability of amino-acids.

In the current trial, RM-Yeast and RM-Koji surprisingly recorded either numerically or significantly higher (in the case of protein ADC) digestibility coefficients than RM. These findings are in line with what has been found in fish enzyme activities. Intestinal enzyme activities (protease,

TABLE 4: Apparent availability coefficients (%) of amino acids in test ingredients for Japanese flounder*.

Amino acids	Ingredients			
	FM	RM	RM-Yeast	RM-Koji
Arginine	91.97 ± 0.84^b	70.81 ± 1.58^a	79.82 ± 8.71^{ab}	77.03 ± 0.12^{ab}
Histidine	89.41 ± 1.29^b	73.79 ± 0.31^a	79.82 ± 4.02^a	76.00 ± 0.52^a
Isoleucine	88.60 ± 3.25^b	67.42 ± 1.26^a	82.33 ± 0.50^b	74.40 ± 1.15^a
Leucine	92.16 ± 1.80^c	71.26 ± 1.28^a	82.32 ± 2.99^b	78.68 ± 0.23^b
Lysine	90.26 ± 1.91^b	68.83 ± 1.17^a	84.03 ± 0.41^b	70.94 ± 3.53^a
Methionine	88.37 ± 1.15^c	65.51 ± 1.05^a	79.87 ± 0.67^b	75.09 ± 3.35^b
Phenylalanine	91.41 ± 0.50^d	66.88 ± 1.68^a	80.98 ± 0.12^c	74.04 ± 0.02^b
Threonine	91.99 ± 0.08^c	69.50 ± 0.50^a	82.14 ± 0.13^b	72.77 ± 2.22^a
Valine	88.70 ± 2.26^b	66.97 ± 0.23^a	81.59 ± 0.79^b	69.99 ± 3.44^a

Values (mean ± SEM, $n = 3$) within a row with nonshared superscripts are different ($P < 0.05$).

TABLE 5: Enzyme activity (U/mg) in the intestine of Japanese flounder fed test diets.

Parameters	Ingredients			
	FM	RM	RM-Yeast	RM-Koji
Protease	0.33 ± 0.02^{ab}	0.27 ± 0.53^a	0.34 ± 0.10^{ab}	0.45 ± 0.04^b
Lipase	0.54 ± 0.05^{ab}	0.37 ± 0.06^a	0.59 ± 0.12^{ab}	0.76 ± 0.07^b
Amylase	0.82 ± 0.03^a	0.59 ± 0.15^a	0.83 ± 0.27^a	1.13 ± 0.06^a

Values (mean ± SEM, $n = 3$) within a row with various superscripts are different ($P < 0.05$).

lipase, and amylase) in juveniles' flounders fed RM recorded minimal values, while the best activities were achieved with RM-Koji, followed by RM-Yeast and then FM, respectively. These positive trends in groups fed RM-Yeast and RM-Koji over those fed simple RM could be attributable to the fermentation process and the reducing ANFs contents in the fermented ingredients. Murooka and Yamshita [46] explained that, during the fermentation process, *Aspergillus oryzae* not only produces a lot of enzymes but also has an extraordinarily good capacity to transform starches, proteins, and lipids into bioactive compounds. The fermentation process reduces the size of peptides, which lowers digestion's secretions level by producing more bioavailable items [11, 47]. According to Zhou et al. [48], peptide absorption in the gut, especially di- and tripeptides, is the main transport route for subsequent nitrogen utilization in fish. Increasing nutrient digestibility in various fermented meals has been already reported previously [21, 22, 49–53]. For precise feed formulation and regulation of aquaculture wastes, a reliable database on nutrient digestibility of various protein sources is essential [54, 55].

It could be concluded that poor nutrient digestibility due to the existence of ANFs is a significant limiting feature when simple RM was implemented as the main dietary protein source for *P. olivaceus*. However, feeding fish with fermented RM results in improved nutrients utilization supported with intestinal enzyme developments. Yet, it is necessary to conduct feeding trials over a longer period to check the performance, feed utilization, and optimum inclusion levels of these feedstuffs in diets for olive flounder. As future work, the most advanced computing models and artificial intelligence [56–60] can be applied to the sustainability of aquaculture.

Data Availability

The data used in this study are available upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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