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Evaluation of corn steep powder as a protein source of Nile tilapia *Oreochromis niloticus* diet

Evaluasi *corn steep powder* sebagai sumber protein pada pakan ikan nila *Oreochromis niloticus*

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ABSTRACT

This study was aimed to evaluate the use of corn steep powder (CSP) as a plant protein source in *Oreochromis niloticus* diet. A commercial feed with 28% protein content and 368 kcal/g energy was used as reference diet, while the test feed consisting of various CSP content, namely 0%, 10%, 20%, and 30% and feed containing soybean meal (SBM) at the level of 20% and 30%. Tilapia were used in the trial with the initial body weight of 6.44 ± 0.29 g, and reared for thirty days in the aquarium at the density of fifteen and fed 3 times daily at a satiation level. All diets were supplied by 0.5% of Cr_2O_3 as an indicator for digestibility measurement. This study applied the completely randomized design experimental method containing six diet treatments and four replications. The result showed that CSP contains 40.27% protein, 26.10% lactic acid, and minerals. CSP is low in crude fiber and anti-nutritional factors. This study results that increasing the level of CSP significantly decreased feed acidity (P<0.05) compared to the control. The addition of CSP 20% increased feed digestibility including protein, lipid, energy, and dry matter digestibility. CSP 20% treatment increased final body weight, specific growth rate and reduced feed conversion ratio significantly (P<0.05) compared to other treatments. In conclusion, CSP can be used up to 20% to improve the growth performance of tilapia.

Keyword: corn steep powder, feed digestibility, growth performance, tilapia

ABSTRAK

Penelitian ini bertujuan mengevaluasi penggunaan *corn steep powder* (CSP) sebagai sumber protein nabati pada pakan ikan nila *Oreochromis niloticus*. Pakan komersial dengan kadar protein 28% dan energi 368 kkal/g digunakan sebagai pakan acuan, sementara pakan uji terdiri atas pakan dengan kandungan CSP sebanyak 0% (CSP0), 10% (CSP10), 20% (CSP20) dan 30% (CSP30) serta pakan dengan kandungan tepung bungkil kedelai (SBM) pada level 20% (SBM20) dan 30% (SBM30) sebagai pembanding. Penambahan Cr_2O_3 0,5 % diberikan sebagai indikator untuk mengukur kecernaan. Ikan nila dengan bobot tubuh rata-rata 6.44 ± 0.29 dipelihara dalam akuarium (95×45×45 cm^3) yang diisi air 100 L dengan kepadatan 15 ekor per akuarium dan diberi pakan tiga kali sehari secara *at satiation* selama 30 hari masa pemeliharaan. Penelitian ini menggunakan desain rancangan acak lengkap dengan enam perlakuan dan empat ulangan. Hasil penelitian menunjukkan bahwa CSP mengandung protein sebesar 40,27%, asam laktat 26,10%, beberapa mineral dan indeks asam amino esensial 0,90. CSP juga rendah serat kasar dan zat antinutrisi. Peningkatan dosis CSP menurunkan pH pakan secara signifikan (P<0,05) dibandingkan dengan kontrol. Penambahan CSP sampai level 20% meningkatkan nilai kecernaan total, kecernaan bahan, kecernaan protein, kecernaan lemak dan kecernaan energi. Di samping itu, perlakuan CSP 20% meningkatkan bobot akhir, laju pertumbuhan harian dan rasio konversi pakan yang signifikan (P<0,05) dibandingkan perlakuan lainnya. Hasil penelitian ini menunjukkan bahwa pemberian CSP 20% dapat meningkatkan kinerja pertumbuhan ikan nila.

Kata kunci: *corn steep powder*, kecernaan pakan, pertumbuhan, ikan nila

INTRODUCTION

Feed is the major aspect in supporting the sustainability of aquaculture and it contributes more than 50% towards the production cost (Ahmad, 2018; Sharma *et al*., 2014; Collins *et al*., 2013). The quality and quantity of the feed are the most faced challenges in aquaculture. Feed with a complete nutrition content, i.e., protein, lipid, carbohydrate, mineral, and vitamin, is surely required to support optimal fish growth (Suprayudi *et al*., 2013). A high-quality feed can be fulfilled through ingredients selection with balanced nutritional content. One of the nutritional aspects that specifically important to be in the feed ingredient and directly determine the cost is protein. Protein is a predominant macronutrient that provides essential and nonessential amino acids, which are later used in protein synthesis and energy in basal metabolism. The energy will be utilized in daily health daily maintenance and growth (Kim *et al*., 2016; Coloso, 2014). The protein quality is determined by various factors, i.e., amino acid compositions, digestion, absorption, and utilization in the metabolism (Nosworthy & House, 2017). Since protein could be costly (Mohseni *et al*., 2013), besides composition, the price tag is one of the considerations in selecting the protein source.

An ingredient will be stated as a potential protein source when it has a particular protein content and amino acids that comply with the requirements of a certain species. Anti nutrition compound is preferably low. For the cost, a relatively low price is still on the top of the list, and sustainability is needed to be maintained (Smiglak-Krajewska, 2020). The most common protein source which is frequently applied in aquaculture is categorized into two different sources, i.e., animal-based and plant-based. The plant-based is more frequently used because of the price, easy access, and the consistency of the nutritional content compared to the animal-based, e.g. fish meal, meat, or poultry by-product (Ayadi *et al*., 2011). Plant-based protein source from the agriculture by-product that commonly used in the fish feed industry is palm kernel meal (Thongprajukaew *et al*., 2015; Agbabiaka & Osuigwe, 2014), dried distiller's grains with solubles (DDGS) (Chatvijitkul *et al*., 2016; Suprayudi *et al*., 2015), corn gluten meal (Al-Thobaiti *et al*., 2018; Potki *et al*., 2018; Bu *et al*., 2017; Nandakumar *et al*., 2017; Herath *et al*., 2016; Saez *et al*., 2014), corn steep liquor (Sukhanandi & Bhatt, 2016), and soybean meal (Bonvini *et al*., 2018a; Trosvik *et al*., 2012).

Soybean meal contains 45–53.5% of protein and 0.5–9% of lipid (Ahmad *et al*., 2020; Zhang *et al*., 2018; Koch *et al*., 2016; Xu *et al*. 2016). However, the utilization of soybean meal in aquaculture competes with human needs, the cattle feed industry, and biodiesel production. It causes the high demand, but it is not supported by the sustainable supply which followed the market price (Sekali *et al*., 2020). Therefore, to guarantee sustainable supply and fish feed production, a substitute protein source should be discovered, especially the protein source that suitable for various aquatic species, easily available, and sustainable supply. These days, most studies about a substitute ingredient lead to waste utilization or agroindustrial by-product. It is believed that we can reduce waste, increase its value, and support the efficiency in natural resource utilization to eliminate the environmental load and support sustainability (Caruso, 2015; Ogello *et al*., 2014).

One of the discovered potential ingredients as a protein source in fish feed is a corn steep powder (CSP). CSP is the dry form of corn steep liquor (CSL) produced using the spray drying method. CSL comes from the corn starch production waste which contains nitrogen, protein, mineral, vitamin, reducing sugar, organic acid, enzyme, crude fiber-free, phytate acid, and trypsin inhibitor. It also contains anti-nutrition agents, e.g., tannin and saponin (Azizi-Shotorkhoft *et al*., 2016; Li *et al*., 2016; Tan *et al*., 2016; Chovatiya *et al*., 2011). The result of CSL production reaches 1000 tons/day or 50 tons/day after the changes into CSP (Tereos, 2016). Chovatiya *et al.* (2011) presented that CSL can substitute the fish meal up to 75% in *Labeo rohita* without inhibiting the growth and it can boost the meat quality of *Labeo rohita.* Nowadays, a specific study about the CSP utilization in aquaculture feed has not existed yet.

This study aimed to evaluate CSP utilization as a plant-based protein source in tilapia *Oreochromis niloticus* feed. Tilapia was chosen as the experimental fish because of its economic value and it is considered as the future leading species in aquaculture (Yue *et al*., 2016) with the runner-up worldwide production after carps species (Prabu *et al*., 2019). It is also the most consumed species in Asia, i.e. China, Indonesia, Taiwan, Thailand, and Malaysia (FAO GLOBEFISH, 2017).

MATERIALS AND METHODS

Experimental design

CSP was obtained from PT. Tereos FKS Indonesia, Cilegon, Banten. Moreover, several tests were conducted towards CSP, i.e., proximate analysis, amino acid, acid detergent fiber (ADF), neutral detergent fiber (NDF), aflatoxin, tannin, saponin, calcium, magnesium, potassium, sodium, phosphorus, chloride, and lactic acid test. A complete randomized design was used in this study, consisted of six treatments with four replications each. Cargill commercial feed (protein 29.51%, lipid 5.27%, ash 13.70, crude fiber 6.32%, and energy 368 kcal/g) was used as reference diet. The experimental feeds were CSP feed 0% (CSP0), 10% (CSP10), 20% (CSP20), and 30% (CSP30) and soybean meal feed (SBM) 20% (SBM20) and 30% (SBM30) as comparator.

Experimental feed

The experimental feed was produced using commercial feed as a reference diet. CSP was added with different level, namely 0, 10%, 20%, and 30%. Feed comparator was also made by adding 20% and 30% of SBM (Carvalho *et al*., 2016). The reference diet, which was pellet was crushed and mixed with the other experimental ingredients and Cr_2O_3 0.5 % (Mmanda, 2020) as the digestibility marker. All ingredients were stirred thoroughly and formed to the pellet (re-pelleting). After that, the proximate analysis was conducted towards the experimental diet. The formulation of the diet for the digestibility test was presented in Table 1.

Fish rearing and data collecting

The experimental tilapia initial weight was 6.44 ± 0.29 g. Fish body weight was measured at the beginning and the end of the study. Before rearing, fish was adapted (acclimated) for 7 days. They were reared in 24 aquaria sized in 95×45×45 cm^3 that equipped with a 24-hour aeration system. The stocking density was 15 fish/aquarium. The feeding was delivered three times a day, i.e. 8.00, 12.00, and 16.00 using at satiation feeding method. Feces collecting was started at 4^{th} day after chromium diet feeding (Watanabe, 1988). Feces collection was performed by siphoning an hour after feeding, after that it was placed in a film bottle. The bottle then was stored in -20°C freezer.

Proximate analysis

The proximate analysis consisted of protein, ash, crude fiber, and water content in CSL, CSP, SBM, feed, and the feces. Protein analysis was done using Kjehdal method. Soxhlet method was applied in lipid analysis, while the 600°C furnaces were used to analyze ash. Strong base and strong acid dilution were conducted to analyze crude fiber and water content was analyzed using a furnace at 110°C for 4 hours (AOAC, 2005).

Table 1. Experimental feed formulation for CSP digestibility test.

Ingredient	Diet (%)					
	CSP0	CSP10	CSP20	CSP30	SBM20	SBM30
Commercial feed	99.2	89.2	79.2	69.2	79.2	69.2
CSP	0	10	20	30	0	0
SBM	0	0	0	0	20	30
PMC	0.3	0.3	0.3	0.3	0.3	0.3
Cr_2O_3	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100
Protein	29.67	31.08	32.89	32.86	34.39	36.10
Lipid	4.82	6.41	5.78	5.14	5.75	5.61
Ash	10.64	12.41	13.17	14.15	12.43	11.17
Crude fiber	7.13	6.76	6.62	5.43	5.13	5.65
BETN	47.74	43.34	41.54	42.42	42.30	41.47
GE (kcal/100 g feed)	407.19	411.97	408.80	406.24	420.05	424.90

CSP = corn steep powder; SBM = soybean meal; PMC = polymethylolcarbamide. CSP0 = reference diet; CSP10 = CSP 10%; CSP20 = CSP 20%; CSP30 = CSP 30%; SBM20 = SBM 20%; SBM30 = SBM 30%. BETN= nitrogen-free extract; GE = gross energy.

Amino acid analysis

Amino acid analysis in CSP was done using HPLC (high-performance liquid chromatography), which consisted of 4 steps, i.e., hydrolysate sample making, drying, derivatization, and amino acid injection (AOAC, 2005).

Mineral analysis

The observed parameters in CSP were Ferrum, calcium, magnesium, potassium, sodium, and phosphorus using AOAC (2005). Phosphorus analysis was done using a spectrophotometer in a 660 nm wavelength. Meanwhile, the Ferrum, calcium, magnesium, potassium, and sodium were analyzed using AAS (atomic absorption spectrophotometer) with different wavelengths, i.e. 248.3 nm, 422.7 nm, 285.2 nm, 766.5 nm, 589 nm, respectively.

Digestive enzyme analysis

The digestive enzyme analysis was conducted on day 30. At the end of the rearing, the fish fasted for 24 hours. As many of 5 fish were collected from each container as samples to be dissected. Digestive enzyme activity was measured from the digestive tract by separating those organs with digestion organs, especially the intestine. The intestine was collected and homogenized (40 mg of tissue/mL) in 0.15 M NaCl. The result of the preparation was centrifuged in 10000 ×g for 10 minutes at 4°C. The sample was diluted ten times in aquadest to obtain the crude enzyme extract to be stored in a -20°C freezer until the analysis started. Protease enzyme was measured using casein as a substrate and tyrosine as the standard (Bergmeyer *et al.*, 1983). Lipase activity was analyzed using plant-based oil as the substrate (Linfield *et al.*, 1984). Amylase activity was measured using starch as the substrate and maltose as the standard (Bernfield, 1955). Meanwhile, the pepsin was analyzed using Worthington (1993) method, and trypsin and chymotrypsin was analyzed using a method by Erlanger *et al.* (1961)

Digestibility analysis

The feces were collected 4 days after the experimental feeding (Watanabe, 1988). The Cr_2O_3 measurement in the experimental diet and feces were managed by drying the sample and the absorbent interpretation on spectrophotometer was done using 350 nm of wavelength (Takeuchi, 1988).

The pH measurement of experimental diet and digestion tract

The pH measurement was done by mixing 5 g of experimental diet into 50 mL of aqua dest and stirring the mixture using a magnetic stirrer for 1 minute (Li *et al.*, 2015). The gastric pH measurement was conducted two hours after feeding, while the intestine pH level was measured six hours after feeding (Castillo *et al.*, 2014). The remaining fish diet in the gastric and intestine as many as 5 g was diluted in the 50 mL of aqua dest (Li *et al.*, 2015). The pH level of the diet, gastric, and intestine were measured using a pH meter.

The growth and digestion performance analysis

The digestion performance was calculated based on Takeuchi (1988), including total digestibility, ingredient, protein, lipid, and energy digestibility. The total digestibility was calculated using the total digestibility (%) = [1-a/a'] × 100, with a is the Cr_2O_3 concentration in the experimental diet and a' is the Cr_2O_3 concentration in the feces. The ingredient digestibility was calculated using the formula: ingredient digestibility (%) = [[ADT – 0.7 × AD]/0.3] × 100, with ADT as the total ingredient digestibility and AD as the total digestibility of the reference diet. The protein and lipid digestibility were calculated using the following formula: nutrient digestibility (%) = [1-a/a'× b'/b] × 100, with a is the Cr_2O_3 concentration in the experimental diet, a' is the Cr_2O_3 concentration in the feces, b is the nutrient compound the fish diet, and b' is the nutrient compound in the feces. The digested energy was calculated using the following formula: digested energy = Ep–Ef × [n/n'], with Ep is diet energy, Ef is feces energy, n is the Cr_2O_3 concentration in the experimental diet, and n' is the Cr_2O_3 concentration in the feces. The energy digestibility was calculated through this formula: energy digestibility (%) = [digested energy/energy diet] × 100.

The essential amino acids index (IAAE) was calculated according to Kirimi *et al.* (2020), i.e. IAAE = (aa1/AA1) × (aa2/AA2) × …× (aan/AAn)$^{1/n}$, with aa1 is the essential amino acid in the experimental diet, AA is the essential amino acid required by the fish, and n is the amount of evaluated essential amino acid. The survival rate was calculated by dividing the final population and initial population then changed it into a percentage.

The growth performance was measured at the end of the study (day 30). The measured parameters were feed consumption, specific growth rate (SGR), and feed conversion ratio (FCR). Feed consumption was calculated using: feed consumption (g/fish) = [∑ consumed diet/ ∑ final population]. SGR was calculated using: SGR (%/day) = [Ln Wt – Ln Wo]/T ×100, with Wt is the final average weight, Wo is the initial average weight, and T is the rearing period. FCR was calculated using: FCR = F/[Wt–Wo], Wt is the final fish biomass and Wo is the initial biomass.

Table 2. Proximate composition, antinutritional compound, and mineral in CSP.

	CSP	CSL[a]	SBM[b]
Water content	5.13	22.75	11.92
Protein (%)	40.27	43.48	44.02
Ash (%)	22.92	10.30	6.34
Lipid (%)	0.82	0.10	1.79
Crude fiber (%)	0.30	0	6.26
Total energy (kcal/100g)	296.42	379.00	416.50
Lactic acid (%)	26.10	20.79	2.38[g]
pH	5.62	4.70	-
Starch (%)	<10	-	5.51
Reducing sugar (%)	0.12	2.76	-
Acid detergent fiber (%)	0.22	0	8.76
Neutral detergent fibre (%)	0.52	0	13.05
Antinutrition compound			
Aflatoxin (µg/g)	ND	ND	1.30[c]
Phytate acid (mg/g)	ND	ND	1,16[d]
Tanin (mg/g)	0.03	0.48	1.93[d]
Trypsin inhibitor (TIU/g)	ND	ND	1.20[d]
Saponin (mg/g)	0.92	0.87	0.60[e]
Mineral (%)			
Ferrum (Fe)	0.2	0.02	0.01[f]
Calcium (Ca)	0.05	0.08	0.31[f]
Magnesium (Mg)	2.21	0.58	0.27[f]
Potassium (K)	4.86	2.94	1.99[f]
Sodium (Na)	1.12	-	0.02[f]
Phosphorus (P)	3.15	1.60	-

Note: [a]Chovatiya *et al.* (2011); [b]Eys (2012); [c]Daga *et al.* (2015); [d]Adeyemo & Onilude (2013); [e]Peisker (2001); [f] Ravindran *et al.* (2014). CSL = corn steep liquor, CSP = corn steep powder, SBM = soybean meal.

Data analysis

The survival rate, specific growth rate (SGR), FCR, total digestibility, protein, lipid, energy, and ingredients digestibility, feed pH, digestive tract pH, and enzyme activity were analyzed using one-way ANOVA with a 95% confidence level and were further tested using Duncan's test. Statistical analysis was conducted using SPSS 24.

RESULTS AND DISCUSSION

Result

CSP composition

Proximate composition, mineral content, and antinutritional compound in CSP was shown in Table 2. The CSP had 40.27% protein and antinutritional compound, such as aflatoxin, phytate acid, and trypsin inhibitor, was not found. CSP was relatively acid with lactic acid levels up to 26.10%. CSP contained several minerals, i.e. Ferrum, calcium, magnesium, potassium, sodium, and phosphorus. Amino acid composition in CSP

Table 3. Amino acid and essential amino acid index in CSP and SBM.

Amino acid	CSP	SBM[*a]
Essential amino acid (%)		
Lysine	2.02	2.7
Methionine	0.62	0.68
Histidine	1.91	1.1
Threonine	1.34	1.79
Arginine	0.92	2.82
Leucine	2.66	3.27
Valine	2.23	2.07
Isoleucine	1.16	2.2
Phenylalanine	1.19	1.37
Tryptophan	0.16	0.6
EAA total	14.21	18.6
EAAI	0.90	0.95
Non essential amino acid (%)		
Aspartic acid	1.78	6.27
Serine	1.61	2.25
Glutamic acid	4.4	10.26
Glycine	1.99	1.98
Alanine	3.14	1.98
Cystine	1.07	0.74
Tyrosine	0.68	1.56

Note: CSP=corn steep powder; SBM=soybean meal; EAAI= essential amino acid index; a= amino acid composition data of SBM from IAFFD (2020).

Table 4. Feed pH, gastric pH, and intestine pH of tilapia

Parameter	Treatment					
	CSP0	CSP10	CSP20	CSP30	SBM20	SBM30
Feed pH	5.58 ± 0.03[e]	5.44 ± 0.02[c]	5.37 ± 0.01[b]	5.33 ± 0.00[a]	5.53 ± 0.02[d]	5.72 ± 0.02[f]
Gastric pH	4.67 ± 0.04[c]	4.63 ± 0.13[c]	4.40 ± 0.06[b]	4.25 ± 0.09[a]	4.25 ± 0.02[a]	4.14 ± 0.10[a]
Intestine pH	7.01 ± 0.06[a]	7.17 ± 0.08[c]	7.26 ± 0.06[d]	7.04 ± 0.06[ab]	7.12 ± 0.03[bc]	7.13 ± 0.13[cd]

Note: CSP0=reference diet; CSP10=CSP 10%; CSP20=CSP 20%; CSP30=CSP 30%; SBM20=SBM 20%; SBM30=SBM 30%. Different superscript in the same row indicated significant differences (P<0.05).

Table 5. Digestive enzyme activity in tilapia fed with various corn steep powder content after 30 days of rearing.

Parameter	Treatment					
	CSP0	CSP10	CSP20	CSP30	SBM20	SBM30
Amylase (IU/mL)	2.19 ± 0.15	2.45 ± 0.32	2.64 ± 0.30	2.25 ± 0.13	2.03 ± 0.53	2.33 ± 0.13
Lipase (IU/mL)	0.25 ± 0.01[a]	0.26 ± 0.01[bc]	0.27 ± 0.01[c]	0.24 ± 0.01[a]	0.25 ± 0.01[ab]	0.26 ± 0.01[bc]
Protease (IU/mL)	0.11 ± 0.01[a]	0.13 ± 0.01[b]	0.22 ± 0.01[e]	0.12 ± 0.02[ab]	0.16 ± 0.01[c]	0.19 ± 0.01[d]
Trypsin (IU/mL)	0.03 ± 0.01[b]	0.02 ± 0.00[a]	0.05 ± 0.01[c]	0.03 ± 0.00[b]	0.03 ± 0.00[b]	0.03 ± 0.00[b]
Chymotrypsin (IU/mL)	0.0010 ± 0.00	0.0007 ± 0.00	0.0007 ± 0.00	0.0007 ± 0.00	0.0007 ± 0.00	0.0008 ± 0.00
Pepsin (IU/mL)	0.091 ± 0.00[a]	0.102 ± 0.00[c]	0.123 ± 0.00[d]	0.091 ± 0.00[ab]	0.093 ± 0.00[b]	0.093 ± 0.00[b]
Ratio T: C	36.65 ± 14.10[b]	21.16 ± 1.91[a]	70.50 ± 13.32[c]	38.77 ± 3.84[b]	42.62 ± 10.61[b]	44.79 ± 1.65[b]

Note: CSP0=reference diet; CSP10=CSP 10%; CSP20=CSP 20%; CSP30=CSP 30%; SBM20=SBM 20%; SBM30=SBM 30%. Ratio T: C=trypsin: chymotrypsin ratio; Different superscript in the same row indicated significant differences (P<0.05).

Table 6. Experimental diet digestibility with various CSP content in tilapia.

Parameters	Treatments					
	CSP0	CSP10	CSP20	CSP30	SBM20	SBM30
Total digestibility (%)	67.87 ± 1.90[a]	69.232 ± 0.12[b]	73.43 ± 0.16[d]	69.53 ± 0.30[b]	70.49 ± 0.13[b]	72.16 ± 0.46[c]
Ingredient digestibility (%)	-	81.47 ± 1.16[b]	95.64 ± 0.80[c]	73.40 ± 0.98[a]	80.97 ± 0.63[b]	82.15 ± 1.52[b]
Protein digestibility (%)	85.78 ± 0.87[a]	87.95 ± 0.95[b]	89.17 ± 0.42[c]	85.99 ± 0.87[a]	88.12 ± 0.32[b]	90.62 ± 0.40[d]
Lipid digestibility (%)	81.98 ± 0.54[a]	86.22 ± 0.56[c]	87.98 ± 0.35[d]	83.80 ± 1.29[b]	85.69 ± 0.61[c]	88.04 ± 1.21[d]
Energy digestibility (%)	78.68 ± 1.10[a]	81.17 ± 0.54[b]	84.28 ± 0.35[d]	82.51 ± 0.23[c]	81.20 ± 0.10[b]	84.08 ± 0.51[d]

Note: CSP0=reference diet; CSP10=CSP 10%; CSP20=CSP 20%; CSP30=CSP 30%; SBM20=SBM 20%; SBM30=SBM 30%. Ratio T: C=trypsin: chymotrypsin ratio; Different superscript in the same row indicated significant differences (P<0.05).

and SBM were presented in Table 3. Valine and histidine in CSP were higher than SBM. IAAE level in CSP was lower than SBM.

Digestion activity

Feed, intestine, and gastric pH levels were presented below in Table 4. The pH level in the experimental feed showed a higher result in CSP0 and SBM compared to CSP10, CSP20, and CSP30 (P<0.05). The lowest pH level in the tilapia gastric was found in CSP30 (P<0.05). On the contrary, the CSP20 treatment presented a higher pH level than the other treatments (P<0.05).

Amylase, lipase, protease, pepsin, chymotrypsin, and trypsin activity were described in Table 5. It was shown that protease, trypsin, and pepsin activity in CSP20 treatment was higher than the others (P<0.05). Meanwhile, the amylase and chymotrypsin did not show a significant difference amongst treatments (P>0.05).

The diet/feed digestibility consisted of total, protein, lipid, and energy digestibility. Those existed in Table 6. The total digestibility was higher in CSP and SBM treatment compared to the control. The CSP20 resulted in the highest ingredients and total digestibility compared with the other treatments (P<0.05). Lipid and energy digestibility in the CSP20 was the same as in the SBM30 (P>0.05), but higher than the CSP0, CSP10, CSP30, and SBM20 (P<0.05).

Growth performance

Feed consumption increased along with the CSP existence in the experimental diet. The CSP30 presented a higher feed consumption than the other treatments (P<0.05) (Table 7). The final weight and SGR in the CSP20 were higher than the rest of the treatments (P<0.05). The survival rate (SR) in all treatments exhibited a similar result, i.e. 100% (P<0.05).

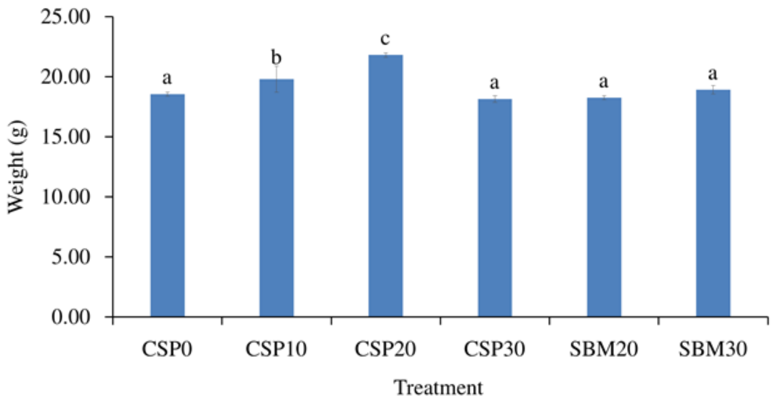

Figure 1. The final weight of tilapia *Oreochromis niloticus* during the rearing period. Different superscript in the same row indicated significant differences (P<0.05).

Table 7. Growth performance of Nile tilapia fed with different corn steep powder content.

Parameters	Treatments					
	CSP0	CSP10	CSP20	CSP30	SBM20	SBM30
Initial weight (g)	6.50 ± 0.08^{b}	6.43 ± 0.08^{b}	6.42 ± 0,04^{ab}	6.50 ± 0.04^{b}	6.36 ± 0.03^{a}	6.43 ± 0.04^{ab}
Final weight (g)	18.56 ± 0.16^{a}	19.82 ± 1.08^{b}	21.83 ± 0.18^{c}	18.16 ± 0.28^{a}	18.26 ± 0.17^{a}	18.92 ± 0.35^{a}
Feed consumption (g)	248 ± 13.76^{a}	249 ± 6.05^{a}	259 ± 7.96^{ab}	276 ± 6.99^{c}	264 ± 1.66^{b}	258 ± 4.23^{ab}
Individual feed consumption (g/fish)	16.59 ± 0.92^{a}	16.63 ± 0.40^{a}	17.27 ± 0.53^{ab}	18.42 ± 0.47^{c}	17.65 ± 0.11^{b}	17.22 ± 0.28^{ab}
FCR	1.39 ± 0.05^{cd}	1.25 ± 0.13^{b}	1.12 ± 0.04^{a}	1.58 ± 0.05^{e}	1.48 ± 0.02^{de}	1.38 ± 0.02^{c}
SGR (%/day)	3.50 ± 0.07^{ab}	3.63 ± 0.08^{d}	3.71 ± 0.04^{e}	3.43 ± 0.04^{a}	3.52 ± 0.01^{bc}	3.60 ± 0.07^{cd}
SR (%)	100	100	100	100	100	100

Note: CSP0=reference diet; CSP10=CSP 10%; CSP20=CSP 20%; CSP30=CSP 30%; SBM20=SBM 20%; SBM30=SBM 30%. FCR= feed conversion ratio; SGR= specific growth rate; SR = survival rate; Different superscript in the same row indicated significant differences (P<0.05).

Discussion

The protein content in CSP was lower than CSL and SBM, but it was higher than the other plant-based protein from agro-industrial by-products, such as DDGS (distillers dried grains with solubles) 28.90–31.30 % (Magalhaes *et al*., 2015; Welker *et al*., 2014), corn gluten feed 21.40–28.70 % (IAFFD, 2020), oilseed meal 17.81% (Obirikorang *et al*., 2015), rubber seed meal 25.40% (Sharma *et al*., 2014), and raw kapok *Ceiba pentandra* seed meal 20–35 % (Wafar *et al*., 2017). CSP contains a lower level of acid detergent fiber (ADF), neutral detergent fiber (NDF), and crude fiber compared to the SBM. Crude fiber is still needed, although it is hardly digested. The low level of crude fiber increased peristaltic movement, thus the digestion process and chemical degradation rate of the diet increased as well (Pandey, 2013; Manullang *et al.*, 2018). The crude fiber level in the tested diet was lower than 15.5%, but it still supported the growth and health performance of the fish (Bonvini *et al*., 2018b). The essential amino acids in CSP showed that this particular ingredient was applicable as a protein source for tilapia. A feed ingredient is categorized feasible as a protein source if it hit 0.9 of the essential amino acid indices. A 0.8 level will be classified as a beneficial protein source and 0.7 will be considered as incomplete (Bunda *et al*., 2015).

A plant-based protein source exploitation as an alternative ingredient of fish diet frequently obstructed by the antinutritional compound. An excessive level of the antinutritional compound will directly affect nutrient intake and digestibility (Bandara, 2018). CSP is an antinutritional-free compound, such as phytate acid, aflatoxin, and trypsin inhibitor. The low level of the antinutritional compound in CSP indicates a fermentation activity by *Lactobacillus* sp. during the submersion process. Lactic acid fermentation can reduce phytate acid, thus the antinutritional compound in CSP is low (Roger *et al*., 2015; Rahimi *et al*., 2017).

CSP addition in fish diet influenced feed, gastric, and intestinal pH differently related to the lactic acid existence in CSP. CSP contains lactic acid higher than CSL. Organic acid content, especially lactic acid, arises from the corn fermentation by lactic acid bacteria in the corn starch industry (Ni *et al*., 2017). Lactic acid is reported to be involved in the glycolytic process

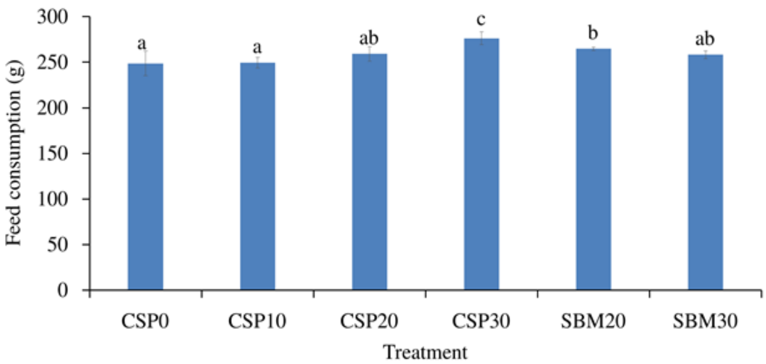

Figure 2. Feed consumption of tilapia *Oreochromis niloticus* during the rearing period. Different superscript in the same row indicated significant differences (P<0.05).

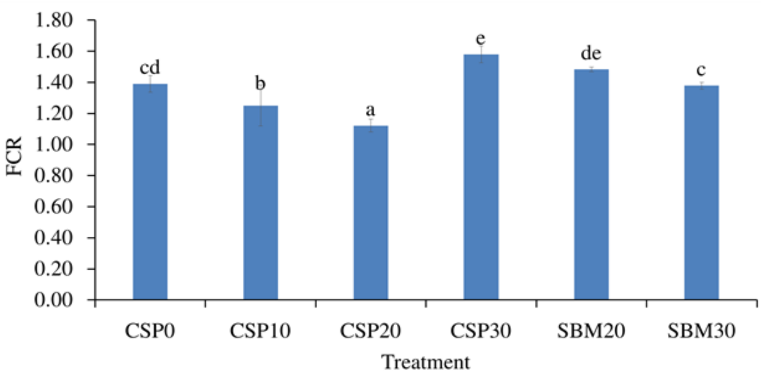

Figure 3. Feed convertion ratio (FCR) of tilapia *Oreochromis niloticus* during the rearing period. Different superscript in the same row indicated significant differences (P<0.05).

because it is known that the quality and quantity of the carbohydrate impacted glycolytic enzyme, such as hexokinase on fish species (Sun *et al*., 2017). The organic acid's role in the digestion tract involved two mechanisms, i.e. decreasing pH level in the gastric, especially in the small intestine, through H^+ transfer and inhibiting Gram-negative bacteria growth through acid dissociation and anion production in the bacteria cell (Castillo *et al*., 2014; Hamidah *et al.*, 2019). Lactic acid bacteria in the fish intestine have a certain mechanism to balance the microbe population. Thus, the digestibility will be increased. It is managed by converting the carbohydrate through an enzymatic reaction into lactic acid that can lower pH levels (Setyawan *et al.*, 2014). It is in line with Castillo *et al*. (2014) who stated that the citric acid administration can reduce pH levels in the gastric from 4.48 to 4.37.

The enzyme activity in the digestive tract is an indicator of digestibility and the ability to utilize the nutrient in the fi'ssh diet. This study showed that CSP0, CSP10, and CSP20 could not inhibit the protease enzyme activity. It was supported by a former study on CSL which reported that protease enzyme activity increased up to 50% of CSL (Chovatiya *et al*., 2011). In addition, trypsin inhibitor is not found in the CSP, because of the existence of antinutritional factor (ANF) which can block the trypsin activity (Lima *et al*., 2019). The acid environment in the gastric is related to the higher activity of pepsin that contributes to boosting protein digestibility (Marquez *et al*., 2012). Several studies used trypsin and chymotrypsin ratio (T: C) as a growth indicator (Rungruangsak-Torrissen & Monoonpong, 2019; Chamchuen *et al*., 2014). In this study, the highest ratio of trypsin and chymotrypsin was in CSP20 treatment and it correlated to the increase of growth performance. Trypsin and chymotrypsin are major digestion enzymes secreted in the anterior part of the intestine. Trypsin hydrolyzes the peptide bond in the carboxyl side of amino acid. Meanwhile, chymotrypsin hydrolyzes the peptide bond in the aromatic amino acid (Moraes & Almeida, 2020). The impact of the endopeptidase activity combination generates a micro protein and peptide fragments on the intestine. Furthermore, hydrolysis is conducted by the exopeptidase secreted by the pancreatic and enterocyte apical membrane. Peptidase in the enterocyte membrane contributes to the final

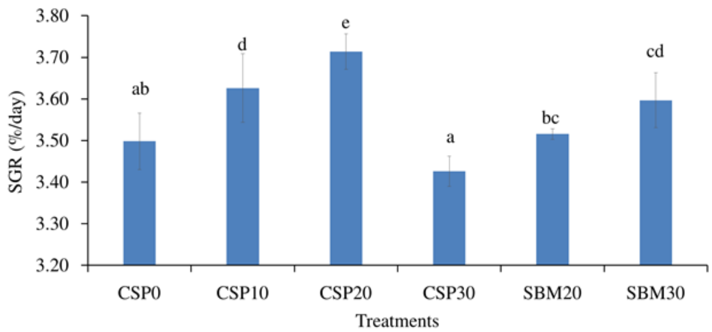

Figure 4. Specific growth rate (SGR) of tilapia *Oreochromis niloticus* during the rearing period. Different superscript in the same row indicated significant differences (P<0.05).

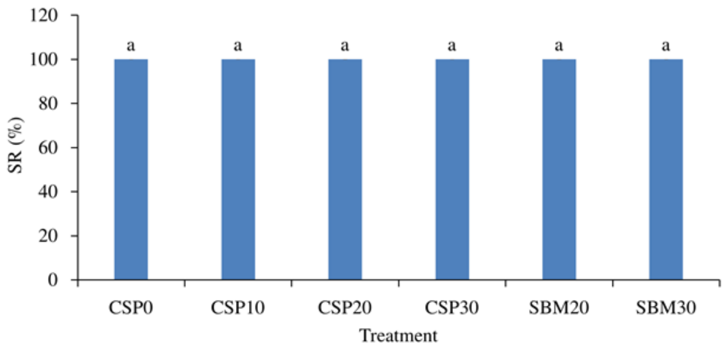

Figure 5. Survival rate (SR) of tilapia *Oreochromis niloticus* during the rearing period. Different superscript in the same row indicated significant differences (P<0.05).

result of the digestion, in form of micropeptide and free amino acid that is easily digested by the intestine. A higher T: C ratio is frequently related to the essential amino acid absorption for protein synthesis and growth performance (Ronnestad *et al*., 2013). Trypsin and chymotrypsin can be used as nutrition indicator of an organism because it represents proteolytic enzyme activity in protein synthesis as a major component in fish diet (Liu *et al.*, 2011; Yulintine *et al.*, 2012).

Digestibility describes the quantity of digestible and absorbable nutrients to support metabolism and growth. A certain ingredient digestibility is influenced by the processing method, life phase, ingredient quality, diet size, and fish activity (Suprayudi *et al*., 2012). Protein digestibility increases along with the CSP20 dosage, but it decreases on CSP30 treatment. It is supported by Chovatiya (2011) who stated that the CSL administration in 25–75% resulted from protein digestibility 89.10–90.87%. The high percentage of protein digestibility could be caused by the low crude fiber content and a high protease enzyme activity which degrades protein so that it facilitates easier peristaltic movement and nutrient absorption (Suprayudi *et al*., 2012; Manullang *et al.*, 2018). Nonetheless, protein digestibility in all treatments was in a tolerable range of around 75–95% (NRC, 2011).

The CSP addition of an inclusive level of 30% in the fish diet reduces lipid digestibility. It was assumed that the saponin compound in the CSP was higher than in the SBM. SBM addition in fish diet could lower the lipid digestibility in greater amberjack *Seriola dumerili* juvenile (Dawood *et al*., 2015). Saponin content in the CSP inhibits bile salt absorption. It is matched with the study by Krogdahl *et al*. (2015) who stated that plant-based ingredients with fiber, phytosterol, phytoestrogen, and saponin compound impacted cholesterol and bile salt absorption. Bile salt takes place indigestion and lipid digestibility. When the bile salt synthesis went lower, the lipid digestibility decreased as well. Bile salt also works in cholesterol and toxin excretion process, such as bilirubin (Romano *et al.*, 2020).

The energy digestibility in CSP20 was higher than CSP0, CSP10, and CSP30. The increase of energy is related to the fish's ability to utilize nonprotein energy sources, such as carbohydrates and lipids. The energy digestibility in this study was higher than the standard determined by Halver (1989), who stated that the energy digestibility from seeds or plant-based ingredients was 70%, whereas animal-based ingredients obtained 85% of energy digestibility. The elevating of feed digestibility was caused by a low crude fiber, high protein, and decrease of the antinutritional compound. Environmental factors, i.e. temperature, dissolved oxygen, and stock density, and the macronutrient in fish diet (Schrama *et al*., 2012; Haidar *et al*., 2016; Phan *et al*., 2019; Schrama *et al*., 2018) impacted energy digestibility.

Feed consumption is directly related to energy digestibility because fish eat to fulfill their energy requirement. A low energy digestibility in the fish diet showed a low energy utilization by the fish. The increase of feed consumption in CSP30 is a response towards various CSP levels in the fish diet. Feed conversion showed efficiency in feed utilization in fish growth. A lower feed conversion indicates efficient feed utilization (Fry *et al*., 2018). The CSP20 had a lower feed conversion compared to the CSP0, CSP10, CSP30, SBM20, and SBM30. It was implied that the experimental diet in CSP20 was easier to digest compared to the other treatments so that the nutrient could be utilized and digested efficiently. The feed conversion in CSP30 was higher than the other treatments. It was assumed that the experimental diet in CSP30 was barely digested and it led to the low nutrient absorption that would be used in daily metabolism. A similar case was also reported by Chovatiya *et al*. (2011) who explained that the increased dosage of CSL could lower the feed conversion in Roho labeo *Labeo rohita.* Lactic acids compound in CSP was assumed to have a certain effect on fish growth. It is in line with Castillo *et al*. (2014) who stated that organic acids' existence in the fish diet boost growth performance in red drum *Sciaenops ocellatus* juvenile because of the pepsin, trypsin, lipase, and amylase compound in the diet. A similar result was shown by Chovatiya *et al*. (2011) who applied CSL which contains 20.79% lactic acid and it could elevate several enzyme activities, such as protease, amylase, and lipase. The overall result stated that CSP could be utilized as a plant-based feed ingredient because of its ability to boost growth performance in tilapia.

CONCLUSION

CSP is a derivative compound from the corn starch industry with 40.27% of protein, 26.1% of lactic acid, and it is free from antinutritional compounds, such as aflatoxin, phytate acid, and trypsin inhibitor. The essential amino acid index presented that CSP is a qualified plant-based feed ingredient. Therefore, CSP could be an alternative plant-based ingredient of the fish diet because of its ability to elevate enzymatic activity, feed digestibility, and growth performance in tilapia. At amount of 20% CSP inclusion was recommended in formulating the tilapia diet.

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