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Evaluation of corn steep liquor on the digestibility and growth performance of juvenile common carp strain Sinyonya

Evaluasi corn steep liquor terhadap kinerja kecernaan dan pertumbuhan juvenil ikan mas Sinyonya

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ABSTRACT

This study aimed to observe the digestibility performance, digestive enzyme activity, and growth performance of the common carp using corn-steep liquor (CSL). The reference diet used commercial feed with 30% protein, and the test diet used CSL at 0% (CSL0), 10% (CSL10), 20% (CSL20), and 30% (CSL30). Common carp (9.38 ± 0.73 g) were stocked at 10 fish per aquarium. Feeding was performed until apparent satiation thrice daily for the 45-day maintenance period. The study used a completely randomized design with four treatments and four replications. The results showed that CSL contained 44.04% protein and an essential amino acid index of 0.80. The additional dose of CSL reduced feed pH and stomach pH compared to the control due to the lactic acid content. In addition, CSL elevated the activity of protease, amylase, and lipase enzymes in the digestive tract of common carp. By applying 20% dose in formulated diet, CSL could increase the total digestibility, ingredient digestibility, protein digestibility, lipid digestibility, and energy digestibility. The high feed digestibility value in the CSL20 treatment was due to the fish ability to utilize carbohydrates and fats as non-protein energy sources, thus producing higher final weight, daily growth rate, and feed conversion ratio. The results of this study indicate that CSL can be used as a source of plant protein for the common carp diet, whereas an administration of CSL at 20% in diet improves the digestibility performance, digestive enzyme activity, and growth performance of common carp.

Keywords: common carp, corn steep liquor, digestibility, enzyme, growth

ABSTRAK

Tujuan penelitian ini untuk melihat kinerja kecernaan, aktivitas enzim pencernaan dan pertumbuhan ikan mas Sinyonya (*Cyprinus carpio*) menggunakan bahan baku *corn steep liquor* (CSL) sebagai sumber protein nabati. Pakan acuan menggunakan pakan komersial dengan protein 30% dan pakan uji menggunakan CSL sebanyak 0% (CSL0), 10% (CSL10), 20% (CSL20) dan 30% (CSL30). Pengukuran kecernaan menggunakan indikator Cr_2O_3 0,5 %. Ikan mas Sinyonya dengan bobot tubuh rata-rata 9,38 ± 0,73 g dipelihara dalam akuarium 60×50×40 cm^3 dengan kepadatan 10 ekor per akuarium. Pakan diberikan secara *at satiation* sebanyak 3 kali sehari selama 45 hari masa pemeliharaan. Penelitian menggunakan desain rancangan acak lengkap dengan empat perlakuan dan masing-masing empat ulangan. Hasil penelitian menunjukkan bahwa CSL mengandung protein sebesar 44,04%, dan indeks asam amino essensial 0,80. Penambahan dosis CSL secara signifikan menurunkan pH pakan dan pH lambung dibandingkan dengan kontrol karena kandungan asam laktat. Sampai dengan dosis CSL 20%, dapat meningkatkan aktivitas enzim protease, amilase dan lipase pada saluran pencernaan ikan mas Sinyonya. Di samping itu, penambahan CSL 20% meningkatkan nilai kecernaan total, kecernaan bahan, kecernaan lemak dan kecernaan energi. Nilai kecernaan pakan yang tinggi pada perlakuan CSL20 disebabkan karena kemampuan ikan dalam memanfaatkan karbohidrat dan lemak sebagai sumber energi non-protein. Disamping itu penambahan CSL sampai dengan dosis 20% meningkatkan bobot akhir, laju pertumbuhan harian dan rasio konversi pakan. Hasil penelitian ini menunjukkan bahwa CSL dapat digunakan sebagai sumber protein nabati untuk pakan ikan mas Sinyonya dan pemberian CSL pada level 20% dapat meningkatkan kinerja kecernaan, aktivitas enzim pencernaan dan pertumbuhan ikan.

Kata kunci: *corn steep liquor*, enzim, ikan mas Sinyonya, kecernaan, pertumbuhan

INTRODUCTION

Common carp (*Cyprinus carpio*) is one of the economically important cultured fish commodities in Indonesia. In 2015 to 2020, the common carp production significantly contributed to Indonesia's fisheries production, with a growth rate of 9.60%. In 2020, the production reached 560,670 tons (KKP, 2022), which included the common carp *strain Sinyonya*. The common carp *strain Sinyonya* is an excellent fish commodity in Banten Province due to high economical value. However, producing the common carp *strain Sinyonya* has drawbacks in fish seed availability and cheap protein ingredients for aquafeed requirements to support their growth.

The development of fish culture business increases the fish demand and feed ingredient from protein source. The combined data from livestock feed entrepreneurs (GMPT) shows an increased feed production in 2008-2016 by 65%, with a total production of 1.63 million tons in 2016. The feed dependency poses a high risk to aquaculture business sustainability, whereas approximately 91.2% of the production costs for common carp culture are absorbed by feed cost (Suprayudi, 2018). To ensure sustainable aquafeed production, alternative feed ingredients are essential as protein source that composes appropriate nutrient contents for fish requirement with adequate quantities and availability.

Potential aquafeed ingredient that can be developed further comes from waste or agroindustrial by-product to regulate efficient resource utilization and support sustainable aquaculture practices (Caruso, 2015). NRC (1993) recommended the protein requirement for common carp based on age size, namely 43-47% for seeds, 37-42% for fingerlings and sub-adults, and 28-32% for adults and broodstocks. To fulfill the protein requirement, several studies have shown that plant-based materials can be used as a protein source in formulated diet for fish (Magbanua & Ragaza, 2024). The agroindustrial by-products that have been applied as plant-based protein aquafeed ingredient are palm kernel meal (Wattanakul *et al*., 2021), dried distiller grains with solubles (Chatvijitkul *et al*., 2016), corn gluten meal (Bu *et al*., 2018), corn steep liquor (Sukhanandi & Bhatt, 2016), and corn steep powder (CSP) (Hermawan *et al*., 2021).

According to Wattanakul *et al*. (2021), replacing dehulled soybean meal with fermented palm kernel meal by 50% could improve the growth performance, feed utilization, and digestive enzyme activities. Chatvijitkul *et al*. (2016) reported that the application of dried distiller's grains with solubles at 300 g/kg feed could regulate the growth performance of hybrid Nile tilapia. Hermawan *et al*. (2021) also stated that the application of CSP at 20% inclusion in formulated diet could improve the digestive enzyme, digestibility, and growth performance of fish. Another potential aquafeed ingredient that has never optimized as plant-based protein ingredient is corn steep liquor (CSL). CSL or corn steep water is a wet milling by-product of corn starch production formed as a brown viscous liquid that contains protein, minerals, vitamins, reducing sugars, organic acids, and enzymes (Tan *et al*., 2016).

CSL is highly available as the production could reach 1000 tons/day (Tereos, 2016). Due to relatively high protein and energy contents with low fiber content, CSL is often utilized as potential feed for ruminants (Jiriaei *et al*., 2020), while minerals and vitamin B in CSL can also be used as a feed binder (Azizi-Shotorkhoft *et al*., 2016). Chovatiya *et al*. (2011) showed that CSL could replace fish meal by 75% in formulated diet for *Labeo rohita* without inhibiting the growth, thus improving the meat nutrient contents, such as protein and lipid. The utilization of plant-based protein material as an aquafeed ingredient often has problems dealing with the anti-nutritional properties and imbalance amino acid profiles.

These anti-nutritional properties tend to disrupt the digestive function, nutrient metabolism, and fish production (Omnes *et al*., 2017). However, CSL is free from phytic acid, aflatoxin, and tripsin-inhibitor. Low anti-nutritional properties in CSL is due to lactic acid fermentation by *Lactobacillus* sp. during the soaking process in corn wet-milling to produce corn starch, thus reducing the phytic acid content (Rahimi *et al*., 2017). Nevertheless, there have been no studies related to the application of CSL as an aquafeed ingredient in common carp *strain Sinyonya*. Therefore, this study investigated CSL as plant-based protein ingredient to improve digestibility, digestive enzyme activities, and growth performance.

MATERIALS AND METHOD

Study design

This study used a completely randomized design with four treatments and four replications in each treatment. The CSL was obtained from

PT Tereos FKS Indonesia, Cilegon, Banten. The composition of CSL was also determined, including nutrient contents (proximate analysis), amino acids, acid detergent fiber (ADF), neutral detergent fiber (NDF), aflatoxin, tannins, saponins, trypsin-inhibitor, and lactic acid.

Diet formulation

The diet was formulated using the commercial feed as a reference diet with 32% protein content. Test diets were formulated with different CSL concentrations at 0, 10, 20, and 30% (Carvalho *et al*., 2016). All diets were produced by grinding the commercial feed, adding different concentrations of test ingredients and Cr_2O_3 at 0.5 % as a digestibility test marker (Mmanda *et al*., 2020). All ingredients were mixed and formed as pellet (re-pelleting method). All diets were analyzed their nutrient contents through proximate analysis. The diet formulation for digestibility test is presented in Table 1.

Fish rearing and data collection

Common carp *strain Sinyonya* as objects in this study were weighed at averagely of 9.38 ± 0.73 g. Before being reared for 45 days, acclimatization process was performed for a week. Fish were reared in 16 units of aquarium at 60×50×40 cm^3 volume equipped with aeration system for 24 hours. Fish were stocked at 10 fish per aquarium. Before feeding, fish were fasted for 24 hours. Fish weight was measured on the initial and final rearing period.

The diets were fed three times a day at 08.00, 12.00, and 16.00 WIB (GMT+7) until apparent satiation. Fish feces were collected after four days of feeding (Watanabe, 1988). Feces were collected in a bottle by siphoning an hour after feeding. Collected feces was kept in a freezer at -20°C.

Proximate analysis

Proximate analysis performed in this study was composed of protein, ash, crude fiber, and moisture contents of CSL, diets, and feces. Protein content was determined using the Kjeldahl method. Soxhlet method and furnace-heating at 600°C were applied to determine lipid and ash contents. Meanwhile, samples dissolved in strong acid-base were performed to measure crude fiber and moisture content was determined through oven-heating at 110°C for 4 hours (AOAC, 2012). As comparative data, the corn steep powder (CSP) proximate analysis results by Hermawan *et al*. (2021) are also presented.

Amino acid analysis

Amino acid analysis of CSL used the HPLC (high performance liquid chromatography) method that contained four steps, namely sample hydrolysate production, drying, derivatization, and amino acid injection (AOAC, 2012). As comparative data, the amino acid analysis results of soybean meal (SBM) by IAAFD (2023) are also presented.

Table 1. Diet formulation and proximate composition.

Ingredient	Diet (%)			
	CSL0	CSL10	CSL20	CSL30
Commercial feed	99.2	89.2	79.2	69.2
CSP	0	10	20	30
PMC	0.3	0.3	0.3	0.3
Cr_2O_3	0.5	0.5	0.5	0.5
Total	100	100	100	100
Protein	32.32	31.51	31.02	31.30
Lipid	5.24	5.52	5.47	5.79
Ash	11.16	11.52	10.60	11.73
Crude fiber	4.93	5.96	5.89	6.15
NFE	40.81	38.74	41.29	39.05
GE (kcal/100 g diet)	416.88	410.20	414.40	410.28

Note: CSL = corn steep liquor; PMC = polymethylolcarbamide. CSL0 = reference diet; CSL10 = CSL 10%; CSL20 = CSL 20%; CSL30 = CSL 30%. NFE = nitrogen-free extract; GE = gross energy.

Digestive enzyme analysis

The digestive enzyme activity was measured on the 45th day of rearing period. During the final day of rearing period, fish were fasted for 24 hours, and three fish were taken from each aquarium. Fish were sedated with Tricaine methane sulfonate (*MS-222*, *Syndel, USA*) at 0.15 g/L (Al-Taee *et al*., 2021). The digestive enzyme activity from the fish digestive tract was determined by separating the digestive organ from the fish body. The intestine was collected and homogenized in 0.15 M NaCl (40 mg tissue/mL). The sample was centrifuged at 10,000 ×g for 10 minutes at 4°C. The sample was dissolved in distilled water 10 times to obtain crude enzyme extract kept in -20°C for further enzyme activity analysis. The protease enzyme was measured using casein as a substrate and tyrosine as a standard (Bergmeyer *et al*., 1983). Lipase enzyme was measured using plant-based oil as a substrate (Linfield *et al*., 1984). Amylase enzyme was determined using starch as a substrate and maltose as a standard (Bernfield, 1955).

Digestibility analysis

Feces were collected after four days of feeding the diets (Watanabe, 1988). The Cr_2O_3 contents in diets and feces were performed by drying and absorbance reading in a spectrophotometer at 350 nm wavelength (Takeuchi, 1988).

pH diets and digestive tract

The pH level was measured by mixing 5 g of diet sample in 50 mL of distilled water until homogenous with magnetic stirrer for a minute (Li *et al*., 2015). The pH level in stomach was measured after 2 hours of feeding and the pH level in the intestine was measured after 6 hours of feeding (Castillo *et al*., 2014). The stomach and intestine contents at 5 g each were dissolved in 50 mL of distilled water (Li *et al*., 2015). The pH levels of diets, stomach, and intestine were measured with pH meter.

Digestibility and growth performances

Digestibility performance was determined based on Takeuchi (1988), namely total digestibility, ingredient digestibility, protein digestibility, lipid digestibility, and energy digestibility. Total digestibility was measured using the formula:

$$\text{Total digestibility (\%)} = [1 - a/a'] \times 100$$

Note:
a = Cr_2O_3 concentration in diet
a' = Cr_2O_3 concentration in feces

Ingredient digestibility was measured with the formula:

$$\text{Ingredient digestibility (\%)} = [[ADT - 0.7 \times AD]/0.3] \times 100$$

Note:
ADT = Apparent (total) digestibility of test diet
AD = Apparent (total) digestibility of reference diet

The protein and lipid digestibility levels were determined with the formula:

$$\text{Nutrient digestibility (\%)} = [1 - a/a' \times b'/b] \times 100$$

Note:
a = Cr_2O_3 concentration in diets
a' = Cr_2O_3 concentration in feces
b = nutrient content in diets
b' = nutrient content in feces

Digestible energy was measured with the formula:

$$\text{Digestible energy} = Ep - Ef \times [n/n']$$

Note:
Ep = Diet energy
Ef = Fecal energy
N = Cr_2O_3 concentration in diet
n' = Cr_2O_3 concentration in feces

Energy digestibility was calculated with the formula:

$$\text{Energy digestibility (\%)} = \frac{[\text{digestible energy /}}{\text{diet energy]}} \times 100$$

Essential Amino Acid Index (EAAI) was calculated based on Kirimi *et al*. (2020):

$$EAAI = (aa1/AA1) \times (aa2/AA2) \times \ldots \times (aan/Aan)^{1/n}$$

Note:
aa1 = Essential amino acid in the ingredient
AA = Essential amino acid requirement
n = Total evaluated amino acids

Survival rate (SR) was determined at the final rearing period with the formula:

$$SR = \frac{\text{total fish in final rearing period}}{\text{total fish in initial rearing period}} \times 100$$

The growth performance of the fish was determined on the final rearing period at 45th day. Parameters for growth performance were composed of feed intake (FI), specific growth rate (SGR), and feed conversion ratio (FCR). Feed intake was measured with:

$$FI\ (g/fish) = \frac{\sum \text{consumed diet}}{\sum \text{total fish in final rearing period}}$$

Specific growth rate (SGR) was calculated using the formula:

$$SGR\ (\%/\ day) = \frac{\ln W_t - \ln W_0}{t} \times 100$$

Note:
Wt = Average fish weight in final rearing period
Wo = Average fish weight in initial rearing period
T = Rearing period

Feed conversion ratio (FCR) was calculated with the formula:

$$FCR = \frac{F}{[Wt - Wo]}$$

Note:
F = Feed intake
Wt = Fish biomass in final rearing period
Wo = Fish biomass in initial rearing period

Data analysis

The SR, SGR, FCR, total digestibility, ingredient digestibility, protein digestibility, lipid digestibility, energy digestibility, pH diet, pH digestive tract, and digestive enzyme activities were all analyzed using a one-way ANOVA at 95% confidence level and continued with the Duncan's test. All statistical analyses used SPSS 24.0 software.

RESULTS AND DISCUSSION

Results

CSL Composition

The proximate analysis and anti-nutritional properties of CSL are presented in Table 2. The protein content in CSL is 44.40%. The total energy

Table 2. Proximate analysis and anti-nutritional properties of CSL.

Proximate Analysis	CSL	CSP[a]
Moisture	49.10	5.13
Protein (%)	44.40	40.27
Ash (%)	15.82	22.92
Lipid (%)	1.79	0.82
Crude fiber (%)	0	0.30
Total energy (kcal/100g)	421.23	296.42
Lactic acid (%)	20.36	26.10
pH	4.50	5.62
Acid detergent fiber (%)	0	0.22
Neutral detergent fiber (%)	0	0.52
Anti-nutritional properties		
Aflatoxin (µg/g)	ND	ND
Phytic acid (mg/g)	ND	ND
Tannins (mg/g)	0.38	0.03
Trypsin inhibitor (TIU/g)	ND	ND
Saponins (mg/g)	0.76	0.92

Note: [a]Hermawan *et al.* (2021). CSL = corn steep liquor and CSP = corn steep powder.

in CSL is twice higher than CSP. Nevertheless, the CSL contains higher tannins and lower saponins than CSP. The pH level of CSL is acidic with the lactic acid content of 20.36%.

The amino acid composition of CSL is presented in Table 3. The EAAI of CSL is 0.8 and lower than soybean meal (SBM). The analysis results indicate that CSL has no cysteine amino acid and generally has lower amino acid contents than SBM, except tyrosine amino acid.

Growth performance

Growth performance of common carp *strain Sinyonya* is presented in Table 4. The results showed that the growth performance of CSL20 treatment was higher than other treatments

Table 3. Amino acid and essential amino acid index of CSL and SBM.

Amino Acids	CSL	SBM[*a]
Essential amino acid (%)		
Lysinet	0.54	2.7
Methionine	0.20	0.68
Histidine	0.35	1.1
Threonine	0.19	1.79
Arginine	0.03	2.82
Leucine	1.06	3.27
Valin	0.56	2.07
Isoleucine	0.22	2.20
Phenylalanine	0.46	1.37
Tryptophan	0.07	0.60
Total EAA	3.68	18.60
EAAI	0.80	0.95
Non-essential Amino acid (%)		
Aspartic acid	0.67	6.27
Serine	0.45	2.25
Glutamic acid	0.68	10.26
Glycine	0.23	1.98
Alanine	1.35	1.98
Cystine	-	0.74
Tyrosine	3.39	1.56

Note: CSL = corn steep liquor; SBM = soybean meal; EAA = essential amino acid; EAAI = essential amino acid index; a = amino acid composition of SBM by IAFFD (2023).

Table 4. Growth performance of common carp *strain Sinyonya* on different corn steep liquor concentrations.

Parameter	Treatment			
	CSL0	CSL10	CSL20	CSL30
Initial weight (g)	9.30 ± 0.18[a]	9.47 ± 0.20[a]	9.20 ± 0.13[a]	9.54 ± 0.15[a]
Final weight (g)	24.28 ± 0.30[a]	26.53 ± 0.20[b]	30.72 ± 0.95[c]	23.34 ± 0.41[a]
FI (g/fish)	241 ± 6.26[a]	251 ± 5.02[ab]	265 ± 8.38[c]	257 ± 5.68[bc]
FCR	1.61 ± 0.04[c]	1.47 ± 0.03[b]	1.23 ± 0.05[a]	1.87 ± 0.09[d]
SGR (%/hari)	2.13 ± 0.04[b]	2.29 ± 0.03[c]	2.69 ± 0.04[d]	1.99 ± 0.04[a]
SR (%)	100	100	100	100

Note: CSL0 = reference diet; CSL10 = CSL 10%; CSL20 = CSL 20%; CSL30 = CSL 30%. FI = feed intake; FCR = feed conversion ratio; SGR = specific growth rate; SR = survival rate. Different superscript letters behind the average value (± SD) on the same line present a significant difference (P<0.05).

(P<0.05). The FI, FCR, and SGR data increased by CSL20, then decreased along with the elevated dosage of CSL.

Digestive performance

5Data on pH levels of diet, intestine, and stomach are presented in Table 5. The results showed that the pH levels of diet and stomach significantly decreased on the CSL20 and CSL30 treatments (P<0.05). However, the application of CSL had no significant difference on pH level of intestine (P>0.05).

The enzymatic activities of protease, lipase, and amylase are presented in Table 6. The results showed that protease, lipase, and amylase activities significantly increased by CSL20, then decreased along with the elevated dosage of CSL (P<0.05).

Digestibility levels, namely total, ingredient, protein, lipid, and energy digestibility are presented in Table 7. The results showed that the CSL20 treatment obtained the highest total, ingredient, lipid, and energy digestibility compared to other treatments (P<0.05). Digestibility increased due to the application of CSL, but then decreased after applying CSL20 treatment.

Discussion

The results present that the CSL protein content is 44.04%, which was similar to Azizi-Shotorkhoft *et al*. (2016) and Ullah *et al*. (2018) at 40–48%. The protein content in CSL is higher than other plant-based protein sources from agro-industrial by-products, such as corn steep powder (Hermawan *et al*., 2021), DDGS (distillers dried grains with solubles) at 26-28% (Abouel *et al*., 2021; Allam *et al*., 2020), rubber seeds at 21.6% (Yusuf *et al*., 2016) and palm oil meal at 17.93% (Sangavi *et al*., 2020). The essential amino acid index of CSL (0.8) is lower than CSP, but can still be categorized as a raw material with a good quality protein source as a source of plant-based protein ingredients for aquafeed (Bunda *et al*., 2015).

Table 5. pH levels of diet, stomach, intestine in common carp *strain Sinyonya*.

pH Level	Treatment			
	CSL0	CSL10	CSL20	CSL30
Diet	5.73 ± 0.17[c]	5.50 ± 0.18[bc]	5.35 ± 0.13[ab]	5.15 ± 0.13[a]
Stomach	3.78 ± 0.13[c]	3.50 ± 0.26[bc]	3.35 ± 0.21[ab]	3.15 ± 0.17[a]
Intestine	7.10 ± 0.08[a]	7.07 ± 0.12[a]	7.06 ± 0.10[a]	7.04 ± 0.07[a]

Note: CSL0 = reference diet; CSL10 = CSL 10%; CSL20 = CSL 20%; CSL30 = CSL 30%. Different superscript letters behind the average value (± SD) on the same line present a significant difference (P<0.05).

Table 6. Enzyme activities of common carp *strain Sinyonya* on different corn steep liquor concentrations.

Parameter	Treatment			
	CSL0	CSL10	CSL20	CSL30
Protease (IU/mL)	0.41 ± 0.01[b]	0.49 ± 0.02[c]	0.57 ± 0.04[d]	0.32 ± 0.03[a]
Lipase (IU/mL)	4.24 ± 0.06[b]	4.40 ± 0.06[c]	4.54 ± 0.04[d]	3.82 ± 0.04[a]
Amylase (IU/mL)	2.12 ± 0.09[b]	2.29 ± 0.02[c]	2.41 ± 0.15[d]	1.87 ± 0.04[a]

Note: CSL0 = reference diet; CSL10 = CSL 10%; CSL20 = CSL 20%; CSL30 = CSL 30%. Different superscript letters behind the average value (± SD) on the same line present a significant difference (P<0.05).

Table 7. Digestibility levels of common carp *strain Sinyonya* on different corn steep liquor concentrations.

Parameter	Treatment			
	CSL0	CSL10	CSL20	CSL30
Total digestibility (%)	70.00 ± 1.78[b]	70.34 ± 0.11[b]	74.59 ± 0.15[c]	66.27 ± 0.35[a]
Ingredient digestibility (%)		73.38 ± 1.12[b]	92.93 ± 0.77[c]	57.57 ± 1.16[a]
Protein digestibility (%)	87.81 ± 0.74[b]	88.54 ± 0.90[b]	89.02 ± 0.43[b]	83.72 ± 1.01[a]
Lipid digestibility (%)	85.80 ± 0.43[a]	86.10 ± 0.57[a]	88.86 ± 0.33[b]	85.47 ± 1.15[a]
Energy digestibility (%)	80.35 ± 1.03[a]	81.40 ± 0.60[b]	84.92 ± 0.34[c]	80.71 ± 0.27[ab]

Note: CSL0 = reference diet; CSL10 = CSL 10%; CSL20 = CSL 20%; CSL30 = CSL 30%. Different superscript letters behind the average value (± SD) on the same line present a significant difference (P<0.05).

The presence of lactic acid in CSL20 and CSL30 significantly decreased the pH levels of diet and stomach. In the digestive tract, lactic acid is known to reduce gastric pH and inhibiting the active transport of excess internal protons, which requires cellular adenosine triphosphate (ATP) consumption, leading to cellular energy depletion (Vieco-Saiz *et al*., 2019). Lactic acid bacteria found in the fish digestive tract cause bacterial balance, thereby increasing feed digestibility by fermenting carbohydrates through an enzymatic reaction and decreasing the pH level (Wang *et al*., 2021). Hermawan *et al*. (2021) also showed that the application of CSP that contained lactic acid up to 30% in formulated diet could reduce the gastric pH from 4.67 to 4.25.

CSL has zero crude fiber content. Crude fiber content in feed below 15.5% can reduce the nutrient intake, increase digestion and absorption capacity, improve feed conversion ratio and digestibility coefficient, support growth performance and fish health (Sun *et al*., 2019; Bonvini *et al*., 2018; Kamarudin *et al*., 2018). In addition, low crude fiber will increase intestinal peristalsis, that can help digest and absorb nutrients, besides increasing the degradation rate of chemical compounds in feed (Manullang *et al*., 2018). The enzyme activity profiles in the fish digestive tract is an indicator of the fish capability to digest and utilize the nutrients in feed.

This study showed that CSL in formulated diet could increase the digestive enzyme activity of common carp *strain Sinyonya*, except protein digestibility, which was insignificantly different with CSL10 and CSL20 treatments. These results are similar to Hoseini *et al*. (2023), who showed that lactic acid administration to common carp could increase the intestinal amylase, lipase, trypsin, and chymotrypsin enzymes by altering the intestinal microbiota. Organic acids have no significant effect on digestive enzymes in the pancreas (Huan *et al*., 2019; Yao *et al*., 2019). However, the anti-nutritional properties in plant-based protein ingredient for aquafeed can decrease digestive enzyme activities in the intestine (Barlaya *et al*., 2021; Willora *et al*., 2022).

The presence of phytic acid inhibits the activity of protease, lipase, and amylase. However, CSL has no phytic acid content. In addition, beneficial bacteria can secrete digestive enzymes in the fish intestines, reducing antinutritional properties and suppressing their negative effects on digestive enzymes (Assan *et al*., 2022). Karatas *et al*. (2023) reported that the presence of lactic acid bacteria that produced phytase could break down phytate level in feed, thereby increasing the activity of digestive enzymes.

Energy digestibility in the CSL20 treatment had a higher value than other treatments, which indicates the fish ability to utilize carbohydrates and lipids as non-protein energy sources through regulating the activity of amylase and lipase. According to Liao *et al*. (2015), digestibility is influenced by the presence of enzymes and activity level of digestive enzymes in digestive tract. Halver (1989) stated that the energy digestibility of fish is 70% for grain-based ingredient materials, while animal-based ingredients is 85%. This study showed that the energy digestibility of feed using CSL is higher than the established standards.

Decreased crude fiber, increased protein level, and decreased antinutritional properties cause the increased digestibility level (Suprayudi *et al*., 2017). Meanwhile, the composition and quality of macronutrients in feed, feed production method, and environmental factors, such as water temperature, dissolved oxygen content, and stocking density are factors that influence energy digestibility in fish (Haidar *et al*., 2016). The amount of feed intake and energy digestibility in fish will be interrelated as fish consume feed to gain energy they need. Feed intake is affected by the feed palatability based on the aquafeed ingredient composition.

Low nutrient digestibility in feed indicates reduced energy availability that can be utilized by fish (Wulaningrum *et al*., 2019). Increasing the CSL concentration in formulated diet causes an increased FI in the CSL treatments. Feed conversion determines the function of feed in supporting fish growth, whereas the lower the value, the more effective the feed utilized (Fry *et al*., 2018). Moreover, feed conversion also indicates the way the feed is digested, as the amount of feed intake that can be absorbed by the body before being converted into body biomass (Afifah *et al*., 2021).

The CSL20 treatment had the lowest feed conversion, which means that the CSL20 formulated diet is easier to digest, allowing good nutrient absorption in digestive tract. In contrast, the CSL30 treatment had a higher feed conversion rate than other treatments. This condition occurred due to low digestibility levels in the CSL30 treatment, which reduced the nutrient availability for subsequent metabolic activities. A similar condition was also reported by Chovatiya *et al*. (2011), which mentioned that increasing the dose of CSL could reduce the FCR value in *Labeo rohita* fish. The lactic acid content in CSL was thought to influence fish growth performance, which was in accordance with Elala *et al*. (2015), who reported that increasing the organic acids in feed could improve the growth performance of fish by regulating the digestive enzyme activities and protein digestibility.

The results indicate that CSL can be used as a source of plant-based protein ingredient for aquafeed that improves growth performance in common carp *strain Sinyonya*. The application of CSL in formulated diet can improve the fish health status based on the blood profiles and phagocytic activity. Blood is responsible for transporting oxygen, carbon dioxide, nutrients, hormones, metabolisms, and antibody production (Aruldoss *et al*., 2014). Hemoglobin and HR values decreased on the CSL20 and CSL30 treatments, but these values could still be tolerated by the fish, due to optimal values of growth performance and feed conversion ratios at these doses.

CONCLUSION

CSL is a corn starch byproduct from agro-industrial processing with a protein content of 44.04%, pH 4.50, and 20.36% of lactic acid. This plant-based ingredient is free from anti-nutritional properties, such as aflatoxin, phytic acid, and trypsin inhibitors. The essential amino acid index value of CSL is 0.8, which indicates as a useful protein ingredient source. Therefore, CSL can be used as a plant-based protein ingredient in aquafeed for common carp *strain Sinyonya*, based on the increased digestive enzyme activity, digestibility performance, and growth performance. The application of CSL at 20% in formulated diet is recommended for further production of common carp *strain Sinyonya* feed.

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REFERENCES

Abouel AFR, Kong F, Tan Q, Zhu Y, Yu H, Yao J, Luo Z. 2020. Effects of replacement of dietary rapeseed meal by distiller's dried grains with solubles (DDGS) on growth performance, muscle texture, health and expression of muscle-related genes in grass carp (*Ctenopharyngodon idellus*). Aquaculture 533: 736169.

Afifah D, Arief M, Al-Arif MA. 2021. The effect of garlic *Allium sativum* and turmeric *Curcuma longa* extract addition in commercial feed on feeding rate, feed efficiency and feed conversion ratio of gouramy fish *Osphronemus gouramy*. *I*OP Conference Series: Earth and Environmental Science 679: 012073.

Allam BW, Khalil HS, Mansour AT, Srour TM, Omar EA, Nour AAM. 2020. Impact of substitution of fish meal by high protein distillers dried grains on growth performance, plasma protein and economic benefit of striped catfish (*Pangasianodon hypophthalmus*). Aquaculture 517: 734792.

Al-Taee SK, Annaz MT, Al-Badrany MS, Al-Hamdani AH. 2021. Biochemical and behavioral responses in carp fish exposed to tricaine methane sulfonate (MS-222) as anesthetic drug under transport conditions. Iraqi Journal of Veterinary Sciences 35: 719–723.

[AOAC] Association of Official Analytical Chemist. 2012. Official methods of analysis of AOAC international, 19th ed. Association of Official Analytical Chemists.

Aruldoss K, Kannan K, Chandira A, Samipillai SS. 2014. Effect of *Cynodon dactylon* on the hematological parameters in the blood of *Oreochromis mossambicus*. International Journal of Modern Research and Reviews 2: 171–177.

Assan D, Kuebutornye FKA, Hlordzi V, Chen H, Mraz J, Mustapha UF, Abarike ED. 2022. Effects of probiotics on digestive enzymes of fish (finfish and shellfish); status and prospects: A mini review. Comparative Biochemistry and Physiology Part B: Biochemical and Molecular Biology 257: 110653.

Azizi-Shotorkhoft A, Sharifi A, Mirmohammadi D, Baluch-Gharaei H, Rezaei J. 2015. Effects of feeding different levels of corn steep liquor on the performance of fattening lambs. Journal of Animal Physiology and Animal Nutrition 100: 109–117.

Barlaya G, Ananda Kumar BS, Huchchappa RC, Basumatary P, Kannur H. 2021. Effect of fish meal replacement with toasted guar meal on growth, food conversion, digestive enzyme activity and final carcass composition of rohu, *Labeo rohita*. Aquaculture Research 52: 5551–5557.

Bergmeyer HU, Grassi M. 1983. Methods of Enzymatic Analysis, Volume 2. Weinheim (DE): Verlag Chemie.

Bernfield P. 1955. Amylase α and β in: Methods in enzymology. New York (US): Academic Press.

Bonvini E, Bonaldo A, Parma L. 2018. Feeding European sea bass with increasing dietary fibre levels: Impact on growth, blood biochemistry, gut histology, gut evacuation. Aquaculture 494: 1–9.

Bu X, Lian X, Zhang Y, Chen F, Tang B, Ge X, Yang Y. 2018. Effects of replacing fish meal with corn gluten meal on growth, feed utilization, nitrogen and phosphorus excretion and IGF-I gene expression of juvenile *Pseudobagrus ussuriensis*. Aquaculture Research 49: 977–987.

Bunda MGB, Tumbokon BLM, Serrano AE. 2015. Composition, chemical score (CS) and essential amino acid index (EAAI) of the crinkle grass *Rhizoclonium* sp. as ingredient for aquafeeds. Aquaculture, Aquarium, Conservation & Legislation International Journal of the Bioflux Society 8: 411–420.

Caruso G. 2015. Use of plant products as candidate fish meal substitutes: An emerging issue in aquaculture productions. Fisheries and Aquaculture Journal 6: 1–3.

Carvalho RAPLF, Ota RH, Kadry VO, Tacon AGJ, Lemos D. 2016. Apparent digestibility of protein, energy and amino acids of six protein sources included at three levels in diets for juvenile white shrimp *Litopenaeus vannamei* reared in high performance conditions. Aquaculture 465: 223–234.

Castillo S, Rosales M, Pohlenz C, Gatlin DM. 2014. Effects of organic acids on growth performance and digestive enzyme activities of juvenile red drum *Sciaenops ocellatus*. Aquaculture 433: 6–12.

Chatvijitkul S, Davis DA, Lim CE. 2016. Lipid extracted distillers dried grains with solubles (LE-DDGS) as a partial replacement for soybean meal in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) diets. Aquaculture 459: 131–136.

Chovatiya SG, Bhatt SS, Shah AR. 2011. Evaluation of corn steep liquor as a supplementary feed for *Labeo rohita* (Ham.) fingerlings. Journal of Aquaculture International 19: 1–12.

Elala NMA, Ragaa NM. 2015. Eubiotic effect of a dietary acidifier (potassium diformate) on the health status of cultured *Oreochromis niloticus*. Journal of Advance Research 6: 621−629.

Fry JP, Mailloux NA, Love DC, Milli MC, Cao L. 2018. Feed conversion efficiency in aquaculture: do we measure it correctly?. Environmental Research Letters 13: 024017.

Haidar MN, Petie M, Heinsbroek LTN, Verreth JAJ, Schrama JW. 2016. The effect of type of carbohydrate (starch vs nonstarch polysaccharides) on nutrients digestibility, energy retention and maintenance requirements in Nile tilapia. Aquaculture 463: 241–247.

Halver JE. 1989. Fish Nutrition. Academic Press, Inc. California. hal 113–149.

Hermawan D, Suprayudi MA, Jusadi D, Alimuddin, Ekasari J. 2021. Evaluation of corn steep powder as a protein source of Nile tilapia *Oreochromis niloticus* diet. Jurnal Akuakultur Indonesia 20: 115–129.

Hoseini SM, Yousefi M, Kordmahalleh AA, Pagheh E, Mirghaed AT. 2023. Effects of dietary lactic acid supplementation on the activity of digestive and antioxidant enzymes, gene expressions, and bacterial communities in the intestine of common carp, *Cyprinus carpio*. Animals 13: 1934.

Huan D, Li X, Yao W, Yang H, Liang G, Leng X. 2018. Effects of organic acids (salt) supplementation in low fish meal diet on growth performance, digestive enzyme activities and nutrient apparent digestibility of *Litopenaeus vannamei*. Chinese Journal Animal Nutrition 30: 4526–4537.

[IAFFD] International Aquaculture Feed Formulation Database. 2023. Feed Ingredient Composition Database v6.0 https://www.iaffd.com/feed.html?v=6.0. [4 September 2023].

Jiriaei F, Kazemi-Bonchenari M, Moradi MH, Mirmohammadi D. 2020. Synchronous feeding of liquid protein source with different grains on performance, digestibility, ruminal fermentation, blood metabolites, and carcass characters in growing lambs. Tropical Animal Health and Production 52: 829–837.

Kamarudin MS, Sulaiman MA, Ismail MFS.

2018. Effects of dietary crude fiber level on growth performance, body composition, liver glycogen and intestinal short chain fatty acids of a tropical carp (*Barbonymus gonionotus* ♀ X *Hypsibarbus wetmorei* male ♂). Journal of Environmental Biology 39: 813–820.

Karatas S, Turgay E, Yıldız M, Kaiza VE, Yardımcı RE, Steinum TM. 2023. Mucosal bacteriomes of rainbow trout (*Oncorhynchus mykiss*) intestines are modified in response to dietary phytase. Aquaculture 574: 739672.

[KKP] Kementerian Kelautan dan Perikanan. 2022. Kelautan dan Perikanan dalam angka tahun 2022 https://perpustakaan.kkp.go.id/knowledgerepository/index.php?p=show_detail&id=1074273&keywords=. [January 2002].

Kirimi JG, Musalia LM, Magama A, Munguti JM. 2020. Protein quality of rations for Nile tilapia (*Oreochromis niloticus*) containing oilseed meals. Journal of Agricultural Science 12: 82–91.

Liao M, Ren T, He L, Han Y, Jiang Z. 2015. Optimum dietary proportion of soybean meal with fish meal and its effects on growth, digestibility and digestive enzyme activity of juvenile sea cucumber *Apostichopus japonicus*. Fisheries Science 81: 915–922.

Linfield WM, Barangkas RA, Sivieri L, Serosta S, Stevenson RW. 1984. Enzymatic fat hydrolysis and Synthesis. Journal of the American Oil Chemists' Society 18: 191–195.

Li X, Cui W, Leng X. 2015. Citric acid substituted the inclusion of inorganic phosphorus in diet of rainbow trout *Oncorhynchus mykiss*. Aquaculture Research 48: 1089–1098.

Magbanua TO, Ragaza JA. 2024. Selected dietary plant-based proteins for growth and health response of Nile tilapia *Oreochromis niloticus*. Aquaculture and Fisheries 9: 3–19.

Manullang Y, Santoso L, Tarsim. 2018. The effect of substitution of fish meal with patin fish head meal *Pangasius* sp. on the growth of catfish *Clarias* sp. Jurnal Akuakultur Rawa Indonesia 6: 129–140.

Mmanda FP, Lindberg JE, Halden AN, Mtolera MSP, Kitula R, Lundh T. 2020. Digestibility of local feed ingredients in tilapia *Oreochromis niloticus* juveniles, determined on faeces collected by siphoning or stripping. Fishes 5: 1–11.

[NRC] National Research Council. 1993. Nutrient requirements of fish. Committee on Animal Nutrition. Board on Agriculture. National Research Council. National Academy Press. Washington DC., USA. p 114.

Omnes MH, Goasduff JL, Delliou HL, Bayon NL, Quazuguel P, Robin JH. 2017. Effects of dietary tannin on growth, feed utilization and digestibility, and carcass composition in juvenile European seabass (*Dicentrarchus labrax* L.). Aquaculture Report 6: 21–27.

Rahimi ZS, Modirsanei M, Mansoori B, Rezaeian M, Farkhoy M, Honarzad J. 2017. The effect of enzymatic pre-treatment of corn or soybean meal on their phytate content under different in vitro conditions. Iranian Journal of Veterinary Medicine 11: 323–334.

Sangavi S, Sawant PB, Ande MP, Syamala K, Chadha NK. 2020. Dietary inclusion of non-conventional palm kernel meal enhances growth, digestive enzyme activities and carcass characteristics of juvenile rohu (*Labeo rohita*). Aquaculture Report 18: 100502.

Sukhanandi SM, Bhatt SS. 2016. Corn steep liquor with lysine and chelated minerals as supplementary feed for *Labeo rohita* fingerlings. International Journal of Fisheries and Aquatic Studies 4: 180–187.

Sun Y, Zhao X, Liu H, Yang Z. 2019. Effect of fiber content in practical diet on feed utilization and antioxidant capacity of loach, *Misgurnus anguillicaudatus*. Journal of Aquaculture Research & Development 10: 1–7.

Suprayudi MA. 2018. Peran ilmu nutrisi ikan dan kemandirian bahan pakan lokal dalam mendukung produksi akuakultur yang berkelanjutan [Orasi Ilmiah]. Bogor: Institut Pertanian Bogor. (In Indonesia).

Suprayudi MA, Alim S, Fauzi I, Ekasari J, Setiawati M, Junior M, Tacon A. 2017. Evaluation of hydrolysed rubber seed meal as a dietary protein source for Nile tilapia *Oreochromis niloticus* L. Aquaculture Research 48: 3801–3808.

Takeuchi T. 1988. Laboratory work-chemical evaluation of dietary nutrient. In Watanabe, T (ed). Fish nutrition and mariculture. Departement of Aquatic Bioscience, Tokyo University of Fisheries. pp. 179–233.

Tan JP, Jahim JM, Wu TY, Harun S, Mumtaz T. 2016. Use of corn steep liquor as an economical nitrogen source for biosuccinic acid production by *Actinobacillus succinogenes*. IOP Conference Series: Earth and Environmental Science 36: 1–5.

Tereos Indonesia, PT. 2016. Corn wet milling industry. Banten, Indonesia. PT Tereos Indonesia.

Ullah Z, Yousaf M, Shami MM, Sharif M, Mahrose K. 2018. Effect of graded levels of dietary corn steep liquor on growth performance, nutrient digestibility, haematology and histopathology of broilers. Journal Animal Physiology and Animal Nutrition 102: 395−402.

Vieco-Saiz N, Belguesmia Y, Raspoet R, Auclair E, Gancel F, Kempf I, Drider D. 2019. Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. Frontiers in Microbiology 10: 57.

Wang Y, Wu J, Lv M, Shao Z, Hungwe M, Wang J, Bai X, Xie J, Wang Y, Geng W. 2021. Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. Frontiers in Bioengineering and Biotechnology 9: 612285.

Watanabe T. 1988. Fish nutrition and mariculture, JICA textbook, the general aquaculture course, Departement of Aquatic Bioscience, Tokyo University of Fisheries. Tokyo. Pp. 233.

Wattanakul W, Thongprajukaew K, Hahor W, Suanyuk N. 2021. Optimal replacement of soybean meal with fermented palm kernel meal as protein source in a fish meal-soybean meal-based diet of sex reversed red tilapia (*Oreochromis niloticus* × *O. mossambicus*). Animals 11: 2287.

Willora FP, Vatsos IN, Mallioris P, Bordignon F, Keizer S, Martınez-Llorens S, Sørensen M, Hagen O. 2022. Replacement of fishmeal with plant protein in the diets of juvenile lumpfish (*Cyclopterus lumpus*, L. 1758): Effects on digestive enzymes and microscopic structure of the digestive tract. Aquaculture 561: 738601.

Wulanningrum S, Subandiyono S, Pinandoyo P. 2019. The effect of various dietary protein content with the E/P Ratio of 8,5kcal/g protein on the growth of Nile tilapia (*Oreochromis niloticus*). Sains Akuakultur Tropis 3: 1–10.

Yao W, Li X, Kabir Chowdhury MA, Wang J, Leng X. 2019. Dietary protease, carbohydrase and micro-encapsulated organic acid salts individually or in combination improved growth, feed utilization and intestinal histology of Pacific white shrimp. Aquaculture 503: 88–95.

Yusuf DH, Suprayudi MA, Jusadi D. 2016. Quality improvement of rubber seed meal diet for Nile tilapia with amino acid supplemetation. Jurnal Akuakultur Indonesia15: 63–69.