The digestibility of improved sugar cane bagasse on Barbonymus schwanenfeldii

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Abstract. The evaluation of fish capacity to digest an alternative ingredient is a critical aspect. The highly digestible ingredients will improve fish growth performance and reducing the production of wastes. This experiment was conducted to determine the digestibility of improved sugar cane bagasse as an ingredient on Barbonymus schwanenfeldii diet. The nutrient contents of improved sugar cane bagasse were crude protein, lipid, ash, crude fiber, carbohydrates (by difference) of 22.27%, 0.68%, 8.03%, 12.75%, and 55.23% (in dry weight), respectively. To measure the digestibility of improved sugar cane bagasse, a test diet in which 30% by weight of the reference diet was replaced with improved sugar cane bagasse. Chromic oxide was used as a marker and added 0.6% to both reference and test diets. Fifteen fishes with an average weight of 10.00±0.53 g were held in 110L aquaria and fed three times daily at satiation level. The digestibility of improved sugar cane bagasse on B. schwanenfeldii was 66.08%, protein 84.35%, lipid 95.26%, and energy 70.49%. The result showed that the nutritive value of improved sugar cane bagasse in this present study was fairly digestible by B. schwanenfeldii and it could be an ingredient in its diet.

1. Introduction
Bagasse is a residue from the processing of sugar cane in a sugar factory. Bagasse produced about 25% of the total sugar cane used as raw material [1]. Sugar factory PG Ngadirjo in Indonesia produces 0.3 tonnes of bagasse for each tonne of sugar cane processed into sugar [2], so bagasse has an abundant quantity. Till now, bagasse has not been widely used. Many sugar factories use bagasse as fuel, but not in a large number. The bulky property of bagasse causes sugar factories to have to allocate a large enough area as storage. There is also concern that the large quantity of bagasse will cause environmental problems. Utilization of bagasse as a fish feed ingredient can be a solution. Apart from reducing the quantity of waste, it also supports sustainable aquaculture policies. Efforts to use waste refer to the need for alternative materials in the context of substituting ingredients that must be imported. Thus the use of alternative materials is expected to be able to reduce the high cost of feed in freshwater fish farming.

Bagasse has nutrients that can be used even though it falls into the category of waste. The nutrients contained in bagasse about 1-3% protein, <3% lipid, 2-8% ash, 25-35% crude fiber and 50-60% NFE, in dry weight [3, 4]. Bagasse is a lignocellulosic material so it requires a multi-stage process to make it be a product that has more value [5]. In general, the processing of lignocellulosic materials is conducted in two stages. The first stage is delignification to remove lignin, and the second one is enzymatic hydrolysis to degrade cellulose into its simpler components. Enzymes role as catalysts that
increase the reaction rate without reacting [6]. Enzymatic hydrolysis has important role in the conversion process of biomass into the desired product and has many advantages [7].

Many efforts to improve waste quality have been made. It is proven to increase nutrient content, in order to use it as a fish feed ingredient without disturbing the fish growth. Processed palm kernel meal which is used as an ingredient in Tilapia (Orechromis sp.) diet, makes the carcass contain higher calcium (Ca) and phosphorus (P) and lower lipid [8]. The utilization of processed rice by-products increases the higher final weight of the Pacific white shrimp, Litopenaeus vannamei [9]. Processed fish processing waste is used as aquaculture feed to increase the yield gain of fish farming [10].

The evaluation of fish capacity to digest an alternative ingredient is a critical aspect. The highly digestible ingredients will improve fish growth performance and reducing the production of wastes. This study aims to determine the digestibility of improved sugar cane bagasse as an ingredient in B. schwanenfeldii diet.

2. Material and Methods
This research was conducted from August to November 2019 in the Laboratory of Chemistry, Laboratory of Microbiology, and Wet Laboratory, Nutrition & Feed Technology, Research Institute for Freshwater Aquaculture and Fisheries Extension (RIFAFE) Bogor, West Java, Indonesia.

2.1 Preparation of sugar cane bagasse meal
Initial sugar cane bagasse (SCB0) was obtained from PT. PG Rajawali II, PG Sindang Laut, Cirebon, West Java, Indonesia. Then the SCB0 was treated to improve the quality. The improved bagasse (SCBi) then oven-dried at 60°C for 24 hours. After the moisture content reaches approx. 6-7%, the SCBi is sieved for particle homogenization, then stored in a tightly closed container for subsequent use. Sufficient samples were taken for analysis such as proximate [11], lignocellulose components [12] and reducing sugar [13].

2.2 Analysis fish and experimental condition
The container used in the digestibility measurement is aquaria 60x50x40 cm, equipped with an aeration system. The experimental fish used B. schwanenfeldii with an initial weight of 10.00 ± 0.53 g obtained from RIFAFE, Bogor, West Java, Indonesia. The fish were stocked at a density of 15 fish per aquarium and acclimatized for seven days. Approx. 30% of the water is changed every morning. The water quality parameters were maintained at optimum range i.e temperature 29-30°C, pH around 7, DO> 3 mg L⁻¹ and NH₃-N<0.05 mg L⁻¹. Measurement of temperature, DO and pH are carried out in the morning before and after the water change, and also in the afternoon.

2.3 Diet preparation
The diet used for the digestibility measurement was prepared from 70% reference diet and 30% SCB0 or SCBi meal [14]. The Chromium Oxide (Cr₂O₃) was used as a marker and added 0.6% both to the reference and test diet. The composition of the reference and test diet is listed in Table 1.

2.4 Fecal collection
Feeding is carried out at satiation level three times a day, at 08.00, 12.00 and 16.00 WIB. Fecal collection starting on the fifth day after the fish were fed, by siphoning the feces and collecting them. The feces are collected immediately after being released by B. schwanenfeldii. The collected feces are put in the oven at 40°C for 24 hours. Then, the feed and feces are analyzed for their chromium and proximate content.

2.5 Analytical method
The SCB0 and SCBi meal each taken as much as 50 g to determine the nutrient content. The water content determined by oven dried at temperature of 105°C for 3 hours or until a constant weight was reached. The protein content is determined by the Kjeldahl method (destruction-distillation-titration).
Lipid content was determined by Soxhlet extraction using petroleum benzene solvent. The ash content was determined by incineration in a furnace at 600°C for 4 hours. Crude fiber content is carried out using acid and alkaline solvents. The digestibility of the diet and ingredient is calculated based on the equation [15, 16].

| Table 1. Composition of the reference and test diet (%) |
|-------------------------------------------------------|
| **Ingredients** | **Reference diet** | **Test diet** |
| Fish meal       | 25.0               | 17.8         |
| Soyabean meal   | 36.0               | 25.1         |
| SCB0/SCBi meal  | 0.0                | 30.0         |
| Pollard         | 25.4               | 17.4         |
| Fish oil        | 3.0                | 2.1          |
| Corn oil        | 3.0                | 2.1          |
| Vitamin & mineral mix | 3.0  | 2.1         |
| Cassava meal    | 4.0                | 2.8          |
| Cr₂O₃           | 0.6                | 0.6          |
| **Total**       | 100.0              | 100.0        |

*a* SCB0 = Initial sugar cane bagasse  
*b* SCBi = Improved sugar cane bagasse

2.6 Data expression
Analysis of each parameter was conducted at three replicates (n = 3). The collected data were entered into Microsoft Excel for Windows 10 Professional Edition and expressed as mean ± standard deviation.

3. Results and Discussion
The nutrient composition of SCB0 and SCBi are shown in figure 1. Improvement quality on bagasse is proven to increase its nutrient content. Protein increased from 1.49% to 22.27%, lipid decreased from 0.82% to 0.68%, ash fell from 9.24% to 8.03%, crude fiber decreased from 32.34% to 12.75% while NFE (Nitrogen Free Extract) increased from 56.11% to 56.27%.

The crude fiber fractions contained in SCB0 and SCBi are shown in figure 2. Improvement quality has succeeded in reducing the crude fiber fraction contained in bagasse. Lignin and cellulose decreased, while reducing sugar (RS) raised. The results of the digestibility measurement of bagasse on *B. schwanenfeldii* are shown in Figure 3. The digestibility of ingredients, protein, lipid, and energy of SCBi was increased, compared to SCB0. This means that SCBi can be digested properly by *B. schwanenfeldii*.

![Figure 1. Nutrient composition of bagasse (% in dry weight) (SCB0 = initial bagasse, SCBi = improved bagasse)](image-url)
The largest component contained in the SCB0 and SCBi were carbohydrate (NFE), 56.11%, and 56.27%, respectively. NFE is a carbohydrate that can be digested by fish. The largest component in NFE is starch, a non-structural carbohydrate, which is an intracellular energy storage [17]. Starch is digested in the anterior part of the fish digestive system and highly dependent on its solubility in digestive juices.

Carbohydrates in SCBi contain more glucose, which is the result of cellulose degradation by cellulase enzymes during the hydrolysis stage (figure 2). The utilization of carbohydrates varies and appears to be related to complexity. More simple the form of carbohydrates, easier to be digested by fish. The digestibility of carbohydrates is also related to the activity of the carbohydrase enzyme. The higher water temperature, the activity of the carbohydrase enzyme will increase [18].

The feed used in fish farming contains carbohydrates in varying amounts, depending on the species being cultivated. The ability of fish to digest carbohydrates is very specific, depending on the anatomy and function of the digestive tract and other related organs. The digestive organs of fish vary widely from simple to complex, reflecting the various nutritional sources. Carbohydrates are used in fish feed as an energy source and binding properties, so they can be added to the feed-in excess of the amount that the fish will use as energy. The development of physiology, economy, and cultivation technology has triggered efforts to use economical feed, by involving carbohydrates in larger quantities.

The second-largest nutrient content in SCBi is protein. Protein is the most important nutrient needed for growth, reproduction, other body functions, and energy source. The higher protein content
and easier to digest and use by fish, the growth will be faster. Fish have very limited ability to synthesize protein, so protein intake from the feed is required. Compared with plant ingredients in general, the protein content in SCBi is quite high (22.27%).

The crude fiber is a component of ingredients that needs attention because crude fiber was reported as an anti-nutritional factor on monogastric animals. The presence of high crude fiber in feed ingredients will affect the digestibility of the material. Crude fiber indicates the amount of fiber that can be digested and affects energy digestibility [19] and produces pellets with weak binding strength and is easily destroyed in water. As a lignocellulosic material, SCB0 has a high fiber fraction. Quality improvement reduced crude fiber (figure 2). The remaining lignin is 1.64%, while the remaining cellulose is 3.35%. Lignin dissolves in the delignification process, while cellulose is degraded by cellulase enzymes into a simpler constituent, which is indicated by the amount of reducing sugar. Figure 2 shows that SCBi has a sufficient number of reducing sugars when compared to SCB0.

The ash content in SCBi fell to 8.03% from the start of 9.24%. The higher levels of ash are not digested well by fish. It will disturb the digestibility of other components. The lipid contained in bagasse is not much because it is not a source of lipid. The results of the proximate analysis showed that SCBi had adequate nutrient composition to be used as an ingredient in B. schwanenfeldii diet. The next step to evaluate non-conventional ingredients is the digestibility measurement of fish. The digestibility ingredient of SCBi was 66.08%. The digestibility of ingredients shows the extent to which the nutrients can be digested by fish [20]. Therefore, determining the digestibility of ingredients is the main priority when it comes to the inclusion of ingredients in a diet, because growth performance will be determined by the efficiency of nutrient digestibility. In addition, it is also influenced by nutrient composition and the capacity of fish to digest and absorb nutrients [21]. Nutrient composition and information on digestibility will be the basis for formulation design and feeding regimes. When compared with the digestibility of other plant ingredients such as corn (52.30%), pollard (45.0%) [22] and cocoa bran (38.1%) [23], the ingredient digestibility of SCBi was fairly high.

The digestibility of protein in SCBi was 84.35%. This result is in line with many studies which reported that the protein digestibility of various plant materials is around 75-95% [24]. Many other plant ingredients such as rice bran, cassava leaves, and cocoa bran are said to have a much lower protein digestibility, 51.6%, 49.8%, and 38.5%, respectively [25]. The protein digestibility is influenced by several factors including drying, temperature, and storage time [25]. In general, the protein quality of feed ingredients is a major factor affecting growth performance, and protein digestibility is the first parameter that must be considered. The protein quality of feed ingredients is mainly related to the composition and digestibility of amino acids. Protein digestibility is an indicator of amino acid digestibility [26]. Furthermore, it is stated that protein digestibility is also affected by amino acid balance [27]. Lack of essential amino acids is known to cause poor utilization of protein which negatively impacts fish growth and feed efficiency.

The digestibility of lipids in SCBi was 95.26%. This result is in line with previous research which states that the digestibility of lipid ranges from 72-97.5% [28]. Although the lipid content in SCBi is low, high lipid digestibility is associated with high lipase activity in fish [29]. The energy digestibility of SCBi in B. schwanenfeldii was 70.49%. These results are consistent with the results of research which states that the energy digestibility is about 39-89% [28]. When compared with many plant ingredients commonly used in fish feed, the energy digestibility of SCBi is lower when compared to pollard (91.3%) and corn (83.9%) [29]. However, it was much higher when compared to cocoa bran (27.10%) [23]. Energy digestibility has a negative correlation with crude fiber content. The energy digestibility is specifically affected by the levels of crude fiber, lipid, and carbohydrates from fish feed ingredients, depending on species and temperature [30].

B. schwanenfeldii used as experimental fish because it is an omnivorous fish that tends to be herbivorous [31]. Fish with these feeding habits need to feed with low protein content and a balanced amount of energy [32]. Overall, the results of the digestibility measurement showed that improved
quality of bagasse is capable to increase the composition and nutrient content, so improved bagasse is adequate for use as a fish feed ingredient in *B. schwanenfeldii* diet.

5. Conclusion
The treatment process was proven to increase the nutrient content in sugar cane bagasse. The largest nutrient contained in improved sugar cane bagasse was NFE 56.27%, followed by protein 22.27%, crude fiber 12.75%, ash 8.03%, and lipid 0.68%, in dry weight. The digestibility of improved sugar cane bagasse on *B. schwanenfeldii* was 66.08%, while the digestibility of lipid, protein, and energy was 95.26%, 84.35%, and 70.49%, respectively. The result showed that improved sugar cane bagasse was fairly digestible by *B. schwanenfeldii*.

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