Improvement quality of sugar cane bagasse as fish feed ingredient

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Abstract. Sugar cane bagasse is a waste from the processing of sugar cane in a sugar factory. Bagasse is obtained about 25% of the total weight of sugar cane which is used as raw material. Nutrients contained in bagasse approximately protein 1-4%, lipid<4%, ash 2-8%, crude fiber 20-38% and NFE 52-61% (in dry weight). Lignocellulosic component found in bagasse were lignin (11-27%), cellulose (26-49%) and hemicellulose (16-33%). This research aimed to improve bagasse into a fish feed ingredient. The study was conducted using a completely random design with four treatments and three replications. The treatment is carried out by mixing pre-treated bagasse with crude enzyme extract from Bacillus subtilis [1/1], [1/0.75], [1/0.5], [1/0.25] w/v which performed at optimum conditions, i.e. 50°C, pH 5.5 and incubation time 72 hours. The results showed that the treatment [1/1] w/v gave the best results. The improved bagasse had a protein content of 24.92%, ash 6.83%, crude fibre 11.56% (in dry weight). Dissolved protein and reducing sugar increased to 417.60 mg/L and 442.63 mg/L, respectively. Lignin decreased to 2.81%. So the improved sugar cane bagasse was feasible to be used as a fish feed ingredient.

1. Introduction

The feed is the main factor that determines the success of freshwater fish farming because the feed cost provides a large contribution to the total cost. The high price of feed is the biggest obstacle to the sustainability of fish farming. Utilization of local plant such as rice polish [1], wheat bran meal [2], palm kernel cake [3] and cassava leaf [4] as an ingredient in fish diet, have done in an effort to reduce the use of commercial feed. However, the use of these materials is limited by their continuity and quantities. Sugar cane bagasse which is produced from the processing of sugar cane in a sugar factory could be a potential candidate.

Bagasse is an organic waste from the processing of sugar cane in a sugar factory. Bagasse produced approximately 25% of the total sugar cane processed [5]. Sulaiman et al. [6] reported that each sugar factory in Indonesia requires about 3,900 tons of sugar cane per day as raw material. Thus bagasse is abundant. This causes a problem for sugar factories because the bulky character of bagasse requires a large storage area. In addition, bagasse is flammable because it contains water, sugar, fibre and microbes. When it accumulates, there will be a fermentation process and heat will be released. Many cases of fires that occurred in sugar factories were caused by this process. The efforts that have been made by sugar factories to reduce the quantity of bagasse, such as using it as fuel and burning, have not been able to
reduce the quantity of bagasse significantly. The use of bagasse as a fish feed ingredient can be an alternative solution to this problem. Even though bagasse is included in the waste category, bagasse contains nutrients such as protein 1-4%, lipid<4%, ash 2-8%, crude fibre 20-38% and NFE (Nitrogen Free Extract) 52-61%, in dry weight [7,8]. Bagasse is easy to obtain, cheap, no hazardous, and biodegradable.

Bagasse is a lignocellulosic material that contains lignin (11-27%), cellulose (26-49%) and hemicellulose (16-33%) [9]. The lignocellulose component and the high content of crude fibre are limiting factors for its utilization as a fish feed ingredient. Xiao et al. [10] stated that the processing of lignocellulosic materials into a higher value product is carried out through a multi-stage process. In general, the processing of lignocellulosic materials consists of two main stages, delignification to remove lignin content and enzymatic hydrolysis for quality improvement. Enzymatic hydrolysis has been widely applied to improve the quality of materials that have a high crude fibre content such as wheat bran [11], rice straw [12], palm kernel cake [13], rice bran [14]; and produce a higher value product. Bagasse that has been processed using enzymatic hydrolysis is expected to have adequate nutrients. This research aimed to improve the quality of sugar cane bagasse through enzymatic hydrolysis using the crude enzyme extract of Bacillus subtilis and evaluate its potential use as an ingredient in fish diet.

2. Material and methods

2.1. Preparation of bagasse meal

Bagasse is obtained at the sugar factory PG Rajawali II, Cirebon, Indonesia. The bagasse is cleaned and washed thoroughly, then sun-dried for 2 days. Furthermore, cut into ±1 cm and pulverized using a grinder machine. The powder is sieved for particle homogenization and then heated using an oven at 105°C until obtaining a constant weight. Bagasse meal then stored in a tightly closed plastic to maintain the quality.

2.2. Preparation of crude extract enzyme from B. subtilis

An one culture of B. subtilis was taken then put into 10 mL Tryptic Soy Broth (TSB), then incubated for 24 hours in an incubator with a temperature of 28°C. The results of the culture were taken 1 mL and then put into 9 mL of TSB and incubated for 24 hours. Furthermore, the culture was centrifuged at 9,000 rpm, 4°C for 30 minutes. The formed supernatant stored in the refrigerator for further use.

2.3. Research procedure

The study was conducted using a completely random design with four treatments and three replications. The treatment is carried out by mixing pre-treated bagasse with crude enzyme extract of B. subtilis [1/1], [1/0.75], [1/0.5], [1/0.25] w/v which performed at optimum conditions 50°C, pH 5.5 and incubation time 72 hours. After the incubation time was completed, the improved bagasse was oven-dried at 50°C for 24 hours until a constant weight was achieved. The parameters observed were protein, ash, crude fiber, dissolved protein, reducing sugar and lignin.

2.4. Chemical analysis and data processing

The nutrient composition of bagasse meal before and after hydrolysis was carried out using the proximate method. Protein was determined through the stages of digestion, distillation and titration. The ash was determined by incineration using a furnace at a temperature of 600°C for 3 hours and the crude fiber was determined by heating using strong acid and strong base solutions. Dissolved protein was determined using Bradford method [15], reducing sugar determined using DNS method and lignin determined using Chesson method [16].

The obtained data were analyzed using One Way Analysis of Variant (ANOVA). If there is a difference, continue with the Duncan difference test. The software used is SPSS Statistical Package version 21.0 for Windows with a significance level of p<0.05.
3. Result and discussion

3.1. Result

The results showed that the quality of bagasse increased significantly after the hydrolysis process using the crude extract of the enzyme from *B. subtilis* for protein, ash and crude fibre content (p<0.05). The protein content increased from 1.57% to 24.92% [1/1], 20.11% [1/0.75], 19.26% [1/0.50] and 18.38% [1/0.25]. Ash content decreased from 9.01% to 6.83% [1/1], 7.25% [1/0.75], 8.07% [1/0.50] and 8.32% [1/0.25]. Crude fiber content decreased from 34.92% to 11.56% [1/1], 13.92% [1/0.75], 15.16% [1/0.50] and 16.49% [1/0.25]. The improved quality of bagasse is linear with the amount of crude enzyme extract added. Treatment [1/1] gave the best results for protein, ash and crude fibre parameters (p<0.05) (Figure 1).

![Figure 1. Nutrient composition of sugar cane bagasse (% in DW)](image)

| Protein | Ash | Crude fiber |
|---------|-----|-------------|
| SCB0    | [1/1] [1/0.75] [1/0.50] [1/0.25] |
| 1.57    | 9.01 | 11.56 |
| 20.11   | 7.25 | 13.92 |
| 19.26   | 8.07 | 15.16 |
| 24.92   | 8.32 | 16.49 |

The results shown in Figure 1 are reinforced by dissolved protein, reducing sugar and also lignin content (Figure 2). The dissolved protein contained in the hydrolyzed bagasse [1/1] increased significantly from 119.07 mg/L to 417.60 mg/L. The reducing sugar increased from 92.17 mg/L to 442.63 mg/L. Lignin decreased from 25.79% to 2.81%. Treatment [1/1] significantly increased the dissolved protein and reducing sugar; on the contrary it reduces the lignin content (Figure 2).

![Figure 2. Dissolved protein (DP), reducing sugar (RS) and lignin content of sugar cane bagasse. Note: SCB0 was Initial Sugar Cane Bagasse](image)
3.2. Discussion

Bagasse is a lignocellulosic material. Processing is required prior to use bagasse as a fish feed ingredient. Enzymatic hydrolysis using crude enzyme extract purposes to degrade cellulose in bagasse into its simpler constituent which is digestible by fish. The quality improvement of bagasse increased with the amount of crude enzyme extract added, and treatment [1/1] w/v gave the best result. This result will be used in the following discussion.

The protein contained in improved bagasse using crude enzyme extract from B. subtilis [1/1] was quite high, >20%. Materials that have a protein content >20%, classified as feed protein contributors. The protein in feed gives a significant influence on fish growth because protein is the main nutrient that determines this one. The higher protein contained in the feed, the higher the potential fish grow. When compared with local plant ingredients commonly used as fish feed such as rice bran (10.74%) [17] or palm kernel cake (17.34%), the protein contained in improved bagasse is relatively high.

Bagasse has a high crude fibre content of 34.92%. Crude fibre that is too high is a problem for fish, because as a monogastric animal, fish has limited ability to digest a high crude fibre. Hydrolysis using crude enzyme extract from B. subtilis reduces crude fibre content to 11.56%. This crude fibre content is relatively low when compared to local plant ingredients, commonly used in fish feed, such as palm kernel cake (23.67%) [18] or rice bran (13.80%) [19].

Improvement quality of bagasse using crude enzyme extract from B. subtilis reduces ash content to 6.83%. Too high ash content in feed will interfere with all nutrient utilization in fish. The ash content contained in the improved bagasse was much lower when compared to rice bran (8.23%) [17]. Improved bagasse has increased the amount of dissolved protein significantly (Figure 2). The dissolved protein is oligopeptides or amino acids which is absorbed by the digestive system easily [20]. The dissolved protein in Figure 2 shows that the enzymatic hydrolysis using crude enzyme extract from B. subtilis has cut the peptide bonds in the inner or middle part and produces short-chain peptide bonds. During the hydrolysis process, the interaction between the enzyme and the substrate has made the peptide bonds simpler so that protein solubility increases. The higher dissolved protein content, more protein can be used by fish for both metabolism process and growth.

The reducing sugar formed in hydrolyzed bagasse increased significantly (Figure 2). Enzymatic hydrolysis plays a role in breaking down cellulose into its constituent (glucose). Increasing reducing sugar in the hydrolyzed bagasse showed that glucose was formed from the degradation of cellulose by crude enzyme extract from B. subtilis. Glucose is the simplest form of carbohydrate so it is easily digested by fish. The lignin level in the improved bagasse was significantly reduced. Lignin is a lignocellulosic component that cannot be digested by fish, so its presence must be minimized. The processing of bagasse is proven to be able to reduce large amounts of lignin.

The utilization of improved waste as fish feed ingredient has been widely used. Iluyemi et al. [21] stated that improved palm kernel cake which is used as an ingredient in Tilapia (Oreochromis niloticus) diet, increases Ca and P in carcass and reduces its lipid content. Bag and Mahapatra [22] reported that processed fish offal waste improve the growth of tilapia and increase its PUFA content. Yanto et al. [23] noted that using improved rice bran in Jelawat (Leptobarbus hoevenii) diet, increase fish growth and feed efficiency.

4. Conclusion

The use of crude enzyme extract from B. subtilis [1/1] w/v significantly increase protein, dissolved protein, reducing sugars and decrease ash, crude fibre and lignin content in bagasse. The overall results of this study indicated that enzymatic hydrolysis using crude enzyme extract from B. subtilis improved the quality of bagasse so it was feasible to be used as a fish feed ingredient.

5. References

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