

Nutritional Value and In Vitro Digestibility of Shrimp Waste Fermented with *Isotericola* sp. A10-1

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ABSTRACT

The study was aimed at indentifying the nutritional value and in vitro digestibility of shrimp waste fermented with *Isotericola* sp. A10-1 bacteria. This types of bacteria was capable in degrading chitin chitinolytic contained in shrimp waste. Chitin has a polymer structure that turned into a monomer structure that was digestible for poultry animal if it was degraded by bacteria. In vitro digestibility was a representation of the poultry digestive system. Samples of 45 fermented shrimp waste were prepared for the content identification of crude protein (CP) and crude fiber (CF). The identifications were conducted by proximate analysis according to the AOAC method. The data obtained in the fermentation results were analyzed by analysis of variance using a randomized block design factorial (5x9) (3 substrate water content, 3 concentration isolates). The next test was Duncan's Multiple Range Test (DMRT) to determine the difference between mean values. The samples used were 10 of the fermented shrimp waste with moisture content of 30% and isolates 15%, compared to the non-fermented shrimp waste. In vitro digestibility conducted was on dry matter, organic matter, crude protein, crude lipid, crude fiber and carbohydrates digestibility. The digestibility analysis results were statistically tested by T test. Shrimp waste fermentation nutrient values were: DM was 61.95 to 73.25%, OM was 55.99 to 56.50%, CP was 26.87 to 27.68%, CF was 20.56 to 21.29 %, and CL was 1.65 to 2.80%. Fermentation with water content of 30% and 15% of isolates decreased 20.30% of crude fiber. Shrimp waste fermentation with *Isotericola* sp. A10-1 enhanced the in vitro digestibility of crude fiber and organic materials, but the digestibility of protein, glucose, dry matter and crude protein was the other way around.

Keywords: Nutrien, *Isotericola* sp., Fermentation, Shrimp Waste

INTRODUCTION

The purpose of this research is to identify the nutritional value and test the shrimp waste in-vitro digestion, which is fermented with *Isotericola* sp. A10-1 bacteria. The *Isotericola* sp. is of the actomycetes family, which is able to hydrolyze chitin (Wu *et al.*, 2010). The chitinolytic bacteria produce chitinase. Research results have shown cloning, sequencing, as well as Is-chiA and Is-chiB gene expressions, which code two exokitinase and endokitinase in *I. jiangsuensis* CLG (Wu *et al.*, 2011).

Shrimp skin is unable to dissolve, which is one of the limitations of liquid fermentation. Applying solid substrate fermentation for enzyme production and other metabolites can

provide better results than liquid fermentation (Pandey, 2003). In addition, the cost used is much lower in solid fermentation, because of the waste usage that can provide added value and be more efficient (Robinson & Nigam, 2003).

A number of microorganisms have the ability to grow in a solid media, such as bacteria, yeast, and mold, but mold possesses the ability to adapt better in a solid media (Khrisna, 2005). Actinomycetes have the ability to penetrate a complex solid substrate and produce various enzymes (Dahiya *et al.*, 2005).

Feed in-vitro digestion is a simulation of the poultry digestive system by controlling the pH and temperature using the same pepsin and tripsin enzymes with the poultry digestive tract (Lafond *et al.*, 2011). An in-vitro test that involves a tested material suspension digestion simulation with a 'specific' digestive enzyme that can be very informative with some animal subjects has been developed (Tibbetts *et al.*, 2017).

MATERIALS AND METHODS

The shrimp waste chemical composition had a proximate analysis according a method (AOAC, 1990). The fermentation results data obtained in the research were analyzed with a variant analysis using a factorial group random design (5x9) (3 substrate water content, 3 isolate concentration). Next, a Duncan's New Multiple Range Test (DMRT) was done to find the differences between average values (Steel, 1997).

The samples used were from shrimp waste fermentation results in 30% water content and 15% isolate content. The in-vitro digestion done involved dry material, organic material, crude protein, crude fat, and carbohydrates (Malathi, 2001).

The in-vitro digestion results were analyzed with a t-test to differentiate the average values between non-fermentation with fermentation.

RESULTS AND DISCUSSION

Fermented Shrimp Waste Chemical Quality

All of the protein which was detected with the Kjeldahl method as nitrogen, actually had complex proteins like amino acids in the fermentation, and the protein value in every treatment tended to be the same, so that it resulted in an insignificant effect.

The primary components which arrange shrimp shell are chitin, minerals, and protein (Table 1).

Table 1. Fermented Shrimp Waste Crude Protein with Different Water Content and Isolate Levels (%)

Isolate content	Fermented water content			Average ^{ns}
	30	40	50	
5	27.94	27.62	26.89	27.48 ±0.45
10	25.87	28.07	27.32	27.09 ±0.49
15	26.80	27.35	27.12	27.09 ±0.40
Average ^{ns}	26.87 ±0.49	27.68 ±0.45	27.12 ±0.39	

Note: ns, non-significant

There was non significantly of the different water content levels on the fermented shrimp waste crude fiber content. The influence of the isolate levels caused significantly ($P \leq 0.05$) towards the fermented shrimp waste crude fiber content. The 15% isolate level had a

significantly with the 5% isolate level, in that it decreased the crude fiber content. However, the 10% isolate level had non significantly with the 5% isolate level. There were no influential interactions between the water content and isolate levels (Table 2).

Table 2. Fermented Shrimp Waste Crude Fiber with Different of Water Content and Isolate Levels (%)

Isolate content	Fermented water content			Average
	30	40	50	
5	23.23	20.25	22.33	21.93 ±0.49 ^a
10	21.13	20.53	20.51	20.72 ±0.49 ^{ab}
15	18.94	20.91	21.04	20.30 ±0.51 ^b
Average ^{ns}	21.10±0.69	20.56±0.37	21.29±0.45	

Note: ns: non-significant,

a, b, c, numbers with different superscripts in the same column reveal significantly ($P \leq 0.05$)

The water content had non significantly on the fermented shrimp waste crude fiber content caused by the chitin having a characteristic of being difficult to dissolve in water. The degree of de-affiliation is defined as molar unit fraction de-affiliation in a polymer chain (Zhang *et al.*, 2005).

The 15% isolate level could reduce the crude fiber content due to having a higher inoculum volume, so that the chitinolytic bacteria cell compactness was able to hydrolyze the shrimp waste chitin higher in the fermentation period.

There was a shrimp waste chemical content in the head and shell. The content of the dry material, crude protein, crude fat, ash, and chitin were $91.6 \pm 0.2\%$; $48.9 \pm 0.1\%$; $5.1 \pm 0.3\%$; $23.1 \pm 0.2\%$; and 16.4 ± 0.4 (Rahman, 2014).

Fermented Shrimp Waste In-Vitro Digestibility

The fermented shrimp waste in-vitro digestibility test results used pepsin, HCl, and pancreatin to represent the digestion of poultry in the digestion and small intestine phase. The test results include the digestion of BK, BO, PK, SK, glucose, and protein compared with non-fermented shrimp waste and fermented shrimp waste.

Table 3. Fermented Shrimp Waste In-Vitro Digestibility (%)

Sample	Protein (mg/ml)	Glucose (mg/100ml)	KcBK	KcBO	KcPK	KcSK
<i>In-vitro</i> LUNF	4.18±0.02	59.79±6.5	37.38 ^b ±6.3	41.82 ^b ±11.2	29.11±0.8	9.31 ^b ±4.6
<i>In-vitro</i> LUF	2.63±0.12	72.78±3.8	39.39 ^a ±2.3	63.32 ^a ±1.3	25.84±0.4	32.81 ^a ±5.6

Note: a, b, numbers with different superscripts in the same column show significantly ($P \leq 0.05$).

LUNF: non-fermented shrimp waste; LUF: fermented shrimp waste; KcBK: dry matter digestibility;

KcBO: organic matter digestibility; Kc,PK: crude protein digestibility; KcSK: crude fiber digestibility.

Crude fiber in-vitro digestibility between LUNF and LUF shows significantly ($P \leq 0.05$) increasing until 23.49%. It shows significantly ($P \leq 0.05$) between dry matter in-vitro digestibility and LUNF organic matter with LUF. It conveys non significantly in in-vitro digestibility in dry material, crude protein, protein, and glucose (Table 3).

There was an increase in crude fiber and organic matter in-vitro digestibility towards shrimp waste that was fermented with the *Isotericola* sp. isolate, A10-1 strain, which reveals

the occurrence of chitinolytic bacteria activity, which is able to survive, even though the low pH still shows high chitinase activity. Chitin has the same structure as cellulose with low digestion. The chitinase enzyme is produced by bacteria, so the polymer chain structure is able to be hydrolyzed into monomers or simple sugars.

The carbohydrate polymer from chitin and cellulose has a condensed form with a strong bond. The chitinolytic bacteria *Serratia marcescens* produces the hydrolase enzyme *chitin-binding protein* (CBP21), which is an enzyme to catalyze the separation of glycosidic bonds in crystal chitin, so that it opens the polysaccharide material which cannot be accessed to be dehydrolyzed by hydrolase to become simple glucose (Vaaje-Kolstad, 2010).

The low value of protein digestion, which is amino acids, is due to the low amino acid content in fermented shrimp waste. Although the glucose in-vitro digestibility has non significantly, the glucose content value is rather high in LUF compared with LUNF. This means that the bacteria are able to change chitin through a fermentation process to become simple sugar. Valette (1993) stated that amino acid digestion does not only depend on the enzyme source but also the substrate.

Next, Bautrif (1990) stated that a high digestion value signifies a high feed quality. The shrimp waste nutritional quality depends on the type of shrimp, body part, and living environment (Rahman, 2014). The shrimp shells near the head has a higher crude protein content compared with the shells part (40.7: 25.9%) and fat (13.9: 2.4%) (Ploydee, 2014). The protein in-vitro digestibility depends on the chitin content and non-digestible amino polysaccharides, which are different in all species (Rahman, 2014).

CONCLUSIONS

Shrimp waste fermentation with *Isoptericola* sp. bacteria is able to hydrolyze chitin, so that it reduces the crude fiber content and increases the crude fiber in-vitro digestibility, so that it is beneficial for poultry feed.

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