



## Developing a standard for authenticating halal gelatine catfish skin: A study on the effect of periodization quarantine (*istihalah*) on gelatin quality in catfish fed with pig-contaminated feeds

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### ABSTRACT

The search for sustainable feed alternatives has led to exploring unconventional sources, including food waste, amidst the growing demand for halal gelatin, which has created a significant need for fish skin by-products. Therefore, this study aimed to authenticate the halal status of catfish skin gelatin by verifying feed origin and determining the contents of pig contaminants. Halal standard was verified using a specific DNA analysis of pig components, conducted at each stage, including feed containing pig, fish skin, and catfish gelatin. Gelatin in catfish skin was predominantly composed of amino acids glycine and proline, and fish enlargement stage which led to a skin yield of  $5.36 \pm 0.75\%$ . Furthermore, the yields of gelatin were 8.67%, 9.94%, and 9.19%, with gel strengths of  $133.4 \pm 1.2$ ,  $129.9 \pm 1.4$ , and  $121.9 \pm 2.8$  bloom, respectively, for the different quarantine periods. The characterization of gelatin using FTIR showed the presence of functional groups, such as amide A, I, II, and III. Real-time PCR detected the presence of pig DNA in feed but not in catfish skin or gelatin. In conclusion, a quarantine period of 0 days for catfish fed pig-containing feed was sufficient to cleanse catfish skin of pig contaminants, with no indication of pig DNA being found.

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### 1 Introduction

Indonesia is the fourth-largest producer of fisheries in the world, with annual aquaculture production reaching 4.2 million tons (FAO 2016). According to Marine and Fisheries Ministry (Kementerian Kelautan dan Perikanan) statistics from 2010-2014, Indonesian aquaculture production increased by 23% per year. The major commodities include seaweed, tilapia, carp, and catfish, accounting for 27%, 21%, 20%, and 29%, respectively (Marine and Fisheries Ministry 2015). In catfish aquaculture, fish farming is typically carried out on a small scale using commercial or artificial feeds. Marine and Fisheries Ministry (2018), reported that fish meal, which is an expensive imported ingredient, is the primary component in the production of feed in catfish aquaculture.

Fish nutritional needs vary by species as herbivorous fish consume feed mixtures that may contain plant proteins (soy, corn), vegetable oils, minerals, and vitamins. In the wild, carnivorous fish, such as salmon feed on other smaller fish. Meanwhile, the increasing demand for catfish has become a sought-after aquaculture commodity and has led to a rise in the demand for catfish feed. Historically, catfish feed has been sourced from protein-rich fish meal, maggots, bran, and natural feed, which tend to be expensive and not sustainable in terms of resources and costs. A new alternative is the use of food waste (Cheng *et al.* 2014), and other animal protein sources, particularly by-products of industrial processes. However, these new sources are controversial due to the potential animal welfare issues, the consistency of the generated quality of feed, health concerns, and religious issues in some groups or countries. A current issue in catfish feed is the protein intake, which is often fraudulently augmented with sources from other animals, such as pork. There are allegations of using pork offal either as a fish meal substitute or directly given to fish as a single food ingredient (Chang *et al.* 2008). Additionally, EU has controversially approved the use of pork and poultry in fish feed. This condition poses health issues and also conflicts with Islamic law regarding halal status. According to Islamic law, livestock that consumes impurities or unclean substances and causes a change in meat smell is called

jallah (Sabiq 2006). Fiqh experts have differing opinions on the legal status of jallah animals. These types of animals are considered disliked (makruh) by Imam Malik, Imam Ahmad, Shafi'i, and Hanafi. According to a previous study, jallah animals were referred to as haram (forbidden) by Hambali (Az-Zuhaili 2011). Jallah animals should be quarantined to ensure consumers can eat clean food (*istihalah*). The length of quarantine for different types of animals varies, such as 3 days for chickens and 40 days for camels, cows, and mammals, according to Imam (Az-Zuhaili 2011). Therefore, understanding the quarantine period for catfish fed with unclean feed (jallah animal derivatives) is important. This condition has significant financial and risk implications for catfish trading.

Catfish by-product processing has been developed as a raw material for the production of gelatin, commonly derived from farm animals, such as cod and salmon, or cows (Arnesen & Gildberg 2006; Arnesen & Gildberg 2007). However, the use of gelatin from cows has several risks, such as the transmission of mad cow disease (bovine spongiform encephalopathy or BSE) and non-compliance with Islamic and Jewish dietary laws. Consequently, there has been a 30% increase in demand for fish gelatin (Karim & Bhat 2009). The development of detection and identification techniques is important in addressing economic, health, and religious concerns related to food products, including the potential for adulteration through the use of less expensive materials or mislabeling (Man *et al.* 2007). Therefore, this study aimed to authenticate halal status of catfish skin gelatin by verifying feed origin and determining the contents of pig contaminants. Ensuring the authenticity of the product is also important to detect any fraud that could impact halal status.

### 2 Methodology

The credibility of halal standard was established through precise DNA analysis of pig DNA using Real-Time Polymerase Chain Reaction, also known as Quantitative Real-Time PCR (qPCR). This analysis was conducted at all stages, including catfish gelatin, feed containing pig, and the skin. Catfish gelatin was evaluated to determine gel strength (bloom)

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properties, and amino acid profile using Ultrahigh Performance Liquid Chromatography (UPLC). The functional group profiles were determined using Fourier Transform Infrared Spectroscopy (FTIR).

### 2.1 Formulation and Characterization of Catfish Feed Containing Pig Contaminants

The formulation of catfish feed containing pig contaminants followed SNI 01-4087-2006 guideline (National Standardization Agency 2006) for artificial feed intended for intensive African catfish rearing. In this study, pig contaminants were derived from pork liver in powder form, presented as flour. The formula was devised with consideration given to the crude protein content of each ingredient, as shown in Table 1. Catfish feed was evaluated for pellet durability index, water activity, and diameter size.

**Table 1:** The catfish feed formula containing pig contaminants

Material	Composition (%)	Crude protein ingredients (%)	Crude protein formulation (%)
Pork offal flour	25	60	15.00
Soybean meal	20	46	9.20
Bran flour	20	8	1.60
Pollard flour	10	15	1.50
Coconut cake flour	10	20	2.00
DDGS	10	30	3.00
CPO	2	8	0.16
Premix	2		
Corn oil	1		0.12
<b>Total</b>	<b>100</b>		<b>32.58</b>

Note: DDGS = distillers Dried grains with solubles, CPO = crude palm oil

### 2.2 Fish Enlargement

The test was conducted in accordance with the guidelines set out in SNI 7774-2013, which pertained to the expansion of catfish (*Clarias spp*) in ponds equipped with a tarpaulin measuring 1 x 1 x 1 m<sup>3</sup>. Catfish seed used in the study had an average length of 8.68 ± 0.76 cm, and the maximum stocking density was 200/m<sup>2</sup>. Furthermore, feeding frequency was twice per day with a rate of 3% biomass. The water in the pond was replaced every 2–6 days to maintain quality, with 10–20% being replaced each time. The enlargement process lasted for 80 days and included feeding catfish contaminants from pig feed.

### 2.3 Periodization of Istihalah

On the first day, fish were fasted and conditioned for 24 hours and subsequently fed with food that was free of pig contaminants for 3 and 6 days. *Istihalah* process was carried out in a pond that had been refilled with new water (Az-Zuhaili 2011). Catfish were transferred from the breeding pond to the quarantine pond. During *istihalah* period, the water in the pond was not refilled or replaced.

### 2.4 Manufacture and Characterization of Catfish Skin Gelatine

Preparation of gelatine from catfish skins was carried out by the immersion method in 50 mmol/L (1:8) acetic acid at 15 °C for 18 hours, followed by the extraction at 45 °C for 7 hours (Liu *et al.* 2008). Characterization of fish skin gelatine consisted of the yield, gel strength (bloom), amino acid composition, and functional groups profile measured by Fourier Transform Infrared (FTIR) method.

### 2.5 Specific DNA Analysis of Pig in Fish Feed, Catfish Skin, and Skin Gelatine

Pig-specific DNA was analyzed using qPCR at the Bogor MUI LPPOM Halal Laboratory, where DNA extraction was carried out on samples of feed, fish skin, and gelatine products from three quarantine periods (*istihalah*). Real-Time PCR was set at a temperature of 95 °C for 10 minutes for the initial denaturation, followed by 95 °C, 42 °C, and 72 °C for 15, 40, and 60 seconds, respectively. Each princess included 35 cycles for the denaturation, annealing, and extension stages. The fluorescence signal was read once at the end of each cycle and the desired result was generated using British English, with strict adherence to spelling, specific terms, and phrases.

## 3 Result

### 3.1 Chemical and Physical Composition of Catfish Feed

The water content of feed affects the durability against mold, thereby prolonging the shelf life. In this study, the maximum shelf life was achieved when feed had the right amount of water. The chemical composition of feed containing pork contaminants was calculated for fish feed. Feed containing pig contaminants used in this study met the quality requirements for African catfish feed, as shown in Table 2. A suitable and standardized feed is an important factor for growth of aquaculture. Table 3 shows the physical characteristics of catfish feed containing pig contaminants.

**Table 2:** Chemical composition of catfish feed containing pig contaminants and quality requirements for catfish feed

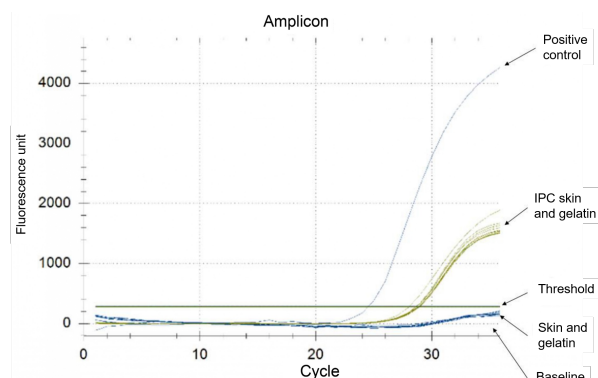
Chemical composition	Value (mean ± SD)	Quality requirements [a-d]
Protein content (%)	29.11 ± 5.37	min. 25
Water content (%)	8.15 ± 1.63	max. 12
Ash content (%)	7.53 ± 0.29	max. 13
Fat level (%)	17.34 ± 0.68	min. 5
Crude fiber content (%)	5.12	max. 6

**Table 3:** Physical characteristics of catfish feed containing pig contaminants

Physical properties	Value (mean ± SD)	Quality requirements [a-d]
Pellet durability index (%)	87.8	85 - 95 [a]
Water activity	0.609 ± 0.012	0.65 - 0.75 [b]
Feed diameter (mm)	3.6 ± 0.43	3 - 4 [c]

### 3.2 Specific DNA of Pigs in Fish Feed

The outcomes of DNA examination showed that catfish feed samples had a positive result for pig DNA, as evidenced by the graph surpassing the threshold (Figure 1). The accuracy of these results was confirmed by the presence of a positive internal control (IPC) in all samples.



**Figure 1:** Graph of DNA analysis results of fish feed in aquaculture with feed contains pig contaminants

### 3.3 Weight Growth and Catfish Aquaculture Survival

Table 4 shows the result of weight measurements and survival rate of fish. The body weight of cultivated catfish reached up to 83.31 ± 34.21 grams during cultivation, which is in the range of quality requirement (75-175 grams). However, survival measurements showed a value of 77% which was below the quality requirements (80-90%).

**Table 4:** Weight and survival rate of catfish during cultivation with feed containing pig contaminants compared to the quality requirements

Fish biology	Value (mean ± SD)	Quality requirements
Body weight (g)	83.31 ± 34.21	75-150 [21]
Survival	77	80-90 [20]

### 3.4 Yield and Strength of Catfish Gelatine Gel

The yield of catfish gelatine gel was not significantly different at the period of *istihalah*, where 0, 3, and 6 days were 8.94 ± 0.38, 9.34 ± 0.84, and 9.25 ± 0.07, respectively. In this study, the highest catfish gelatine gel strength was achieved by *istihalah* period of 0 days (132.5 ± 1.7), followed by 3 (130.2 ± 1.2) and 6 days (122.7 ± 2.8) bloom, respectively. Table 5 shows the result of yield and gel strength of gelatine.

**Table 5:** Gelatine characteristics at different periods of *istihalah*

Gelatine characteristics	The period of <i>istihalah</i>		
	0 days	3 days	6 days
Yield (%)	8.94 ± 0.38	9.34 ± 0.84	9.25 ± 0.07
Gel strength (bloom)	132.5 ± 1.7	130.2 ± 1.2	122.7 ± 2.8

### 3.5 Amino Acid Composition of Catfish Gelatine in Compliance with Halal Gelatine Standards

Table 6 shows that amino acid composition of catfish skin gelatine at different *istihalah* periods had different profiles. The highest glycine score was gelatine with *istihalah* 0 days (213.77 ± 2.21 mg/g) followed by a sample of 3 (217.45 ± 2.27 mg/g), and 6 days (264.26 ± 4.47 mg/g). The proline values from *istihalah* 0, 3, and 5 days were 125.96 ± 1.41 mg/g, 113.94 ± 1.29 mg/g, and 112.42 ± 2.05 mg/g, respectively.

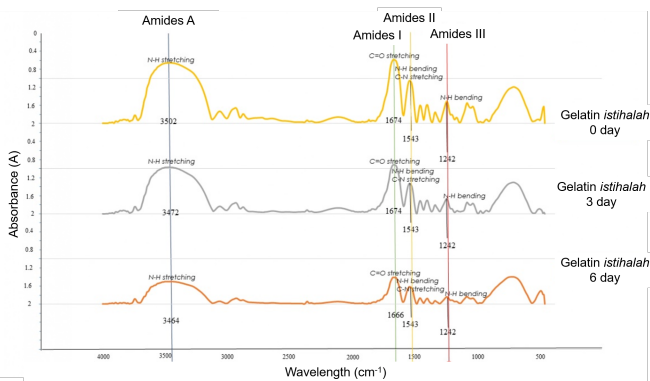
**Table 6:** Weight and survival rate of catfish during cultivation with feed containing pig contaminants compared to the quality requirements

Parameter	Quarantine periode ( <i>istihalah</i> )		
	0 days (mg/g)	3 days (mg/g)	6 days (mg/g)
Histidine*	7.41 ± 0.08	7.86 ± 0.13	12.79 ± 0.19
Threonine*	25.30 ± 0.21	29.28 ± 0.34	40.44 ± 0.71
Leucine*	22.45 ± 0.12	26.62 ± 0.25	31.12 ± 0.61
Lysine*	31.83 ± 0.24	37.49 ± 0.38	29.23 ± 0.52
Valine*	21.40 ± 0.16	25.20 ± 0.26	28.59 ± 0.52
Isoleucine**	12.66 ± 0.08	14.93 ± 0.13	17.49 ± 0.34
Phenylalanine*	16.24 ± 0.19	17.97 ± 0.15	28.53 ± 0.60
Aspartic acid	46.96 ± 0.37	48.16 ± 0.54	39.41 ± 0.51
Glutamic acid	82.23 ± 0.68	86.54 ± 0.97	74.02 ± 1.25
Serine	34.76 ± 0.68	39.28 ± 0.41	50.69 ± 1.58
Glycine	213.77 ± 2.21	217.45 ± 2.27	264.26 ± 4.47
Arginine	70.05 ± 0.60	83.15 ± 0.93	117.69 ± 1.21
Alanine	77.10 ± 0.60	89.22 ± 0.95	82.04 ± 1.49
Proline	125.96 ± 1.41	113.94 ± 1.29	112.42 ± 2.05

Note: \*significantly (P<0.05), \*\*significantly (P<0.01)

### 3.6 Functional Groups of Catfish Skin Gelatine in Compliance with Halal Gelatine Standards

The functional groups that characterize gelatine were amide A, I, II, and III with absorption regions of 3600-2300 cm<sup>-1</sup>, 1670-1636 cm<sup>-1</sup>, 1560-1335 cm<sup>-1</sup>, and 1300-1200 cm<sup>-1</sup>, respectively (Muyonga *et al.* 2004). The infrared gelatine spectrum and absorption peak wave numbers at each period are shown in Figure 2 and Table 7, respectively.



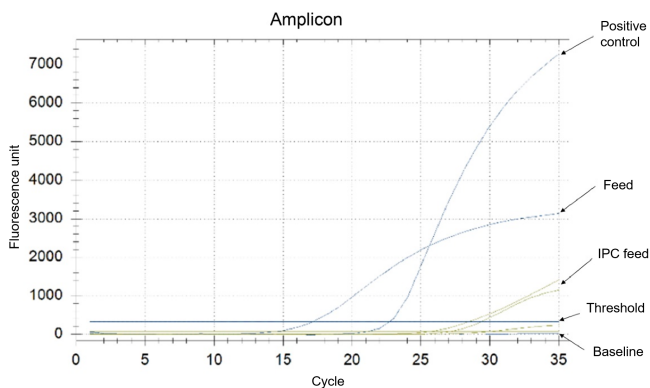
**Figure 2:** Graph of DNA analysis results of fish feed in aquaculture with feed contains pig contaminants

**Table 7:** Gelatine characteristics at different periods of *istihalah*

Absorption area	Absorption peak wave number (cm <sup>-1</sup> )			Material
	0 days	3 days	6 days	
Amida A	3448	3479	3464	NH stretching
Amida I	1674	1674	1666	C=O stretching

### 3.7 Specific DNA of Pigs in Fish Skin and Catfish Skin Gelatine

The results of DNA analysis showed that samples of fish and catfish skin gelatine gave undetectable (negative) results of pig DNA. This is because fish skin chart and catfish skin gelatine did not cut the threshold, as shown in Figure 3.



**Figure 3:** Graph of DNA analysis results of fish feed in aquaculture with feed contains pig contaminants

## 4 Discussion

The durability of 4 mm pellets was strong, with an endurance of 85-95% (Jónsson *et al.* 2007). This result suggests excellent properties for optimal cultivation. However, the accumulation of pellet damage can negatively impact fish weight and feed conversion (Haetami *et al.* 2017). In this study, feed had a time-saving water activity of 0.65-0.75, which met the quality requirements for African catfish feed. The diameter of catfish feed was suitable for growth rate (Hossain *et al.* 2000), which was associated with the size of feed used. The weight of catfish reflects the fulfillment of the farming requirements for growth in tarpaulin ponds. A lack of separation treatment for fish by size during cultivation can cause uneven feed consumption among fish. Additionally, diverse fish weights during harvesting can be caused by low water quality. This condition is also attributed to the absence of water treatment or recirculation systems. Poor water quality can affect the appetite of fish, thereby reducing feed intake of catfish (Alfia *et al.* 2013).

Survival measurements obtained a value of 77%, which fell below the desired quality standards of 80-90%. This difference may be attributed to the absence of any size-based separation process during the expansion process, where only one aquaculture pond was used. Consequently, the risk of cannibalism in catfish and growth of diverse weights in fish are increased significantly. According to a previous study, the yield of catfish skin gelatine was 10% (Liu *et al.* 2008). The rendering of gelatine may be due to the loss of collagen during extraction, specifically due to incomplete hydrolysis or when washing. Hydrolysis imperfections may occur due to the presence of insoluble collagen resulting from partial cross-linking during acid or base treatment, which could prevent gelatine formation (Jamilah & Harvinder 2002). In this study, the strongest catfish gelatine gel was achieved with *istihalah* period of 0 days, followed by 3 and 6, respectively. The strength of catfish gelatine was generally low compared to mammals, such as pig and cow skin. The range of pig gelatine strength was 326.47 ± 0.07 - 415.10 ± 1.21 bloom, higher than cow (193.49 ± 2.09 to 270.35 ± 8.02 bloom) at all pH levels (Hafidz *et al.* 2011). This result shows that pig gelatine is more rigid than beef, and contains a strength of approximately 71-426 bloom (Karim & Bhat 2009).

The proline content of fish gelatine was generally lower than mammals. This study showed that the highest amino acids composition from catfish skin gelatine were glycine (216.9 mg/g) and proline (116.5 mg/g) (Mostafa *et al.* 2015). In a study by Mostafa *et al.* (2015), catfish skin gelatine was found to have the highest levels of glycine (216.9 mg/g) and proline (116.5 mg/g). However, gelatine from cows, pigs, and fish show similar chromatogram spectra, as reported by Widyaninggar *et al.* (2012). To determine the origin of gelatine raw materials, studies analyzed the amino acid profile or composition (Azilawati *et al.* 2015). A proportion of amino acid was used to establish the difference in species, with a threshold of 0.6% or higher, suggesting a significant difference. The result showed a significant difference in pig and cow gelatine when compared to fish with respect to specific amino acids. Furthermore, the concentrations of serine, threonine, and methionine were higher in fish gelatine than in cows and pigs. The levels of glycine and proline in fish gelatine were relatively lower than in pig skin and cowhide. In general, amino acid composition of fish skin gelatine was lower than mammalian skin, such as pig and cow (Azilawati *et al.* 2015). In this study, the characteristic functional groups in gelatine included amide A, I, II, and III, with an absorption region of 3600-2300, 1670-1636, 1560-1335, and 1300-1200 cm<sup>-1</sup>, respectively (Muyonga *et al.* 2004). A low intensity of amide III band was associated with collagen denaturation into gelatine and changes in the triple helical structure (Muyonga *et al.* 2004). Amide I uptake region showed the presence of the α-helix component (Kong & Yu 2007). There was difficulty in differentiating pig and cow gelatine due to both having similar spectra. However, fish gelatine had distinctly different spectra, with significant differences in the range of 1000-1100 cm<sup>-1</sup> (amide III), compared to others (Cebi *et al.* 2016). Amide III absorption peak in catfish skin gelatine was at 1242 cm<sup>-1</sup>. Differences between cow and pig gelatine could be observed in the spectra range of 3290-3280 cm<sup>-1</sup> and 1660-1200 cm<sup>-1</sup>. The analysis was based on the use of bovine and pig gelatine spectra, followed by testing with Principal Component Analysis (PCA) and Cooman plot visualization (Hashim *et al.* 2010).

DNA analysis of catfish fed with pig offal showed the presence of pig DNA under controlled conditions for up to 36 hours, as reported by Wan Norhana *et al.* (2012). This result was due to contamination in the larger pool and water system used in the process. *Istihalah* process in 0-day transition from aquaculture ponds to quarantine ponds, along with a 24-hour conditioning treatment, had a significant effect on the undetectable pig DNA in catfish skins. Specifically, this treatment resulted in the absence of pig DNA in catfish skins after 3 and 6 days in quarantine, as well as in gelatine shell. The 24-hour fish control was implemented to empty the stomach of feed, as suggested by Agustono (2014).

## 5 Conclusion

In conclusion, this study explicitly showed that *istihalah* period of 0 days was adequate to remove pig contaminants from catfish skin, as evidenced by the absence of pig DNA in subsequent analyses. This result

was particularly significant because of the indication that the metabolic processes of catfish could quickly purge the skin of specific dietary impurities, which has considerable implications for halal food industry. Furthermore, this result increased the potential for using catfish by-products in halal food production and provided a vital insight into the efficiency of *istihalah* practices. By verifying that a zero-day quarantine could effectively rid catfish skin of pig DNA, the results of this study provided more streamlined and cost-effective protocols in halal aquaculture. The result also contributed to the ongoing efforts to ensure that halal food standards were rigorously maintained, thereby reinforcing consumer trust and compliance with religious dietary laws.

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## Conflict of Interest

The authors declare no conflict of interest.

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