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Authentication Propriety Standard Halal Gelatine Catfish Skin From Periodization Quarantine (Istihalah) In Cultivation With Feed Containing Pig Contaminants

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ABSTRACT

The limitations of fish meal as a feed ingredient were addressed by utilizing food waste, specifically pig innards. Concurrently, the rising demand for halal gelatin in the market generated a substantial need for fish skin by-products. This study aimed to determine the authentication propriety of halal standards for gelatin derived from catfish skin that had been fed with fish meal containing pig components over three different quarantine periods (istihalah): 0, 3, and 6 days after harvesting. The verification of the halal standard was conducted through the specific DNA analysis of pig components. This testing was performed at each stage, including the feed containing pig, the fish skin, and the catfish gelatin. The gelatin in the catfish skin was predominantly composed of the amino acids glycine and proline. The fish enlargement stage resulted in a skin yield of 5.36 ± 0.75%. The yields of gelatin were 8.67%, 9.94%, and 9.19%, with gel strengths of 133.4 ± 1.2 bloom, 129.9 ± 1.4 bloom, and 121.9 ± 2.8 bloom for the 0, 3, and 6 days of istihalah, respectively. Gelatin characterization using FTIR indicated the presence of functional groups such as amide A, amide I, amide II, and amide III. Real-time PCR detected the presence of pig DNA in the feed; however, it was not detected in the skin and gelatin of the catfish. Ultimately, a quarantine period of 0 days for catfish fed with pig-containing feed was sufficient to cleanse the catfish skin of pig contaminants, with no indication of pig DNA being found.

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1. Introduction

Indonesia is the fourth-largest fisheries producer in the world, with aquaculture production reaching up to 4.2 million tons/year (UN FAO, 2016)the chemical compound which causes cigarette addiction, could be responsible for some lung cancers. Evidence for genetic influence on smoking behavior and nicotine dependence has prompted a search for susceptibility genes. Furthermore, assessing the impact of sequence variants on smoking-related diseases is important to public health. Recently, the locus containing two genes encoding nicotine acetylcholine receptor subunits, CHRNA3 and CHRNA5, were shown to be associated with lung cancer risk. Here we performed to directly test whether variants at codon 398 (D398N. KKP fisheries statistics from 2010-2014 showed a 23% increase in aquaculture production per year with major commodities being seaweed (27%), tilapia (21%), carp (20%), and catfish (29%) (Kementerian Kelautan dan Perikanan, 2015). Catfish is one type of fish with an omnivorous feeding pattern, meaning it consumes a variety of foods. Fish farming in catfish aquaculture is generally carried out on a small scale with commercial or artificial feeds. The primary ingredient in the manufacture of fish feed is fish meal, an imported and costly commodity (Kementerian Kelautan dan Perikanan, 2018).

Fish nutritional needs vary by species. Herbivorous fish consume a feed mixture that may contain plant proteins (e.g., soy, corn), vegetable oils, minerals, and vitamins. In the wild, carnivorous fish such as salmon eat other fish. With the increasing demand for catfish, it has become a sought-after aquaculture commodity, which has also led to a rise in the demand for catfish feed over time. The feed has historically 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 for feed ingredients is the utilization 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 their potential animal welfare

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issues, the consistency of the generated quality of feed, health concerns, and religious issues in some groups or countries. One of the current issues in catfish feed is the protein intake, which is often fraudulently augmented with sources from other animals, one of which is 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; Kanchanaree et al., 1995). Additionally, the EU has controversially approved the use of pork and poultry in fish feed. This condition not only poses health issues but also conflicts with Islamic law regarding halal status. According to Islamic law, livestock that consumes impurities or unclean substances and causes a change in its meat smell is called jallalah (Sabiq, 2006). Fiqh experts have differing opinions on the legal status of jallalah animals. Imam Malik, Imam Ahmad, Shafi'i, and Hanafi consider it disliked (makruh). Hambali deems it haram (forbidden) (Az-Zuhaili, 2011). Jallalah animals should be quarantined to ensure the consumers can eat clean food (istihalah). The quarantine period varies for each animal: 3 days for chicken (poultry) and 40 days for camels, cows, and mammals according to Imam (Az-Zuhaili, 2011). Therefore, information about the quarantine period of fish fed with unclean feed (jallalah animal derivatives) has become the basic understanding for determining the quarantine of catfish. This affects both the financial implications and the risks associated with catfish trading.

Catfish by-product processing has been developed as a raw material for gelatin. Commercial gelatin is generally derived from farm animals or cold-water fish such as cod (Arnesen & Gildberg, 2006) and salmon (Arnesen & Gildberg, 2007). Although gelatin from cows is preferred (Cho et al., 2005), it carries the risks of mad cow disease (bovine spongiform encephalopathy-BSE) and the non-fulfillment of Islamic and Jewish dietary laws. These concerns have led to an increased demand for fish gelatin by up to 30% (Karim & Bhat, 2009). The detection and identification techniques for determining the species origin in food products have been developed with regard to economic, health, and religious issues. The challenges arise from food adulteration issues, such as the replacement with less expensive materials, masking product appearance, and mislabeling (Che Man et al., 2007). Hence, this research aimed to authenticate the halal status of catfish skin gelatin by verifying the feed origin, specifically whether it contains pig contaminants. The authenticity test of the product is essential to detect any fraud that has implications for the halal status of the product.

2. Methodology

The authenticity of the halal standard was verified through specific DNA analysis of pig DNA using Real-Time Polymerase Chain Reaction, also known as Quantitative Real-Time PCR (qPCR). This test was conducted at each stage, including the feed containing pig, the fish skin, and the catfish gelatin. The catfish gelatin was characterized for its gel strength (bloom) properties, amino acid profile using Ultrahigh Performance Liquid Chromatography (UPLC), and functional group profiles using Fourier Transform Infrared Spectroscopy (FTIR).

A. Formulation and Characterization of Catfish Feed Containing Pig Contaminants

The composition of catfish feed containing pig contaminantwas formulated as followed to SNI 01-4087-2006 guideline about the artificial feed for intensive African catfish. The pig contaminant was from pork liver in powder form as flour. The formula was designed based on the crude protein content in each ingredient as presented in Table 1. The fish feed was characterized for pellet durability index, water activity, and diameter-size.

Table 1 The catfish feed formula containing pig contaminant

Material	Composition (%)	Crude protein ingredients (%)	Crude protein formulation (%)
Pork offal flour	25	60	15
Soybean meal	20	46	9.2
Bran flour	20	8	1.6
Pollard flour	10	15	1.5
Coconut cake flour	10	20	2
DDGS	10	30	3
CPO	2	8	0.16
Premix	2		
Corn oil	1	12	0.12
total	100		32.58

B. Fish Enlargement

The test refers to SNI 7774-2013 about the enlargement of catfish (Clarias spp) in fiber ponds equipped with a tarpaulin (1 x 1 x 1m3). The size of catfish seed was 8.68 ± 0.76 cm with a maximum stocking density of 200/m2, a frequency of feeding of twice/day, and a feeding rate of 3% biomass/day. The water in the pond was refilled every 2-6 days to maintain water quality with 10 - 20% of water replacement. The enlargement process was carried out for 80 days in parallel to give feeding containing pig contaminants.

C. Periodization of Istihalah

The day-0 was conditioning the fish for 24 hours. The day-3 and day-6 were aimed to feed the fish without pig contaminants for 3 and 6 days after 24 hours of fasting. The istihalah period was carried out in different pond refilled with new water (Az-Zuhaili, 2011): catfish was removed from the rearing pond and transferred into the quarantine pond. During the istihalah period, the water was not refilled or replaced.

D. Manufacture and Characterization of Catfish Skin Gelatine

Preparation of gelatine from catfish skins was carried 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 the Fourier Transform Infrared (FTIR) method.

E. Specific DNA Analysis of Pig in Fish Feed, Catfish Skin, and Skin Gelatine

Pig specific DNA analysis was performed by using qPCR at Bogor MUI LPPOM Halal Laboratory. The DNA extraction was carried out on feed samples, fish skin, and fish gelatine products of three quarantine periods (istihalah). Real-Time PCR setting temperature and time were 95°C (10min) of 1 cycle as initial denaturation, 95°C (15s), 42°C (40s), and 72°C (60s) of 35 each cycle for the denaturation, annealing, and extension stage. The fluorescence signal reading was done once at the end of each cycle.

4. Results

A. Chemical and Physical Composition of Catfish Feed

Fat is one of the main sources of energy needed by fish and plays a role in feed storage. The water content of the feed plays a role in the durability of the feed against mold so that the feed has a long shelf life and shelf life maximum. Calculation of the chemical composition of feed is carried out on fish feedcontaining contaminants in pork. Based on its chemical composition, the catfish feed containing pig contaminants used in this study has met the quality requirements of African catfish feed (see Table 2). Suitable and standardized feed becomes an important factor for the growth of aquaculture. Physical characteristics of catfish feed containing pig contaminants were presented in Table 3.

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
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]

B. Specific DNA of Pigs in Fish Feed

The results of DNA analysis showed that catfish feed samples had a positive result of pig DNA, which was indicated by a feed graph cutting the threshold, as can be seen in Figure 1. The results were valid because the internal positive control (IPC) of all samples indicated positive responses.

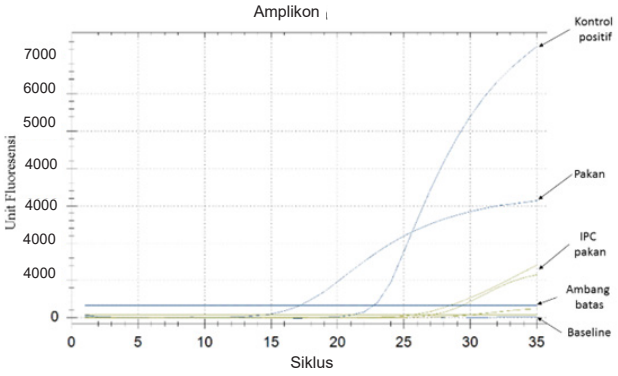

Figure 1 Graph of DNA analysis results of fish feed in aquaculture with feed contains pig contaminants

C. Weight Growth and Catfish Aquaculture Survival

The result of weight measurements and the survival rate of fish was presented in Table 4 below.

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]

D. Yield and Strength of Catfish Gelatine Gel

The results of yield and gel strength of gelatine were presenter in Table 5.

Gelatine Characteristics	The period of istihalah		
	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

E. Amino Acid Composition of Catfish Gelatine in Compliance with Halal Gelatine Standards

The amino acid composition of catfish skin gelatine at different istihalah period resulted in different profiles and values as pr sented in Table 6 below. The highest glycine score was the gelatine with istihalah 0 days (213.77 ± 2.21 mg/g) followed by, sample of istihalah 3 days (217.45 ± 2.27 mg/g), and istihalah 6 days (264.26 ± 4.47 mg/g). The proline values from istihalah 0 days, 3 days, 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. Amino acid composition of gelatine from catfish skin at three quarantine periods (istihalah)

Parameter	Quarantine Period (istihalah)		
	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

F. Functional Groups of Catfish Skin Gelatine in Compliance with Halal Gelatine Standards

The functional groups that characterize gelatine were amide A (absorption region of 3600 to 2300 cm-1), amide I (1670 to 1636 cm-1), amide II (1560 to 1335 cm-1), and amide III (1300 to 1200 cm-1+) (Muyonga et al., 2004). Absorption peak wave numbers at each period were presented in Table 7. The infrared gelatine spectrum was presented in Figure 2.

Table 7. Functional groups characterization of catfish gelatine at three quarantine periods (istihalah)

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

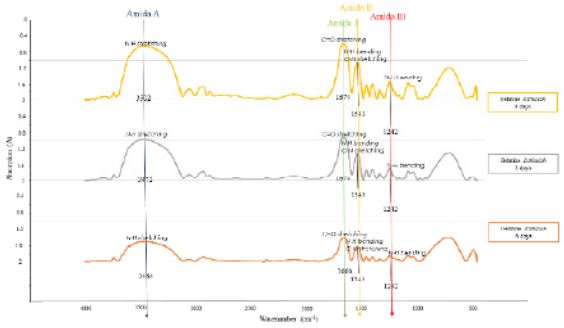

Figure 2 Infrared spectrum of catfish gelatine at three quarantine

G. Specific DNA of Pigs in Fish Skin and Catfish Skin Gelatine

The results of DNA analysis showed that saples of fish skin and catfish skin gelatine gave undetectable (negative) results of pig DNA in dicated by fish skin chart and catfish skin gelatine did not cut the threshold, as can be seen in Figure 3.

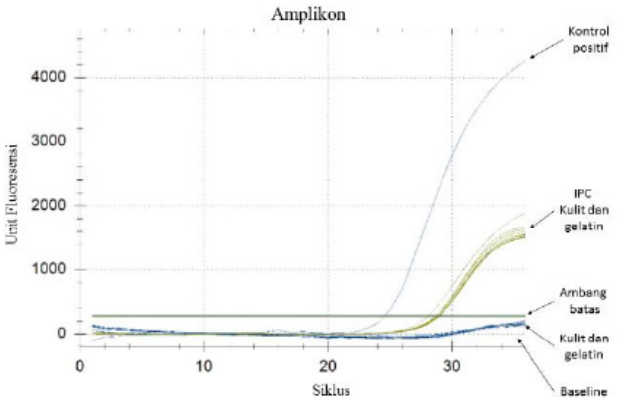

Figure 3 DNA analysis results of catfish and gelatine skin from quarantine periodization

5. Discussion

The endurance of 4mm-size pellets was 85-95% (Jónsson et al., 2007) that represented strong durability properties for an optimum cultivation process. However, the accumulation of pellet damage can affect fish weight and feed conversion (Hae tami et al., 2017). The pellet water activity (aw) of 0.65 - 0.75 showed that the feed had a time-saving. The diameter of the catfish feed in this study had met the quality requirements of African catfish feed. The growth rate of catfish was as sociated with the size of the feed used (Hossain et al., 2000). Suitable and standardized feed becomes an im portant factor for the growth of aquaculture. The weight of fish indicates the fulfillment of catfish far ing requirements for enlargement in tarpaulin ponds. The absence of fish separation treatment according to size at the time during cultivation will result in an unequal consumption of feed by fish. Diverse fish weights during harvesting can also be result from low water quality. It might be caused by the ab sence of water treatment or recirculation systems. Decreas ing water quality can affect the appetite of fish, then af fect the feed intake of catfish (Alfia et al., 2013).

Survival measurements showed a value of 77%. This val ue is below the quality requirements (80-90%). This differ ence might occur because of the utilization of only one aquaculture pond during the enlargement process with out any separation process based on sizes. This in creased the potential (risk) of cannibalism in cat fish and the growth of more diverse fish weights. The yield of catfish skin gelatine was 10% (Liu et al., 2008). The rendering of gelatine might be caused by the loss of collagen during extraction, especially a series of wash ing steps or imperfect hydrolysis. Hydrolysis imperfec tions can be caused by the partial cross-linked struc ture during acid or base treatment resulted in the presence of insoluble collagen and could not form gelatine (Jamilah & Harvinder, 2002). The highest catfish gelatine gel strength was achieved by is tihalah 0 day period, followed by 3-day and 6-day istihalah, respectively. The strength of the catfish gelatine is generally lower than gelatine from mammals i.e. pig and cow skin.

The strength of pig gelatine ranged from 326.47 ± 0.07 - 415.10 ± 1.21 bloom or higher than cattle (193.49 ± 2.09 to 270.35 ± 8.02 bloom) at all pH (Raja Nhari et al., 2011). It showed that pig gelatine was more rigid than beef gel atine. The strength of fish gelatine gel was approxi mately 71 - 426 bloom (Karim & Bhat, 2009).

The proline content of fish gelatine was generally lower than gelatine from mammals. 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). Gelatine from cows, pigs, and fish are known to have a similar chromatogram spectrum (Widyaninggar et al., 2012). The amino acid composition can characterize the origin of gelatine raw material by com paring amino acid profile or composition (Azilawati et al., 2015). The approach of determining raw material origin was carried out through a proportion of amino acid difference. The amino acid difference should be divided by the fac tor of 10 (more than 0.6% of proportion indicates a sig nificant difference of species). These resulted in sig nificantly different pig and cow gelatine compared to fish gelatine for particular amino acids. In addition, the composition of serine, threonine, and me thionine were higher in fish gelatine compared to cow and pig gelatine, whereas the glycine and proline in fish gel atine were lower than the pig skin and cowhide. The ami no acid composition of fish skin gelatine was gen erally lower than of mammalian skin gelatine, such as pigs and cattle (Azilawati et al., 2015). The functional groups that characterize gelatine were amide A (absorption region of 3600 to 2300 cm-1), amide I (1670 to 1636 cm-1), amide II (1560 to 1335 cm-1), and amide III (1300 to 1200 cm-1+) (Muyonga et al., 2004). The low intensity of amide III band was associated with the collagen denatur ation into gelatine and the triple helical structure chang es (Muyonga et al., 2004). The amide I uptake region showed the α-helix component (Kong & Yu, 2007). Pig and cows gelatine had a similar spectrum that might it would be difficult to be distinguished (Hashim et al., 2010). However, fish gelatine was easily distinguished with signifi cantly different spectra of 1000 - 1100 cm-1 (amide III) com pared to others (Cebi et al., 2016). The amide III absorption peak in catfish skin gelatine was at 1242 cm-1 of wavelength. Differences in cows and pig gelatine could be found in the spectrum range of 3290 - 3280 cm-1 and 1660 - 1200 cm-1. The principle of this analysis was the utilization of bo vine gelatine and pig gelatine spectra, followed by test ing with substantial component analysis (PCA) and Cooman plot visualization (Hashim et al., 2010).

The DNA analysis on catfish be ly part fed with pig offal showed the presence of pig DNA under controlled conditions up to 36 hours (Wan Norhana et al., 2012). The result might be caused by the mastery process in the same enlargement pool and water that causes contamination. The istihalah 0 days with separation from aquaculture ponds to quarantine ponds and 24-hour conditioning treatment had an effect on the undetectable pig DNA in catfish skins. This results also had an impact on the absence of pig DNA in the skins of catfish within 3 days and 6 days of quarantine period and the gelatine shell 3 gelatine during periods. Fish control for 24 hours was aimed to empty the stomach from feed (Agustono, 2014).

6. Conclusion

The study conclusively demonstrated that an istihalah period of 0 days was sufficient to eliminate pig contaminants from the catfish skin, as evidenced by the absence of pig DNA in sub sequent analyses. This finding is particularly noteworthy be cause it suggests that the catfish's metabolic processes are capable of rapidly purifying the skin from specific dietary im purities, which has significant implications for the halal food in dustry. Not only does this bolster the potential for utilizing catfish by-products in halal food production, but it also provides a crucial insight into the efficiency of istihalah practices. By con firming that zero-day quarantine can effectively cleanse catfish skin of pig DNA, the results of this research could pave the way for more streamlined and cost-effective protocols in halal aqua culture. This contributes to the ongoing efforts to ensure that halal food standards are rigorously upheld, thereby reinforc ing consumer trust and compliance with religious dietary laws.

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