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PCR-Based DNA Detection of Bovine on Medium of Cultured Meat for Consumption: Religious Perspectives

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Abstract

Food technology advancements have led to the development of cultured or artificial meat produced in a laboratory setting. Recently, Israel and Singapore have built factories specifically for cultured meat production. Malaysia is also informed about a cultured meat processing facility, which is expected to be completed in 2024. This alternative is a viable solution to meet the boosting needs and demand for meat-based food products while minimising the environmental impact of traditional livestock farming. Nevertheless, despite its potential benefits, cultured meat faces diverse challenges, including religious concerns for faiths with specific dietary requirements. Therefore, this research intended to determine the detection of DNA of bovine on the cultured medium by using polymerase chain reaction (PCR) analysis. The polymerase chain reaction analysis was conducted by targeting the mitochondrial DNA of the cytochrome oxidase II (COII) gene sequence and produced an amplicon size of 165 bp. The PCR was obtained by using the sample of medium mix with different concentrations (10-20%) of Foetal Bovine Serum (FBS) (Capricorn Scientific, Ebsdorfergrund, Germany), and the cell was harvested on different days. For DNA extraction, GENEAID Blood/Cell DNA mini kit (Taipei, Taiwan) was used. The findings demonstrate that DNA concentration in foetal bovine serum content was detected on the cultured medium. The presence of DNA contradicts religions such as Islam and Judaism that have strict standard dietary practices known as halal and kosher respectively. This study serves as a reference for the consumption of cultured meat for the consumer, particularly for Muslim and Jewish communities.

Keywords: *Cultured Meat, Polymerase Chain Reaction, Cultured Medium, Halal, Kosher.*

INTRODUCTION

According to Ramani et al. (2021), most of the world's population consider meat as the primary protein source in their daily diet. Meat products are also made into luxury foods and have dedicated fans (Chriki & Hocquette 2020). In fact, along with the growth of the human population, the demand for meat-based products also grew drastically (Kashim et al. 2022). One of the measures to meet the needs of meat is to diversify methods and analyses linked to protein production from meat (Aiking 2014). Recently, innovation in food technology that produces cultured meat is becoming famous worldwide (Awang 2021). The early history of cultured meat began around 1930 as an alternative to conventional meat by Frederick Edwin Smith and Winston Churchill (Arshad et al. 2017). In 2000, the National Aeronautics and Space Administration (NASA) developed a laboratory to

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explore cultured meat to grow myoblasts in suspension culture as a supply system for long-term flight and use in the space station (Wolfson 2002; Zhang et al. 2020). Ultimately, cultured meat was successfully produced by scientist Mark Post in 2013 (Zhang et al. 2020). This world's first cultured meat burger patty, costing thousands of dollars and made from at least 10,000 individual pieces of muscle (Kadim et al. 2015), was introduced in 2013 (Choudhury et al. 2020), and it honoured the beginning of the commercial availability of cultured meat.

Through the Food and Drug Administration (FDA), the American government has yet to give favourable support to cultured meat-based products. Until now, no cultured meat-based products have been marketed in America (Kateman 2021). In 2022, Israel declared the world's first cultured meat industry and factory capable of delivering 500 kilograms of cultured meat per day. Future Meat Technologies in Rehovot, Israel, plans to market its products to surrounding countries and become a substantial producer. In addition, the Singapore government approved the first regulations for cultured meat in 2020. Eat-Just, a cultured meat producer that works with the Singapore government, has marketed the first cultured meat in the world through a restaurant (Lucas 2020). Singapore has built a 30,000-square-foot factory expected to be operational in 2023. It follows Singapore's desire to become Asia's primary cultured meat producer (Begum 2022). In general, cultured meat delivers eclectic advantages in replacing conventional meat and solving financial, animal welfare, ethical, resource scarcity, and general health issues (Stephens et al. 2018; Zhang et al. 2020). Nonetheless, Zhang et al. (2020) maintained that although cultured meat could help provide meat needs that previously depended on conventional meat, various technology-related challenges must be resolved. It includes challenges and debates from a religious perspective (Hamdan et al. 2017; de Weele & Driessen 2013).

Singapore's desire to market cultured meat in Southeast Asia has stirred apprehension among Muslim-majority countries such as Malaysia, Indonesia and Brunei (Kashim et al. 2023). Currently, Malaysia is also informed about a cultured meat processing facility, which is expected to be completed in 2024. Islam in Southeast Asia is paramount; Indonesia has the most significant number of Muslims worldwide, representing more than 200 million people (World Population Review 2022). According to Houben (2003), Islam plays a prominent role in lifestyle, law and politics. Furthermore, Islam in Southeast Asia also influences the economy and consumerism, media, culture and identity (Williams & Kamaludeen 2017). Hence, the marketing of cultured meat products in Southeast Asia is related to Islamic religious components with specific conditions and guidelines for food consumption. Blood-derived products are prohibited in some religions, including Islam and Jews (Fischer 2016), while vegetarians and vegans refrain from consuming them out of ethical concerns (Hopkins & Dacey 2008). A large part of the population prefers not to consume blood-derived products for health reasons, either due to allergens in the blood or their belief that the blood contains toxic metabolites that make it unsafe for human or animal consumption (Kashim et al. 2017). Consumer trust in consuming these products will increase as a result of clear labelling and regulatory action.

The drastic blossoming of innovative marketing of cultured meat used as a food product is taken very seriously by Muslims (Awang 2021). This innovation has raised a big question for Muslims about whether cultured meat is *halal or* consumable. Consuming *halal* food is obligatory and must be followed by every Muslim. Whereas for many Jews, keeping kosher is more than just a matter of health or food safety. It has to do with respect and obedience to religious custom. This study is a preliminary investigation to develop a reference for religious or vegan consumer to decide the status of cultured meat. This study is essential because it is a proactive step as the primary reference for Muslims, Jewish or vegan, especially regarding the latest technology. Through this technology, one can solve the perplexity of the consumers, especially in Southeast Asia. Accordingly, this study aimed to examine the status of cultured meat by detecting the specific DNA of bovine from cultured medium. In addition, this study will serve as a reference source for the general public such as for Muslims, Jewish, or vegan who are particular and selective in their dietary practices and other nations that maintain similar values.

What is Cultured Meat?

By definition, 'cultured meat', also known as cultivated meat, cell-based meat, slaughter-free meat and clean meat (Gaydhane et al. 2018) is in vitro, synthetic or lab-grown meat which refers to meat produced in a bioreactor through tissue engineering technology (Bhat, Kumar & Fayaz 2015; Zhang et al. 2020). Cultured meat can be distinguished in several ways based on its source, manufacturing process, medium, and final

product (Qotadah et al. 2022). To produce meat without conventional animal husbandry, animal cells are often extracted and cultivated in a laboratory. Cells, culture medium, proteins, growth factors, scaffold and circumstances are vital components in the laboratory synthesis of cultured meat (Gaydhane et al. 2018). Although it is referred to as synthetic meat since it does not involve the slaughter of animals, cultured meat is made from actual animal cells. Since most edible meat consists of skeletal muscle tissue, the postnatal/post-hatch skeletal muscle cells (satellite cells) or embryonic myoblasts are the most suitable cell sources for producing cultured meat (Edelman et al. 2005).

Furthermore, there are several other essential cells with distinct positions that should be considered to reflect the natural environment, textures and structures as closely as possible to the actual meat, including (i) adipocytes for fat production, (ii) endothelial cells for vascularisation and (iii) chondrocytes and/or fibroblasts for connective tissue production (Datar & Betti, 2010). However, since its cells, especially the muscle cells, cannot synthesise nutrients (Kadim et al., 2015), it must obtain its nutrients from the growth culture media. For the consumption of cultured meat to be acceptable, several requirements must be met, including mimicking the characteristics of conventional meat, a high rate of protein synthesis in skeletal muscle and a good efficiency ratio (Pandurangan & Kim 2015).

According to Bhat et al. (2015), cultured meat is yielded outside the animal's body by producing cells from specialised stem cells. The stem cells and tissue are then placed in an appropriate medium for their growth and maturation into muscle fibres, which are the main components of meat. The medium used must also include all the nutrients and sufficient substrate to allow cells and tissues to reproduce and mature well (Bhat et al. 2015). In contrast to conventionally raised meat, cultured meat exhibits distinct physical characteristics, resembling minced meat rather than a typical meat cut (Qotadah et al. 2022). In short, the production of cultured meat is shown in Figure 1.

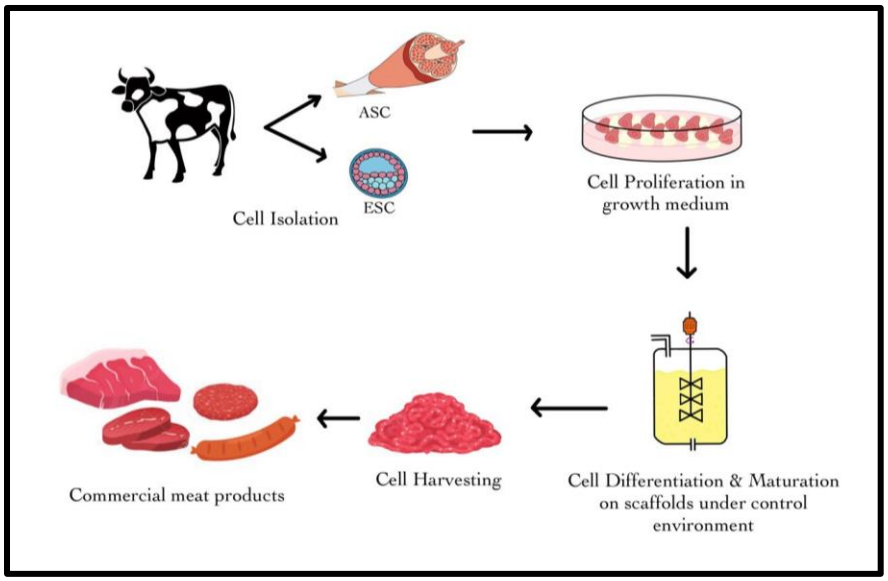

Figure 1. Cultured meat process flow

Two standard techniques are used in cultured meat production, i.e., scaffolding and self-organising (Edelman et al. 2005; Bhat et al. 2015b). Scaffolding technique, which uses the cell culture technique, is a process where the scaffold, commonly used microcarrier beads and collagen-based meshwork as a carrier of satellite cells or embryonic myoblasts derived from animals through biopsy fitting for producing boneless and boneless meats, such as sausage and burger patty. Whereas self-organising, which uses the tissue culture technique, includes all tissues and growth conditions in specific proportions that mimic the *in vivo* situation and is suited for generating highly structured meat such as steaks (Edelman et al. 2005).

However, with advanced technologies nowadays, the techniques for producing cultured meat are broadened into newly developed ones such as (i) organ printing, i.e. a process of spraying cell balls or live cells in layers

over the gel that functions as a printing paper, (ii) biophotonics, i.e. a process of using laser light to keep cells in place and enable them to interact and develop in an orderly framework, and (iii) nanotechnology, i.e. a process of developing assemblers or nanorobots that can selectively arrange similar molecules or atoms to form the whole structure (Bhat et al. 2015b; Gaydhane et al. 2018).

Advantages of Cultured Meat

Cultured meat renders many advantages, causing various parties to expect this technology to be widely used, especially in the food industry. First, cultured meat could provide benefits in terms of health and safety to humans (Zhang et al. 2020; Chriki & Hocquette 2020). It is because cultured meat is produced *in vitro* in a sterile environment. Thus, the spread of disease-causing pathogens is thereby prevented. The production of cultured meat is not exposed to harmful pathogens such as E. coli, Salmonella or Campylobacter, which contribute to diseases that often infect humans (Shapiro 2018). Besides, infectious diseases like swine flu, bird flu, and other zoonotic illnesses that can spread to humans can thrive in large-scale animal farming. Many animals receive antibiotics and vaccines to stop the spread of these diseases, endangering human health and contributing to antibiotic resistance (World Health Organization, 2017).

An advantage of cultured meat is its capacity to reduce animal suffering. To supply meat to the human population, billions of farm animals are slaughtered yearly (Zhang et al. 2020). As an alternative, cultured meat technology is an option to produce meat through *in vitro* techniques to meet global needs (Bhat & Bhat 2011; Kashim et al. 2022). In theory, each parent cell concerned with culturing can multiply meat. In addition, the number of animals required to prepare tissue samples is much lesser than the number of animals involved in conventional meat production. The final advantage of cultured meat technology is its contribution to sustainability and environmental protection. It is possible as this technology uses less land than conventional meat (Post 2012; Lynch & Pierehumbert 2019). Lynch and Pierehumbert (2019) proposed that cultured meat technology would reduce global warming compared to conventional animal husbandry caused by methane emissions from the digestive tract of herbivorous animals. In addition, cultured meat technology does not require large pastures. Accordingly, it could help mitigate deforestation and land degradation, major environmental issues associated with conventional agriculture (Giacalone & Jaeger, 2023). Therefore, cultured meat can be categorised as environmentally friendly and sustainable technology.

Cultured Meat Issues

According to a study attended by Chriki and Hocquette (2020), apart from the challenges from a technical point of view, several issues related to cultured meat were highlighted. Firstly, the problems related to the use of serum in cultured meat. The production of cultured meat is an alternative to creating a slaughter-free environment, but the serum used is a medium based on the blood of dead calves. According to Jochems et al. (2002), FBS was collected from the blood of fetal cows in the second and third trimesters of the pregnancy of pregnant cows. In addition, there are also some scientific controversies regarding the use of FBS in culture systems. This is due to the fact that FBS is a combination of compounds believed to contain contaminants such endotoxin, mycoplasma, virus, or prion protein as well as numerous unidentified chemical constituents (Fang et al. 2017). However, issues have been raised about the biological safety of blood obtained from slaughtered animals, such as the transmission of spongiform encephalopathy (Ofori & Hsieh 2011). Its limited use in food applications is also a result of religious regulations and negative consumer perceptions of blood for consumption.

Next, cultured meat raises ethical and religious issues (Kashim et al. 2023). The production of unnatural cultured meat and an FBS medium based on animal blood is unethical. It is prohibited from a religious point of view, especially in Islam and Jewish, which emphasises the issue of *halal* and kosher in food. Halal food is defined as food that is permitted for consumption by Muslims according to the Al-Quran (Kashim et al. 2023). Kosher, which means fit or proper to eat, is defined by the laws of Torah which is the compilation of the first five books of the Hebrew Bible. Both halal and kosher food laws clearly prohibit the use of blood, but these prohibitions arise from various philosophies. Islam forbids consuming animal blood according to Quran and it is believed to contain various microorganisms and metabolic products that are potentially harmful and toxic (Al-Qabisy

2012; Eliasi & Dwyer 2002). Meanwhile, Jewish practitioners are prevented from eating blood because blood is considered synonymous with life (Eliasi & Dwyer 2002).

The techniques recommended for slaughtering animals in accordance with halal and kosher rules and regulations are designed to ensure that the animal's blood is discarded as much as possible. Additionally, just like Seventh Day Adventists, vegetarians and vegans refrain from consuming any animal products or blood for a variety of reasons, such as their religious beliefs, personal preferences, support for animal rights, health, and the environmental benefits derived from eating plants (Alvaro 2019).

An alternative plant-based medium that is suitable for use on a large scale that is comparable in effectiveness to FBS is still being tested by scientists (Kashim et al. 2022). Besides, the long-term effect of consuming cultured meat on health remains a question. Mere assumptions cannot decide the benefits or harms of cultured meat because the process is controlled in a laboratory (Hasim et al. 2020). This ethical issue is also closely related to the views of Islam and Jews since there is no decisive proof regarding the safety of consuming cultured meat. Therefore, through the issues discussed, it is vital to stress that it is necessary to ensure that the claims of producers who promote cultured meat as environmentally friendly meat are valid.

METHODS

Samples for Analysis

The medium was obtained from cultured 3T3 cell by previous research. The cell was cultured in a high glucose (4.5g/L) Dulbecco's Modified Eagle's Medium (DMEM) with stable glutamine and sodium pyruvate (Capricorn Scientific, Ebsdorfergrund, Germany). The medium was mix with different concentration (10-20%) of Fetal Bovine Serum (FBS) (Capricorn Scientific, Ebsdorfergrund, Germany) and the cell were harvested on different day. The medium from the harvested cultured cell was seperated and stored at -20◦C. As a positive control, commercial bovine genomic DNA (Novagen®, Darmstadt, Germany) was used.

DNA Extraction

A GENEAID Blood/Cell DNA mini kit (Teipei, Taiwan) was used for DNA extraction from cultured medium. For each extraction, the following materials were used: 300 μL RBC lysis buffer, 200 μL GB buffer, 200 μL 100% (v/v) ethanol, 400 μL W1 buffer, 600 μL Wash buffer and 100 μL pre-heated elution buffer (GENEAID, Teipei, Taiwan). Sample volumes of 300 μL were used for extraction. Extracted DNA was stored at −20 ◦C until further analysis. From each source, all DNA samples were extracted in duplicate.

Oligonucleotide Primers

A pair of species-specific primers were used in each PCR assay for bovine (*Bos taurus*) detection. Bovine-F/Bovine-R primers were used to detect bovine DNA in the following sequence: Bovine-F, 50 -CAT CAT AGC AAT TGC CAT AGT CC-30 and Bovine-R, 50 -GTA CTA GTA GTA TTA GAG CTA GAA TTA G-30 (Corona et al. 2007). These primers targeted the mitochondrial DNA of the cytochrome oxidase II (COII) gene sequence and produced an amplicon size of 165 bp. All primers were supplied by Apical Scientific (Seri Kembangan, Selangor, Malaysia).

Polymerase Chain Reaction (PCR)

The PCR simplex amplification technique using COII primers targeting mtDNA was performed at a final volume of 25 μL; the volumes of each PCR mixtures are shown in Table 1. A mastercycler® gradient thermal cycler (Eppendorf, Westbury, NY, USA) was used to run the PCR with a temperature program as stated in Table 2. The detection limit reported for the analysis was as low as 0.1 ng DNA (ref)

Table 1 Volume of PCR mixture

PCR Mixture Material (µL)	**Sample**	**Positive Control**	**Negative Control**
DreamTaq parent mix™ green PCR (2X)	12.5	12.5	12.5
Primer (front)	0.5	0.5	0.5
Primer (rear)	0.5	0.5	0.5
DNA template	5.0	-	-
DNA samples	-	2	-
Nuclease-free water (NFW)	6.5	9.5	11.5
Total volume (µL)	**25.0**	**25.0**	**25.0**

Table 2. PCR program for bovine DNA detection

Primer	Method	Temperature (°C)/Time (min)	Reference
Bovine-F/Bovine-R	Initial denaturation	95/2	Corona et al.
	Denaturation	94/1	
	Annealing	55/1	
	Elongation	72/2	
	Annealing	55/1	
	Elongation	72/2	
	Final elongation	72/10	

Using a 1.5% (w/v) agarose gel in a 1 TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) stained with MaestrosafeTM nucleic acid (V-BioScience, Kuala Lumpur, Malaysia), the amplification products were electrophoresed at 100 V for 30 minutes (Ismail 2016). As a positive control, commercial bovine DNA NovaGen® (Merck, Darmstadt, Germany) was used. The PCR product was electrophoresed in agarose gel using a GeneRulerTM 100-bp DNA ladder (Fermentas, Vilnius, Lithuania) as a molecular size marker and was visualised with a UV gel documentation system (Syngene, Cambridge, UK).

RESULTS AND DISCUSSIONS

Based on the mass media, cultured meat has not yet penetrated the market of countries other than Singapore and Israel. Singapore, for example, has made a statement to focus on the cultured meat market, which is expected to be widely marketed in Southeast Asia. One must take this matter earnestly by conducting a detailed study and research to avoid doubtful matters arising in the ruling context (Kashim et al. 2021). This matter of doubt is crucial in Islam. Islam continually educates people to stay vigilant and confident in all actions, conversations, nutrition and other aspects of human life. When the context of cultured meat production is still debated, especially concerning the uncertain process and medium use, some doubtful issues arise. A recent study piloted by Yamanaka et al. (2023) titled "Development of serum-free and grain-derived-nutrient-free medium using microalgae-derived nutrients and mammalian cell-secreted growth factors for sustainable cultured meat production" has succeeded in producing cultured meat without the need for a medium. However, the study has used growth factors based on cultured rat liver to culture the cultured meat. It raises doubts in Islam because rats are pests and are forbidden in Islam.

The purpose of this study was to detect bovine (Bos taurus) DNA in cultured medium. The cytochrome oxidase II (COII) gene sequence for bovine species was targeted by bands at the 165 bp position in the current study. There was no statistical analysis used, and samples were examined in duplicates (D) for different FBS concentrations of 5%, 10%, and 15%. Figure 1 shows the amplicons for medium with 5% FBS samples, labelled as D1 and D2, and medium with 10% FBS samples, labelled as D3 and D4. Medium samples with 15% of FBS were labelled as D5 and D6.

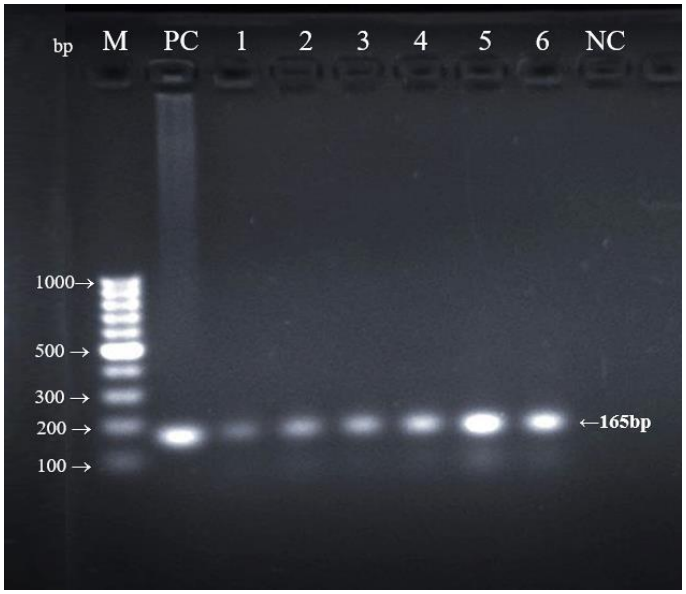

Figure 1. PCR amplification results for used culture medium samples. M lane: 100 bp marker; PC lane: positive control (165 bp); lane 1: D1; lane 2: D2; lane 3: D3; lane 4: D4; lane 5: D5; lane 6: D6.

The positive control, denoted as PC, yielded 165 bp amplicons. The absence of DNA resulted in the absence of a DNA band in the negative control (NC). The PC on the agarose gel was consistent with all samples, demonstrating a similar molecular size. The findings are consistent with those of Kashim et al. (2022) who detected bovine DNA in serum. Amplicons produced have various intensities. Figure 1 illustrates that the band's intensity rises from lanes 1-6, showing that the DNA intensity and DNA concentration were in line. A greater FBS concentration in the medium results in a higher amount of DNA being extracted and, consequently, a higher intensity. The outcome is consistent with research by Shahimi et al. (2022) and Kashim et al. (2022), who found that thicker bands indicated that there was more DNA accessible for primer amplification. The findings demonstrate that differences in DNA concentration correspond to differences in foetal bovine serum content in the medium. This is because the cell uses or consumes the serum during their growth to support cell proliferation and differentiation. Corona et al. (2007) previously reported using the mitochondrial DNA (mtDNA) COII as the targeted gene. So, through the results of this study, it is clear that DNA on used culture medium can still be detected through PCR. This indirectly shows that blood DNA is still present in the medium thus making it not meet halal and kosher standards.

For compliance with halal and kosher food standards, evidence of the use of serum in meat culture is necessary. Mixing blood or its derivatives in Halal and Kosher food is a serious matter because it is not allowed by the respective religious laws. Knowledge of dietary laws for halal and kosher is important for Muslim and Jews consumers who abide to these laws and to food companies that intended to market to this population as well as to consumers who are interested even if they do not abide to these laws.

In the study by Zhu et al. (2022) titled 'Production of cultured meat from pig muscle stem cells', they have proven that FBS is an effective medium to be used in meat culture. Nevertheless, there are doubts about using the Foetal Bovine Serum (FBS) medium in meat culture, consisting of blood elements classified as *haram* (prohibited) in Islam. Moreover, in a recent study, Kashim et al. (2022) concluded that using bovine serum in producing foods such as cultured meat is *haram*. As a result, bovine DNA in bovine serum can still be detected through the polymerase chain reaction (PCR) test. This establishes no process of *istihalah tammah* (perfect transformation) and causes animal DNA to still exist in foods that use bovine serum. Accordingly, cultured meat producers must replace the medium from a *halal* source to ensure there is no doubt about the medium's status.

In Islam, halal law comes from the Quran and Hadith, the tradition of the Prophet Muhammad. Kashim et al. (2022) noted that views related to cultured meat demand the opinion of contemporary religious scholars

because it is not presented in the Quran and hadith. The scholars agreed that food declared *halal* by Allah SWT is *halal* to eat, while food is considered forbidden when there is proof that it is presented. The analysis of some laws and jurisprudence expressed by contemporary religious scholars should be used to determine or state the appropriateness of laws or views on this cultured meat (Hamdan 2015). The debate involves issues about cultured meat are also often highlighted from a Jewish point of view. The kosher dietary laws need to determine which foods are "fit or good" for Jews and deal primarily with three issues: permitted animals, the prohibition of blood, and the prohibition of mixing milk and meat. This law comes from the Torah and the oral law received by Moses on Mount Sinai (Talmud As for the Kosher law, there are animals that are allowed as well as the prohibition of taking blood (Regenstein et al. 2003). As a result, identifying the detection of DNA is especially important in order to preserve the sanctity and rules set by certain religions or beliefs regarding blood.

CONCLUSION

Ergo, cultured meat is one of the long-term alternatives for humans's consumption. It promises to meet life's demands and equipment aspects more effectively, cleanly and environmentally friendly. Production measures also greatly influence and impact the livestock and food industries. Since cultured meat is one of the latest technologies that will significantly impact society and a precise ruling determination, the consensus of views from contemporary scholars must agree in solving the issue of cultured meat. The use of any component derived from blood is prohibited in Islam and Jewish. The use of FBS in the culture medium is the main concerns for consumers because serum is derived from blood. Muslims and Jews are only permitted to consume foods that are in compliance with their religion rules and regulation. Whereas, vegan has the rights to know the origin of each components used to produce the cultured meat and its product so that they can assess and has the right to choose for consumption. Cultured meat producers are also advised to release the methods and ingredients involved in meat culture honestly and transparently to ensure that consumer, especially Muslims, Jews and vegan, can accurately decide the ruling of cultured meat. Hopefully, this deconstruction will become a reference for authorities, industry, society and countries with different faiths on dietary practices.

Conflict of Interest

The researchers declare no conflict of interest.

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