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Review Article

Serum markers for beef meat quality: Potential media supplement for cell-cultured meat production

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ABSTRACT

As the global population continues to grow and food demands increase, the food industry faces mounting pressure to develop innovative solutions. Cell-cultured meat involves cultivating cells from live animals through self-renewal methods or scaffolding and presents a promising alternative to traditional meat production by generating nutritionally rich biomass. However, significant research is still needed to overcome challenges such as developing serum-free media, identifying suitable additives to support cell growth, and ensuring the quality of cell-cultured meat closely resembles that of traditional meat. Meat quality, which is influenced by various sensorial factors (color, texture, and taste), tenderness, and nutritional values, is determined by the level of intramuscular fat deposition, which significantly influences both meat yield and quality. This paper offers a concise overview of serum markers used to assess beef quality and yield and potential additives currently used in culture media for cell-cultured meat production. We also proposed the potential of using serum markers as additives in the culture media to enhance production of cell-cultured meat. Overall, this review highlights the significance of cell-cultured meat production as a viable solution to address the challenges posed by increasing food demands.

1. Introduction

Meat is a key source of protein globally, and global meat consumption reached 286 million tons in 2013 and projected to increase to 486 million tons by 2050 (Valin et al., 2014). As the global population grows from 7.5 billion in 2015 to approximately 10 billion by 2050, current agricultural and meat production systems may struggle to meet the rising demands owing to limited resources and arable land (Fig. 1) (Sans et al., 2015; Henchion et al., 2017). Traditional meat production is also resource-intensive and linked to environmental harms, animal welfare concerns, and public health risks. It is associated with various zoonotic diseases, antibiotic resistance, and climate change, with livestock operations accounting for 14%–51% of global greenhouse gas emissions (Gerber et al., 2013; Goodland et al., 2009). These challenges make scaling up conventional livestock farming an inadequate solution to growing global demands for meat.

To address these challenges while sustaining a growing population, more efficient and sustainable meat production methods are being explored. A promising solution is cell-cultured meat, which is a potential alternative to traditional meat. Cell-cultured meat is produced by growing edible biomass through the in vitro culture of cells derived from the muscle tissue of live animals (Kumar et al., 2021). Compared with traditional methods, this technology offers benefits such as improved public health, nutritional security, and more ethical production processes while reducing water usage and greenhouse gas emissions (Tuomisto et al., 2011).

Research in cell-cultured meat primarily focuses on improving meat quality and yield. Meat quality is commonly defined by characteristics such as tenderness, intramuscular fat (IMF) deposition (marbling), flavor, juiciness, and color, which are highly valued by consumers (Muchenje et al., 2009a). In traditional meat production, these parameters are affected by animal species, sex, age, diet, and pre-and post--slaughter conditions. Despite the potential of cell-cultured meat, it remains in its early stages and is still costly compared with conventional

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methods. The high cost is largely due to the use of fetal bovine serum (FBS). However, FBS presents several issues, such as ethical concerns and batch-to-batch variabilities (Gottipamula et al., 2013).

To overcome these challenges, efforts are focused on developing alternative culture media by replacing FBS that can perform similarly (O'Neill et al., 2022). To enhance meat quality, the potential utilization of serum markers as dietary supplements in cell culture media for cultured meat production has been explored. Serum markers are traditionally been used to assess meat yield and quality in livestock and could provide valuable insights when applied to cell-cultured meat production. These markers offer a deeper understanding beyond conventional quality indicators such as tenderness, juiciness, and IMF deposition, providing information on meat composition, nutritional content, and overall quality.

This review aimed to provide an update of the serum markers that influence meat quality and yield in traditional meat production. It also explores the potential of these markers as additives in the culture media for enhancing the quality and yield of bovine cell-cultured meat.

2. Serum markers for beef meat quality

The assessment of beef quality involves a range of sensory attributes, such as taste and juiciness, and textural characteristics, particularly tenderness. Among these, consumers highly value tenderness and fat content, specifically IMF and marbling score (MBS), and this review primarily focus on these aspects. IMF accumulation in beef results from adipogenesis, a process involving the expansion and differentiation of mature fat cells. This process is regulated by key factors, such as peroxisome proliferator-activated receptor gamma (PPARγ), which are crucial in fat cell development. The MBS and the final market value of the carcass have consistently demonstrated a strong correlation.

Despite the importance of IMF in determining beef quality, relatively few studies have focused on blood and genetic biomarkers that could predict meat quality and yield before slaughter. Understanding these biomarkers offers potential not only for traditional meat production but might play important role for the improvement of cell-cultured meat. By identifying and leveraging these biomarkers, IMF deposition in cell-cultured meat may be replicated or enhanced, improving both quality and consumer appeal. Herein, we aimed to update current knowledge on serum markers that influence beef quality, particularly on IMF deposition (see Table 1).

2.1. Leptin

Leptin, an adipocyte-derived hormone, is critical in regulating food intake, energy metabolism, and body composition in mammals. Leptin concentrations are strongly associated with adipocyte mass because an increase in adipocyte size typically leads to higher leptin synthesis and secretion (Goldberg et al., 2009; Jindřichová et al., 2007). Blood leptin

Table 1
Serum markers for beef meat quality assessment and their role as media additives in enhancing adipogenesis.

Serum markers	Serum levels	Correlation	References
Vitamin A/ Retinol	128 μg/L	High retinol content (>1.10) poorly/ negatively associated with meat MBS. Retinol concentration positively correlates with meat color.	Torii et al. (1996)
Non-esterified fatty acid (NEFA)	2.237 ± 0.12 μEq/10 mL	Palatability and IMF positively correlate with non-esterified FA content.	Moon et al. (2018)
Total cholesterol	128.03 ± 6.00 mg/mL	The amount of total blood cholesterol was strongly/positively connected with carcass weight and MBS.	(Moon et al., 2018) (Otani, 2006) (Litwińczuk, 2015)
Growth hormone (GH)	3.8 ng/mL (Body weight dependent- 509 kg)	Negatively correlated with carcass fat and positively associated with muscle mass.	Trenkle et al. (1978)
Paraoxonase 1 (PON1)	60–100 mg/mL (dependent on gender and meat quality grade)	PON1 levels are positively correlated with the MBS and are higher in females and castrated males than in males, indicating a sex-dependent difference.	Park et al. (2019)
Insulin	1.49 ± 2.37 ng/ mL (Dependent on body weight)	Positively correlated with meat MBS	Trenkle et al. (1978)
Leptin	6.31 ± 1.32 ng/ mL	Leptin is positively correlated with meat MBS.	Geng et al. (2020)
Aspartic acid transaminase (AST)	0.54 ± 0.0324 U/ 10 mL	AST is positively connected with meat color and negatively correlated with the yield index and meat MBS.	Moon et al. (2018)
Alanine transaminase (ALT)	0.51 ± 0.043 U/ 10 mL	ALT is positively correlated with the yield index and meat MBS and negatively correlated with meat color.	Moon et al. (2018)
Total protein (TP)	1.1 ± 0.04 g/10 mL	TP is positively correlated with the yield index and meat MBS.	Moon et al. (2018)

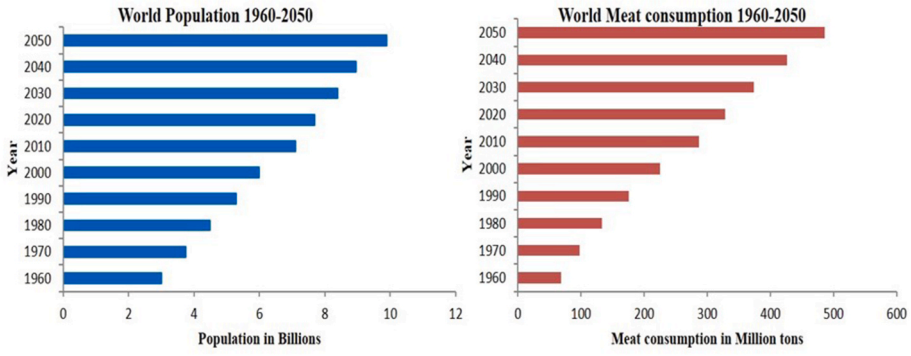

Fig. 1. Worldwide meat consumption and anticipated increase in population by 2050.

levels positively correlate with the MBS; IMF deposition; fat depth; kidney, pelvic, and heart fat; and overall meat quality and are negatively associated with cholesterol content (Geng et al., 2020; Geary et al., 2003). Studies have indicated a positive relationship between plasma leptin levels and lipid content in Wagyu cattle (Wegner et al., 2001). Furthermore, polymorphisms in the leptin gene have shown association with variations in serum leptin levels and body fat content (Liefers et al., 2002). Daix et al. observed that Belgian Blue cattle had lower body fat, IMF, and serum leptin concentrations, along with reduced leptin gene expression in adipose tissue, than Limousine and Angus breeds (Daix et al., 2008). Conversely, a significant positive correlation was found between leptin levels and back fat thickness in Hanwoo steers, whereas leptin was negatively correlated with the carcass yield index (Chung et al., 2017).However, the role of leptin in adipogenesis varies across species and remains somewhat controversial. Zhang et al. investigated the effect of exogenous bovine leptin supplementation on bovine adipogenesis and demonstrated that leptin inhibits triglyceride deposition in bovine cells, indicating a regulatory role in adipocyte differentiation (Zhang et al., 2010).

2.2. Growth hormones (GHs)

GHs interact with its receptor, the growth hormone receptor (GHR), to regulate growth and metabolism. The plasma GH concentrations in 24 crossbred steers were measured and negatively correlated to carcass fat but positively related to carcass muscles (Trenkle et al., 1978). Gene polymorphisms in GHs were examined in different cattle breeds, which established GHs as a potential molecular marker for meat quality. For instance, a study on Wagyu cattle revealed that a single nucleotide polymorphism (SNP) resulting in the substitution of leucine (L) for valine (V) at position 127 (L127V) led to a reduction in shorter fatty acids (FAs), an increase in the proportion of long-chain FAs, and increased subcutaneous fat thickness. Another SNP, involving the substitution of threonine for methionine at position 172 (T172M) in the *GH* gene, is linked to carcass traits such as carcass weight, rib thickness, subcutaneous fat thickness, and firmness (Matsuhashi et al., 2011). Further research on the GH1: c.457C > G SNP in Australian feedlot cattle proposed that this genetic variation is strongly associated with traits such as rump fat thickness (measured at the junction of the gluteus medius and superficial gluteus medius muscles), eye muscle area, and carcass weight (Barendse et al., 2006).

The effect of recombinant GH was also checked on the bovine adipogenesis, which suggested the inhibitory effect of GH on the bovine adipogenesis with inhibition of C/EBP α expression (Zhao et al., 2023; Li et al., 2022).

2.3. Insulin

Insulin is vital in lipid metabolism by promoting lipogenesis and inhibiting lipolysis. In cattle, blood insulin levels tend to increase with age and body weight (Trenkle, 1970; Martin et al., 1979). A study showed that plasma insulin concentrations were positively correlated with carcass fat and lipid content in the longissimus muscle but were negatively correlated with carcass muscle mass (Trenkle et al., 1978). In a study comparing insulin secretion and glucose response between Japanese Black heifers, known for their high IMF deposition, and Holstein heifers, Japanese Black heifers exhibited higher insulin secretions after reaching sexual maturity than Holsteins. Although both breeds show similar glucose responses to insulin, increased insulin secretion in Japanese Black heifers may contribute to their higher IMF levels (Shingu et al., 2001). In another study, plasma insulin concentrations, metabolites, and carcass composition were measured across five Japanese Black, five Japanese Brown, and four Holstein steers. Plasma insulin levels were significantly higher in Japanese Black steers than in other breeds, and carcass analysis revealed a higher proportion of fat and less bone than Holsteins, despite similar feeding conditions (Matsuzaki et al., 1997). A study on Hanwoo steers indicated that plasma insulin concentrations were the highest during the growth phase and decreased as the cattle reached the finishing phase (Moon et al., 2018). These studies indicate insulin is a good marker of beef quality.

2.4. Total protein

The serum total protein (STP) refers to the total amount of protein in the blood, including albumin and globulins.The serum albumin/globulin ratio was measured in Japanese Black beef cattle and showed significantly high concentrations in the high MBS group of cattle. Total protein levels were positively correlated with carcass weight, springiness, gumminess, chewiness, and drip loss (Yu et al., 2021). STP also correlates with lipid content, which influences meat quality (Wang et al., 2020). In stem cell development, STP in the culture media provides energy, enhancing cell division, regeneration, and proliferation. It is essential for metabolic processes and affects meat yield grade, yield score, and quality (Moon et al., 2018). However, its role at the cellular level is complex due to the variety of proteins it contains. Albumin, a key component, supports cell proliferation and might also enhance adipogenesis in cultured meat by aiding fatty acid uptake. Despite its importance in cell culture, albumin is not a direct marker of beef quality Therefore, considering total protein as media additive is quite complicated.

2.5. Paraoxonase 1 (PON1)

PON1 is a calcium-dependent serum antioxidant enzyme associated with high-density lipoprotein. It is crucial in preventing the oxidation of low-density lipoprotein (LDL) and is synthesized in the liver before released into the bloodstream. PON1 is an important marker in the early diagnosis of liver damage, including fatty liver disease in cattle. During bovine in vitro oocyte maturation, PON1 enhances blastocyst development rates, highlighting its significance in female fertility (Rincón, 2016). In Hanwoo cattle, a Korean breed, PON1 levels are notably higher in females, particularly during parturition. Conversely, PON1 levels are lower in males than in females and castrated males, reflecting sex-dependent variations. This variation is associated with meat quality, as PON1 levels are lower in grade3 meat than in those in grades 1 and 2 (Park et al., 2019). Specific polymorphisms of PON1, such as PON1/*Eco*RV and PON1/*Alu*I loci, are positively associated with carcass weight, tenderness, and meat color (Ji et al., 2008).These evidences suggest that PON1 would be a useful serum marker for beef meat quality, fatty liver diagnosis, and parturition changes analysis.

2.6. Aspartic acid transaminase (AST)and Alanine aminotransferase (ALT)

AST is widely distributed in the liver, heart, skeletal muscles, and brain. It is critical in cell survival by promoting growth and preventing mitochondrial disintegration through the regulation of intracellular calcium release. AST levels are commonly measured to assess liver health. They are negatively correlated with MBS, rib-eye muscle area, and meat quality and yield grades (Moon et al., 2018), suggesting that AST could serve as a biomarker for both meat quantity and quality (Moon et al., 2018).

ALT is a crucial marker for liver dysfunction and participates in gluconeogenesis in the liver and glycolysis in the muscle tissue. High ALT levels indicate liver dysfunction, which can negatively affect meat quality, marbling, and yield index. ALT levels are positively correlated with AST, non-esterified FAs, and albumin and negatively correlated with leptin, cholesterol, and glucose. These relationships can affect meat color and overall quality (Moon et al., 2018).The roles of AST and ALT in cell culture level remain insufficiently understood and require investigation under in vitro conditions.

2.7. Vitamin A (VA)/retinol

VA (also known as retinol) is a fat-soluble vitamin essential for normal bone growth, vision, development, and various physiological functions in cattle. It is crucial in IMF deposition by influencing adipocyte development and fat accumulation (Peng et al., 2021). VA deficiency is associated with increased IMF content. A study on Wagyu cattle established a negative correlation between serum retinol and IMF deposition during the fattening period, suggesting its antiadipogenic activity on preadipocytes (Torii et al., 1996). In Angus steers, a study using a cereal-based diet low in β-carotene and VA for 308 days demonstrated that supplementation led to a 19% reduction in MBS. The IMF content in the longissimus dorsi muscle was 35% lower in supplemented steers than in controls (Gorocica-Buenfil et al., 2007). Similarly, in Japanese Black cattle, 15-month VA supplementation significantly decreased the MBS. A high correlation coefficient (−0.38) was observed between serum VA levels and marbling just before slaughter (Adachi et al., 1999). Plasma retinol was identified as a potential biomarker for meat quality in Hanwoo steers, which showed a notable negative correlation between blood retinol levels and MBS (Moon et al., 2018). Previous research showed that retinoic acid inhibits adipocyte differentiation by activating the cellular retinoic acid-binding protein 2-II/RAR pathway and suppressing the expression of late marker genes of adipocytic differentiation such as PPAR-γ(Berry et al., 2012).

2.8. Non-esterified fatty acids (NEFA)and total cholesterol

On average, IMF in beef meat comprises different fatty acids (FAs) approximately 50%, 45%, and 5% saturated, monounsaturated, and polyunsaturated FAs, respectively. Fat deposition is an important aspect of meat quality and is a complex of phospholipids, cholesterol, and triacylglycerides, which are important energy reserves in muscles. The composition of beef marbling fat significantly affects carcass value, palatability, and overall meat quality (Kazala et al., 1999; Schumacher et al., 2022; Ladeira et al., 2018). Marbling fat, deposited in the longissimus muscle (rib-eye section between the 12th and 13th ribs), is composed of >20 FAs, with oleic, palmitic, stearic, linoleic, palmitoleic, and myristic acids making up >92% of the total content. Marbling also includes unique FAs such as conjugated linoleic acid, which refers to various isomers of linoleic acid (cis-9, cis-12 octadecadienoic acid) (Duckett, 2005). The FA composition of beef, particularly the amount of polyunsaturated FAs, is influenced by the animal's diet, whereas ketones, saturated aldehydes, FAs, and unsaturated aldehydes form the flavor. Stearic acid (18:0) is a key determinant of fat hardness that affects the lipid melting point (Chung et al., 2006). The conversion of stearic acid to oleic acid through diet can enhance fat softness and overall beef palatability. The age, diet, and breed type of the animal are the three main factors that influence the FA composition of beef. Age and breed type specifically affect the monounsaturated FA concentration by influencing the expression and activity of the stearoyl-CoA desaturase gene.

Total cholesterol composed of LDL, very-low, low- and high-density lipoproteins (VLDL and HDL), and triglycerides and an essential component of meat, influencing its nutritional quality and health aspects. It plays a key role in cell membrane structure and function, serves as a precursor for hormone synthesis, and provides an energy source. In meat, cholesterol is stored in lipid droplets, contributing to its nutritional profile (Muchenje et al., 2009b).Total blood cholesterol levels positively correlate with carcass weight and rib thickness (Chung et al., 2017; Kato et al., 2011), its relationship with MBS in most studies correlated positively except few (Moon et al., 2018; Otani, 2006; Litwińczuk, 2015). LDL and HDL individually were also used as serum markers for marbling score in cattle. In Japanese Black beef cattle, LDL showed a positive correlation with MBS, while HDL was negatively correlated (Noro et al., 1995).

3. Development of serum-free media formulation for cell-cultured meat production

The culture medium significantly contributes to the cost of cell-cultured meat. Culture media comprises vitamins, amino acids, carbohydrates, organic and inorganic salts, and other nutrients needed for cell proliferation and differentiation. Most media are sourced from animals, such as FBS. It renders cultured meat generated from the serum media inappropriate for vegan and specific religious consumption (Halal& Kosher) (Stephens et al., 2018; Chen et al., 2022). Therefore, serum-free media optimized with nutrient components that are cost effective, not animal-derived and also food compatible are needed to support the growth of various cells. However, transitioning to serum-free media remains a challenging task, requiring extensive research and effort to identify appropriate medium formulations. Moreover, serum-free media typically exhibit lower efficiency in promoting cell growth compared to serum-based alternatives (Miki et al., 2015). To address this, gradual advancements have been made by incorporating key components and systematically adapting cells to serum-free conditions, as discussed in detail below. This adaptation process involves the stepwise replacement of serum with essential nutrients, growth factors and also food compatible components.

The development of serum-free media is significantly progressing for cultured meat production. Earlier studies have shown that single proteins such as sericin, a major component of silk, support the proliferation of mammalian cell lines, including myoblasts, potentially replacing FBS in cultured meat applications (Terada et al., 2002). Cyanobacteria also stands out as one of the prospective food sources for facilitating muscle cell growth in cultured meat production (Ghosh et al., 2023). Plant-based media rich in amino acids derived from maitake mushroom extracts are optimal for fish explant development and surface area expansion (Benjaminson et al., 2002).Studies using a serum-free medium supplemented with additional proteins have revealed positive findings, and innovative media such as Ultroser-G and AIM-V have been developed (Fujita et al., 2010; Helinski et al., 1988).Dulbecco's modified eagle medium derivatives are the most commonly used media in cultured meat research because of their effectiveness in mammalian cell culture. Essential 8™, a chemically defined medium, consists of DMEM/F12 and eight additives, including transforming growth factor beta, insulin, fibroblast growth factor, minerals, vitamins, and buffers. This formulation supports the growth of pluripotent stem cells (iPSCs) for at least 6 days (Kolkmann et al., 2020). The serum-free medium B8, developed for culturing human iPSCs, reduced the need for serum required for growing human iPSCs and marked a promising step toward developing a fully serum-free formulation for cultured meat production (Kuo et al., 2020). B8 was subsequently improved by adding recombinant albumin and renamed Beefy-9, tailored for growing and maintaining bovine satellite cells (Stout et al., 2022). However, recombinant albumin in the Beefy-R formulation was later replaced by rapeseed protein isolate to reduce the production cost (Stout et al., 2023). A recent study revealed that replacing certain components of DMEM with food-grade alternatives, such as using L-arginine instead of L-arginine HCl, resulted in the comparable growth and differentiation of C2C12 cells and bovine skeletal muscle-derived cells to those observed with standard DMEM (Kanayama et al., 2022). In a different approach, a nutrient medium derived from *Chlorella vulgaris* (CVNM) showed enhanced growth of bovine myoblasts with higher insulin like growth factor-2 (IGF-2) levels than DMEM (Yamanaka et al., 2023). Despite these advancements, both food-gradeDMEM and CVNM still exhibited improved proliferation rates when supplemented with FBS, emphasizing its continued importance. Further innovations include the use of a serum-free medium incorporating black soldier fly larvae hydrolysate into B8. This medium achieved comparable proliferation efficiency to the FBS-containing media (Garg, 2024). Another chemically defined serum-free medium was developed, which replaced FBS with ingredients such as albumin and α-linolenic acid, attaining 97% growth

and differentiation efficacy of traditional FBS-containing media (Kolkmann et al., 2022).

Mosa Meat, a Dutch startup, has developed a serum-free medium that effectively induces muscle differentiation, and this advancement represents significant move toward commercialization. This medium was formulated using transcriptomics and proteomics, included sodium bicarbonate, ascorbic acid 2-phosphate, epidermal growth factor 1, minimum essential media amino acids, and serum albumin, and supplemented with insulin, transferrin, glucagon, and lysophosphatidic acid (Messmer et al., 2022). The IntegriCulture'sCulNet® system also facilitates enhanced cell growth by circulating a medium with "feeder cells" that secrete serum-like components. This leads to enhanced duck and chicken liver cell proliferation compared with FBS-containing media (IntegriCulture, 2022). Multus'sProliferum® M is recently designed as a complete FBS replacement that demonstrates comparable proliferation efficiency to FBS-containing media and complies with ISO22000 food safety standards for cell-cultured meat production (Multus, 2024). A Knockout™ Serum Replacement (KSR) is another alternative to animal serum, which is used to culture embryonic stem cells and iPSCs from different species. KSR includes essential and nonessential amino acids, antioxidants, transferrin, insulin, and trace elements and supports cell stemness and proliferation in a manner similar to the FBS-containing media (Knockout™ Serum Replacement, 2021).

4. Potential serum marker as media additives for cell-cultured meat production

4.1. Insulin

In cell-cultured meat production, insulin is the most extensively studied hormone as a media additive for promoting muscle cell proliferation and differentiation. It enhances myoblast differentiation by upregulating myosin heavy chain (MHC) isoforms and myogenin, while also supporting cell survival, myotube hypertrophy, and the self-renewal of muscle satellite cells (Rhoads et al., 2016; Godoy-Parejo et al., 2019). Insulin has been widely studied as an adipogenesis inducer in murine, and human models often using FBS (Gregoire et al., 1998; Dufau et al., 2021). However, lipid metabolism differs between these monogastric species and ruminants like cows. In ruminants, lipogenesis occurs mainly in adipose tissue, using acetate from enteric fermentation as the primary carbon source, while in monogastric species, it occurs in the liver using glucose-derived acetate (Nayananjalie et al., 2015; Laliotis et al., 2010). The standard adipogenic cocktail does not work efficiently for differentiation of bovine cells as it does for murine and human preadipocytes. There's also little research on growing these cells without serum, which is important for cultivated meat. However, recent findings demonstrated that insulin, combined solely with rosiglitazone (without dexamethasone or IBMX), successfully induced adipogenesis in bovine stromal vascular cells under serum-free conditions, outperforming serum-containing media. This simplified protocol is effective across multiple species, including cows, sheep, pigs, and mice (Mitić, 2023). Additionally, another study on bovine adipocyte stem cell spheroids developed a differentiation protocol using bovine insulin, rosiglitazone, and dexamethasone (excluding IBMX), achieving successful adipocyte differentiation in both 2D and 3D cultures (Klatt et al., 2024).

4.2. Non-esterified fatty acids (NEFA)

NEFAs offer a promising solution as food-compatible components for inducing bovine adipogenesis, paving the way for fully edible, cell-cultured meat that closely resembles traditional meat. In a study, the addition of oleic acid, combined with insulin and ciglitazone, to bovine satellite cell cultures in myogenic differentiation medium successfully induced adipogenesis, indicating the trans differentiation of myoblasts into adipocytes (Kim et al., 2019). More recently, six different free fatty acids, including oleic acid, phytanic acid, myristoleic acid, elaidic acid, and palmitoleic acid, were incorporated into culture media to induce adipogenesis in bovine adipose stem cells, producing cell-cultured meat comparable to Wagyu beef (Louis et al., 2023).

4.3. Total cholesterol

Total cholesterol, comprising HDL, LDL, and triglycerides, plays a crucial role in adipogenesis across bovine, murine, and human models. Studies have demonstrated that VLDL, LDL, and HDL can induce the differentiation of 3T3-L1 cells, bovine stromal vascular cells, and human preadipocytes into adipocytes, with LDL showing the strongest effect (Wu et al., 2000; Stanton et al., 1997, 1998). Additionally, serum-free media enriched with bovine serum lipids (mainly cholesterol) has been found to enhance adipocyte differentiation more effectively than fetal bovine serum-containing media (Sandhu et al., 2017). However, cholesterol depletion enhances differentiation of chick myoblasts confirming its role in early stage of myogenesis (Mermelstein et al., 2005).

4.4. Paraoxanose 1 (PON1)

PON1 shows a positive association with the FA composition of the adipose tissue (Kulka et al., 2016). PON1 level was positively associated with meat quality, supplementation of PON1 may increase the fatty acid composition and adipose tissue formation which may enhance the quality of cultured meat. However, its role at the cellular level remains underexplored. Few in vitro studies have examined the effect of PON1 on adipogenesis, with existing research primarily focusing on murine adipogenesis, where PON1 levels are negatively correlated with obesity and inhibit adipocyte differentiation (Seres et al., 2010; Park et al., 2013). Despite the inhibitory effect in murine adipogenesis, the effects of PON1 on bovine adipogenesis have yet to be investigated. Given its positive association with FAs, IMF and beef quality, PON1 may hold potential as a media additive to enhance adipogenesis in the production of cell-cultured meat, requiring further study.

5. Limitations and overcoming solution of using serum free culture media

The goal of cell-cultured meat production is to replicate the color, texture, flavor, and nutritional content of traditional meat using cost-effective, food-grade, and animal-free media additives (O'Neill et al., 2021; Post et al., 2017). Traditional meat primarily consists of skeletal muscle, which develops in long, thin fibers interspersed with fat deposits. While most research in cell-cultured meat has focused on replicating skeletal muscle, fat deposition plays an equally critical role in enhancing meat quality. Reproducing this intricate structure in cultured meat will require extensive investigation (Bhat et al., 2015). As discussed earlier (Section 3), serum-free formulations with food-grade additives have been developed for muscle cell proliferation and myogenesis. However, specific formulations for adipogenesis remain underdeveloped. To replicate the sensory attributes and nutritional profile of traditional meat, we proposed to incorporate serum markers associated with beef quality, particularly those positively correlated with MBS and studied for their effects on adipogenesis and myogenesis at the cellular level. Among various serum markers, insulin, NEFAs, PON1, and total cholesterol could be a significant potential as culture media additives to improve the quality of cell-cultured meat (Fig. 2). However, limitations such as the cost, animal-derived origins, and food compatibility of certain additives, including insulin and cholesterol, present challenges. To address these issues, recombinant systems using fermentation offer a promising solution for the cost-effective, large-scale production of insulin and other proteins from various species. Yeast-based expression systems are particularly favored for producing recombinant insulin on a large scale (Thim et al., 1986; Kjeldsen, 2000). However, while insulin produced through these systems is approved for

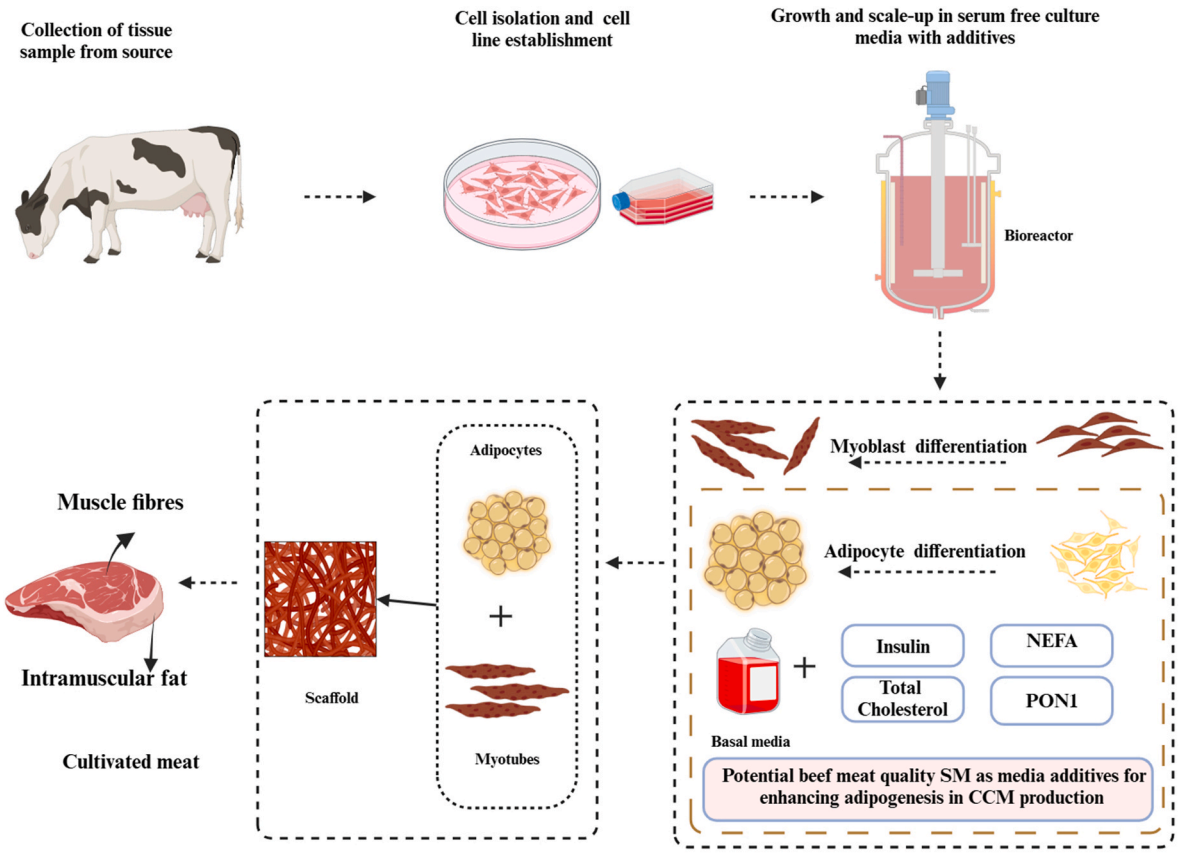

Fig. 2. Schematic representation of future prospects in cell-cultured meat production, emphasizing the role of beef serum markers as crucial additives in culture media to improve meat quality. Abbreviations: NEFA – Non-esterified fatty acids; PON1 – Paraoxanase 1; IMF – Intramuscular fat; SM- Serum markers; CCM- Cell-cultured meat. The illustration is created using BioRender.

pharmaceutical use, it remains unsuitable for food applications, similar to other adipogenesis-inducing components such as rosiglitazone, dexamethasone, and IBMX. To overcome these challenges, alternative strategies such as the use of plant-derived NEFAs are being explored. These plant-based substances could facilitate the development of food-compatible protocols for adipocyte differentiation.

In Table 2 we summarized the specific serum markers hold potential as media additives for cell-cultured meat production. In summary, given the critical role of insulin in cell proliferation, adipogenesis as well as in, myogenesis, it is an essential component for the production of high-quality cell-cultured meat, while NEFA and total cholesterol are also important for induction and promoting adipogenesis. PON1, a promising marker of beef quality, remains underexplored in vitro but could emerge as a valuable media additive with further research. However, challenges such as cost-effectiveness and food compatibility still need to be addressed.

Table 2
Potential serum markers for beef meat quality as media additives for cell-cultured meat production.

Serum markers as potential media additives	Function as media additive	Correlation with IMF and meat quality	References
Insulin	Enhances adipogenesis across all animal species (bovine, murine, human and porcine) and myogenesis	Positive	(Rhoads et al., 2016) (Godoy-Parejo et al., 2019) (Gregoire et al., 1998) (Dufau et al., 2021) (Mitić, 2023) (Klatt et al., 2024)
Non-esterified fatty acids (NEFA)	Promote bovine adipogenesis	Positive	(Kim et al., 2019) (Louis et al., 2023)
Total cholesterol (LDL, HDL, VLDL)	Promotes adipogenesis (bovine, murine and human)	Positive	(Wu et al., 2000) (Stanton et al., 1998) (Stanton et al., 1997) (Sandhu et al., 2017)
Paraoxanase 1 (PON1)	Inhibits murine adipogenesis. Effect on bovine adipogenesis yet not explored.	Positive	Park et al. (2013)

6. Conclusions

Cell-cultured meat presents a promising solution to global meat shortages, enhanced food security, and reduced environmental impact compared to traditional meat production. However, the technology is still in its early stages and not yet suitable for large-scale production. To address challenges such as high production costs, researchers are focusing on optimizing culture conditions and developing serum-free media formulations. Although media formulations like Beefy 9, Beefy-R, KSR, and AIM-V have made progress, none have yet proven to be optimal for large-scale cultured meat production. In this review we provide a comprehensive summary of key serum markers related to marbling score and quantity of beef meat that might be used as additives for culture media. Among various serum markers insulin, NEFA, PON1, and total cholesterol could be promising options as media additives. These components, which also influences adipogenesis/myogenesis or both, could improve the efficiency and quality of cell-cultured meat, making it more similar to traditional meat in texture and nutritional value. Moreover, current cultured meat production technologies are not entirely free of animal-derived components. Therefore, advancing the

use of plant-based or food-grade synthetic substitutes is crucial for the industry's development. Additionally, further research is needed to clarify the roles of serum markers in adipogenesis and myogenesis and to develop more viable alternatives. Also, addressing ethical concerns and fostering greater consumer awareness and acceptance will be essential for establishing cell-cultured meat as a viable alternative to conventional meat products.

CRediT authorship contribution statement

Sana Iram: help in the investigation and writing original draft, writing–review, and editing. **Amar Akash:** help in the investigation and writing original draft. **Chandra Sekhar Kathera:** writing–review, and editing. **Kye Won Park:** review and supervision. **Yoon Shin Cho:** review and supervision, All authors have read and agreed to the published version of the manuscript. **Jihoe Kim:** study design, review, and editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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