



# A Spotlight on Archaea in Humans, Livestock and Poultry: A Review

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#### **ABSTRACT**

The microbiota includes prokaryotes (archaea and bacteria) and eukaryotes. Archaea are single-celled prokaryotes and essential part of gut microbiome. Researches on archaea in ruminants and humans are more than mono-gastric. The low abundance of archaea in the gut depends on the method used (metagenomics or meta-transcriptomic) and age of people or poultry. The lack of complete recognition of archaea is due to their small number and method of identifying them (16S rRNA gene primers). The uses of archaea include analytical kit, reduce oil pollution, archaeosomes or drugs production, vaccines agents, lipid carriers in the pharmaceutical industry and molybdenum extraction in the nuclear industry. The nutritional functions of methanogenic archaea including feed utilization (ruminants) and efficiency, hydrogen reducing (human), fat deposition and enhancement of energy harvesting in mice, CAZymes genes, cecal fermentation, syntrophic potential, carotenoid source and improved transit time and appetite and SCFAs production. Archaea acting as antibiotics (produce archaeocins, sulfolobicins and halocin KPS1) and as probiotics (archaeobiotics) can reduce TMAU (trimethylaminuria) disease, cardiovascular diseases (CVDs), and atherosclerosis, brain abscess, cancer, colorectal cancer, inflammatory bowel disease (IBD), constipation, obesity, food allergies, asthma and anti-inflammation which can be prevented by using archaea, and other functions include energy homeostasis, heat shock protein (HSP) production and reducing aging.

## 1 | Introduction

The sciences of nutrition, microbiome, genome, neuroscience, omics and NBIC (Nanotechnology, Biotechnology, Information Technology and Cognitive science) will have tremendous popularity in the future. Other sciences have been instrumental in advancing our knowledge of the microbiome. The microbiome of poultry, human and livestock is made up of bacteria, fungi, viruses, parasites, protozoa and archaea that coexist both inside and outside the body. The archaeal microbiome (archaeome) in poultry nutrition is not being given sufficient attention because

of the limited number of archaea in the bird's digestive system. The archaea are typically found in the lower parts of the bird's digestive system. Moreover, archaea lived in hot springs and deep seas with a high salinity, high temperature and acidity (Fogel et al. 1999), isolated from the human's digestive (Nottingham and Hungate 1968) and nose, vagina, skin and oral cavity (Kohl et al. 2014). The first isolation of archaea producing-methanogens was from the droppings of chickens, geese and turkeys by Miller's and collogues in 1986. However, this topic has not taken a great concern in poultry sciences like those in humans and animals sciences.

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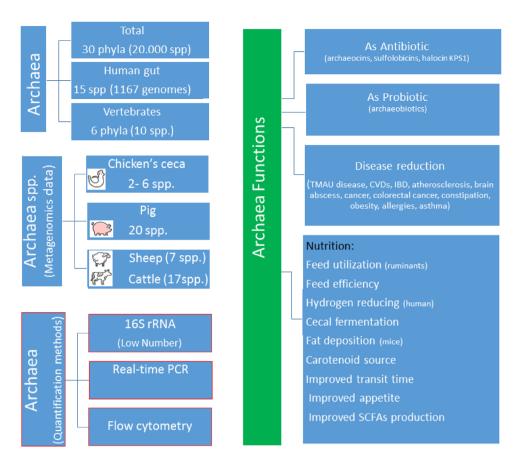


FIGURE 1 | Archaea contribution and functions in livestock rumen, chickens and human gut microbiome and the number of archaea phyla and species.

The world-wide archaea were estimated as 30 phyla and 20.000 spp. (Sprott et al. 1997), 15 spp. with 1167 genomes in the human's gut (Chibani et al. 2021), 6 phyla and 10 spp. in vertebrates (Wen et al. 2021). The metagenomics data stated that the relative abundance of archaea in chicken, pig, sheep and cattle were, respectively, 2, 20, 7, and 17 spp. from 100 archaeal taxa (Patel et al. 2010). The reported archaea spp. in the chicken's ceca were 2 (Patel et al. 2010), whereas they were 6 in the other study (Dittoe et al. 2018) (Figure 1). The metagenomics data stated that the relative abundance of archaea in chicken, pig, sheep and cattle were, respectively, 2, 20, 7 and 17 spp. from 100 archaeal taxa (Patel et al. 2010). The reported archaea spp. in the chicken's ceca were 2 (Patel et al. 2010), whereas they were 6 in the other study (Dittoe et al. 2018).

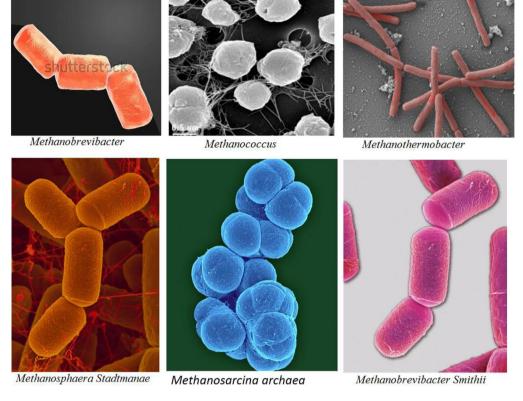
Archaea are single-celled prokaryotes that are similar to bacteria and eukaryotes with some differences. Archaebacteria include aerobic and anaerobic bacterial spp. that are capable of methanogenesis (methane production) and classified into three types: Crenarchaeota (tolerate high temperature *Sulfolobus*), *Euryarchaeota* (tolerate salt water, producing gas and methane, such as *Methanococcus*) and Korarchaeota (inhibit warm water, such as *Nanoarchaeota*). The detection and quantification of the gut methanogenic archaea are based on some recent techniques, including 16S rRNA gene analysis, real-time polymerase chain reaction, the most probable number, a statistical method, autofluorescence-based flow cytometry (Miller, Wolin, and Kusel 1986) and whole-genome shotgun sequencing. However, the

16S rRNA gene primers cannot identify all types of achaeal lineages until now (Bønløkke et al. 2019). All archaea producing methanogens in chicken's ceca have one or two copies/cell (Fogel et al. 1999), whereas there are one or four copies/cell of the 16S rRNA genes in human's *Methanosphaera stadtmanae* (Rieu-Lesme, Delbès, and Sollelis 2005).

Archaea are active and functional in mono-gastric animals despite their low abundance in the gut (Patel et al. 2010). In the human's gastrointestinal tract, archaea were represented about 1.2% of the microbial community (Kumpitsch et al. 2021), and they comprised between 0.1% and 21.3% of the total intestinal microbiota (Kim, Lee et al. 2020; Homayoni Rad et al. 2012). The recent shotgun sequencing analysis of the healthy human's revealed that the archaeome was 7.2% and the Methanobacteriota phylum was more than 50%. The dominant methanogenic taxa in the human's gut include Methanobrevibacter smithii, M. stadtmanae and Methanomassiliicoccales (Chibani et al. 2021; Minnebo et al. 2024). Moreover, the predominant archaea were Methanomethylophilus in cattle, Methanobrevibacter in sheep and chickens and Methanosphaera in pigs (Patel et al. 2010). Moreover, the abundance of Methanosphaera cuniculi and M. stadtmanae in meta-transcriptomic dataset is, respectively, 30 and 27 fold higher than the abundance of metagenomics data in pig's gut (Patel et al. 2010).

The microbiome analysis of the gut microbiota in the healthy Aseel cross breed of chickens showed that the predominant phyla

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**FIGURE 2** | Some types of methanogenic archaea in chicken (*Methanobrevibacter*, *Methanococcus*, *Methanothermobacter* and *Methanosarcina* sp.) and pig digestive (*Methanobrevibacter smithii*, *Methanobrevibacter* sp.) and poultry house (*Methanobrevibacter woesei*).

were Firmicutes (50%), Cyanobacterial (26%), Proteobacteria (17%) and archaea (0.14%) (Sowmiya et al. 2022). Moreover, Samanta et al. (2013) stated that the rumen's fluid contained a total of 10<sup>8</sup>–10<sup>9</sup> archaea, 10<sup>10</sup>–10<sup>12</sup> bacteria and 10<sup>2</sup>–10<sup>4</sup> fungi/mL content. In chicken's ceca, the most common detected archaea spp. were Methanobrevibacter, Methanococcus, Methanothermobacter, Methanosphaera (Figure 2), Methanobacterium, Methanopyrus and Methanothermus (Dittoe et al. 2018). It has been observed that the methanogenic archaea such as Methanobrevibacter woesei and other strains could be involved in the fermentation process in the intestinal environment of chickens (Rieu-Lesme, Delbès, and Sollelis 2005). The 16S ribosomal RNA gene amplification of chicken ceca and goose faeces samples revealed that the methanogenic archaea phylum had a 99% similarity to M. woesei (Rieu-Lesme, Delbès, and Sollelis 2005).

The aims of this article were introducing the general functions of archaea and spotlight on archaeome in the digestive system to emphasize their nutritional roles in human, livestock and poultry.

# 2 | Materials and Methods

The methods used in this article are systematic review frameworks and Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) in order to answer the main questions of this research. In the PRISMA method, the frameworks for collecting information were defined, the sources were searched, and the desired criteria were extracted from the published articles, consolidated and then presented (Page et al. 2021).

Selection criteria of articles: In this research, the authors tried to collect articles related to the types or species of archaea and their effects on the intestinal health and digestion of humans, livestock and poultry. The desired articles were extracted from Science Direct, MagIran, Google Scholar and PubMed databases. Keywords including archaea, gut health, human, livestock and poultry were used as main keywords, whereas caecum and species were used as secondary keywords. The collected articles were listed and then their abstracts were studied. Only articles that related to the article topic were selected, categorized and reviewed.

## 2.1 | Dataset Development

A systematic literature search was conducted using the four Science database. This literature funnel is preferred for systematic reviews and meta-analyses based on the PRISMA flow diagram (Figure 3). At the first stage, out of 1211 collected articles from the abovementioned databases, 443 sources were used for screening. After reviewing the articles, only 149 articles were selected due to the low quality, lack of relevance of the article abstract to the topic of the systematic review article and lack of access to the full text of the article. In the final screening, the lack of connection between the text and the purpose of the work done caused that only 124 articles were selected, and the rest were excluded due to non-compliance. From 1986 to 2022, only six articles were found to be specific to archaea and poultry. Therefore, it is not possible to compare the results of these reports in the form of 'PRISMA table'.

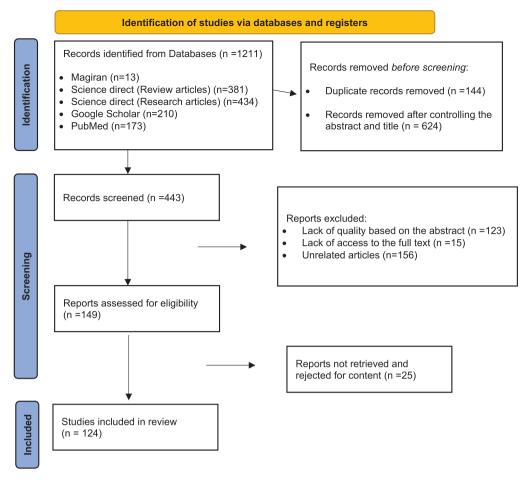


FIGURE 3 | Steps of selecting articles from databases and meta-analyses (PRISMA) diagram.

## 3 | Results and Discussion

## 3.1 | Potentials Uses of Archaea

There are many potential uses of archaea. Archaea could be applied such as blood tests (*Sulfolobus solfataricus* with a glucose dehydrogenase activity) (Einat et al. 1996), oil, nuclear and pharmaceutical industries.

Archaea could be administrated as drugs and have antimicrobial effects due to their ability to produce archaeocins, halocins, KPS1 halocin and cationic antimicrobial peptides (CAMP) (Aparici-Carratal et al. 2023). Archaeosomes which are extracted from archaea have more encapsulation efficiency than liposomes and used in vaccinology as good immuno-stimulators (Hansen et al. 2007) because they could be more easily phagocyted than liposomes, cancer treatment of mice, gene transfection of the mammalian cells (Rani, Balamurugan, and Ramakrishna 2017) and drug delivery. The archaeal gas vesicles (GVs) may be stable for a long period and resist the enzymatic degradation, so they have been used in to the vaccine delivery. These GVs are produced by Halobacterium salinarum (Hbt. Salinarum), Haloferax mediterranei, Halorubrum vacuolatum and Haloquadratum walsbyi (halophilic archaea) (Peng et al. 2022).

**TABLE 1** The dominant archaea spp. in animal's types as identified by 16S rRNA.

Species of archaea	Animal's type
Methanobrevibacter sp.	Pigs and cattle
Methanobrevibacter smithii	Pigs
Methanogenic archaeon	Pigs, cattle and chicken
Methanosarcina sp.	Cattle and chicken
Halorubrum sp.	Pigs and cattle
Archaeon 26-a134	Pigs
Archaeon 26-5a1	Pigs
Archaeon 26-4a6	Pigs

# 3.2 | Methane Production and Archaea

Methanogenic archaea had been detected in the bioaerosols of chickens, pigs and cattle houses (Table 1) (Blais Lecours et al. 2011). The predominant archaea producing methanogens in the human's gut include *Methanobacteriales* (*M. smithii* and *M. stadtmanae*) and *Methanomassiliicoccales* (Candidatus, Methanomassiliicoccus and Candidatus Methanomethylophilus)

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(Kumpitsch et al. 2021). The main groups of archaea in human's intestine are the methanogenic (*Methanosarcina*) (Samuel et al. 2007), halophilic (*Halobacteriaceae*) and thermophilic (*Sulfolobus*) group (Rastädter et al. 2020; Hoffmann et al. 2013).

They can produce and emit methane in an amount of 0.35 L/day (Polag et al. 2019). The *Methanobrevibacter* spp. (McLoughlin et al. 2020) and *Methanoculleus* spp. (Barret et al. 2013) were common in pig breeds and Canadian pigs, respectively. *Methanosphaera*, *Methanobrevibacter*, and *Thermoplasmatales* were also identified in the ceca and faeces of herbivores rodents (Kim, Whon et al. 2020). In sheep, the methanogenesis leads to a reduction of 6%–10% in the gross energy (Pinares-Patiño et al. 2013), whereas in cattle, the anti-methanogenic supplementation caused a 30% (Deng et al. 2021) and a 30%–82% reduction in methane production (Kim, Lee et al. 2020).

The methane production by microbes could be affected by vitamin  $B_{12}$ , fats and dietary fibres. The *Methanobrevibacter* count had negatively correlated with the total fats (R = -0.435),  $B_{12}$  (R = -0.439), vit D (R = -0.345), but positively correlated *Blautia* with vitamin  $B_{12}$ , and protein (R = 0.422) (Kumpitsch et al. 2021).

#### 3.3 | Nutrition and Archaea

The health, diseases and nutrition of humans and animals can be affected by the archaeal spp. Archaea have low nutritional requirements and a high metabolic diversity (Aparici-Carratal et al. 2023). The archaea functions in the gastrointestinal tract of livestock are crucial as they can reduce the hydrogen (Patel et al. 2010), the expression of carbohydrate-active enzymes genes in pigs (Deng et al. 2021), and the decrease in hydrogen and the increase in energy and fat storage by the methanogenic archaea have been also reported in mice (Samuel and Gordon 2006). The quantity of archaea can be influenced by the food intake by humans. For instance, Koreans who consume more fermented seafood have a higher population of *halophilic archaea* (haloarchaea) (Kim, Lee et al. 2020).

The feed efficiency and cecal fermentation were affected by the syntrophic interaction between archaea and bacteria (Ufnar et al. 2006). Chicken feed efficiency is linked by cecal microbial hydrogen cycling and *Methanobrevibacter* spp. abundance, SRBs (archaeal syntrophic partners, e.g., *Desulfovibrio* spp.) and mcrA genes that are indicators for archaeal methanogenesis (Primec et al. 2019). Moreover, the *Methanobrevibacter* spp. and *Deltaproteobacteria* spp. have a syntrophic potential (Sowmiya et al. 2022). The fowl-associated archaea are very specialized symbionts in avian caca (Moran and Bennett 2014). Moreover, haloarchaea spp. are considered a good source of carotenoid with an excellent antioxidant and photo-protective characteristic (Busquet et al. 2006).

The archaea spp. including *Methanomassiliicoccales* and *M. smithii* may induce a better feed utilization in the rumen of dairy cattle (Li et al. 2017; 2019) and sheep (McLoughlin et al. 2020). Moreover, the administration of garlic oil in ruminants may result in an anti-methanogenic effects (Brugère et al. 2014).

Haloarchaea spp. are considered a good source of carotenoid with an excellent antioxidant and photo-protective characteristic (Busquet et al. 2006).

High levels of *Methanobacteriales smithii* were observed in human patients who have anorexia (Armougom et al. 2009), transit or chronic constipation (Lecours et al. 2014), and colorectal cancer. In poultry, there is no information about the effects of intestinal archaea on anorexia induced by diseases conditions, dysbiosis or low quality feed and water.

## 3.4 | Archaea as Probiotics

The next-generations of the probiotics compounds could improve the gut health, immune response and performance and reduce the pathogenic microorganisms (Salahi and Abd El-Ghany 2024). The methanogenic archaea (archaeobiotics) have probiotics characteristics and prevented of cardiovascular diseases (CVDs), cancer and obesity (Amabebe et al. 2020). The probiotic preparations containing *Methanomassiliicoccus luminyensis* could be used in the treatment of trimethylaminuria (TMAU) disease, cerebrovascular diseases and atherosclerosis (Borrel et al. 2020; Aparici-Carratal et al. 2023). Comparing the effectiveness of probiotics produced by archaea requires more extensive research in humans, livestock and poultry.

Low *Bacteroidetes* and high *Firmicutes* counts have been detected in obese people intestine. Moreover, the supplementation of probiotics and prebiotics is an effective way for the prevention of obesity through stimulation of beneficial bacteria, enhancing the production of SCFAs, modulation of energy homeostasis, increasing the secretion of satiety hormones such as peptide YY I and glucagon-like peptide 1 and 2, and decreasing the hunger hormones such as ghrelin (Homayoni Rad et al. 2012).

The uses of *extremophilic* archaea in food production, protein synthesis (Aparici-Carratal et al. 2023), as well as enzyme production (protease, amylase, lipase and cellulase), have been studied (Littlechild et al. 2015).

## 3.5 | Heat Stress and Archaea

Except viruses, the heat shock protein (HSP) is present in archaea, bacteria and eukaryotes. In poultry, the HSP is classified according to the molecular weight into; large weight (HSP 110, 90, 70 and 60) and small HSPs (B1 and B10) (Balakrishnan, Ramiah, and Zulkifli 2023). It helps in the protection of avian cells during stressors by promoting the HSP expression, immune system and protein folding response (Marques et al. 2019). Exposure to heat stress (2 h or more) increased the levels of HSP 90, 70, 29 and 27 (Dridi, Raoult, and Drancourt 2011), whereas exposure to cold stress decreased HSP 90 and increased HSP 70, 60, 40 and 27 levels (Zhao et al. 2013).

Glutamine has a crucial role in the production of glutathione which is important for cells oxidation, HSP 70 production and feed consumption. Improving the ecology of the digestive system and increasing the bird's resistance to heat or cold stresses using archaea still needs more investigation.

## 3.6 | Immunity and Archaea

Archaea producing methane have shown pulmonary inflammation, accumulation of leukocytes and histopathological changes in mice (Blais Lecours et al. 2011). It has been found that archaea membrane lipids have immunogenic characteristics (Schiraldi, Giuliano, and De Rosa 2002) that may be used as vaccines adjuvants to stimulate the immune response (Page et al. 2021). The archaeosomes, a new generation of stable liposomes, were extracted from methanogenic archaebacteria and showed a drug delivery ability. Nano-archaeosome encapsulation (Cisplatin) could deliver a high dose of drug to the target cells for the treatment of breast and prostate cancer.

The role of *M. stadtmanae* in the development of human's intestinal autoimmune diseases such as inflammatory bowel disease (IBD), allergy and asthma has been discussed as its occurrence frequency is increased under diseases conditions. Moreover, *M. smithii* is the most frequent archaea which is commonly observed in the gastrointestinal tract in severe systemic infections and also *Methanobrevibacter oralis* is found in periodontal diseases (Ding and Schloss 2014). To our knowledge, there is no studies on the immunogenic role of archaea in domestic animals and poultry.

#### 3.7 | Diseases Preventions and Archaea

The methanogenic archaea in the GIT of humans are a reliable indicator of gut methanogen overgrowth in various diseases. Archaea are found in 42%–100% of human's faecal samples (Kim, Lee et al. 2020). In a celiac disease of children (celiac sprue or gluten-sensitive enteropathy), *Euryarchaeota* showed a positive role in reducing the anti-inflammatory factor (Primec et al. 2019). Furthermore, archaea are able to eliminate the unfavourable conditions such as reducing the odours by oxidizing ammonia secreted from the skin (Mohammadzadeh et al. 2024). *M. stadtmanae* could reduce asthma in 6–10 years old children, and eczema (Barnett et al. 2019).

Methane gas, the end product of microbial fermentation or archaea, has been usually associated with IBD, colorectal cancer, bloating, abdominal pain (Kumpitsch et al. 2021), transit constipation (Lecours et al. 2014), bacterial overgrowth and decreasing in the gastrointestinal motility. It has been reported that 20% of the healthy Western humans produced higher methane gas (>5 ppm) which may effect on their health, whereas those have a higher emitting capacity (5–75 ppm) showed 1000-fold increase in *M. smithii* count (Kumpitsch et al. 2021).

Before the methanogenic archaea have been identified, the IBD (human ulcerative colitis [UC] and Crohn's disease [CD]) is attributed to many infections by viruses, fungi, helminths or bacteria (Youngblut et al. 2021). Scanlan et al. (2008) confirmed that patients with IBD showed low counts of methanogenic archaea as the prevalence of *M. smithii* in healthy people as well as CD and UC infected people was 48%, 30% and 24%, respectively. Recently, Cisek et al. (2024) concluded that the methanogenic archaea may affect the incidence of IBD in both children and adults. The number of total methanogens in UC and CD significantly lower than control group.

Regarding diseases prevention, archaea have a potential as antimicrobials or antibiotics due to their ability to produce archaeocins (Atanasova, Pietila, and Oksanen 2013), sulfolobicins (thermoacidophiles), halocins (haloarchaea), KPS1 halocin (Just et al. 2013) and CAMP (Nehmé et al. 2009). The halocin H6 of archaea showed cardio-protective characteristics, and it has been applied as Na<sup>+</sup>/H<sup>+</sup> exchanger hyperactivity for the control of blood pressure and the prevention of myocardial damage (Lee et al. 2013) and heart diseases (Barret et al. 2013). An *Hbt salinarum* strain showed cytotoxic effects on the cancer cells of human's prostate (Saengkerdsub, Herrera et al. 2007). It is important to note that the haloarchaea GVs and *Hbt. halobium* are indicators for oncology (Aparici-Carratal et al. 2023).

## 3.8 | The Age and Archaea

The prevalence and diversity of archaea producing methanogens in humans have been increased with increasing the age (Ramírez et al. 2022). They constitute about 90%–99% of the total human's intestinal archaea (Kohl et al. 2014; Mohammadzadeh et al. 2024) and 10% of the total anaerobic community (Samuel et al. 2007). The occurrence frequencies of *Methanobrevibacter* spp. in newborn children and young child (6 months to 2 years old) were 98% and 96%, respectively, and the *M. smithii* made up 88% (neonates) and 97% (children) (Ramírez et al. 2022). By increasing the age (school-ages), the archaea spp. including *M. smithii* (78%–88%), *M. stadtmanae* (8%–11%), *M. luminyensis* (1%) (Dittoe, Ricke, and Kiess 2018; Barnett et al. 2019), *Methanomassiliicoccales* (40%) (Mi et al. 2019), *M. luminyensis* (4%) (Dittoe, Ricke, and Kiess 2018) and *Methanomassiliicoccus* (1%–26%) (Cisek et al. 2024) could be detected.

The aging of human's body can change the composition of archaea producing methanogen. For instance, the prevalence of *Methanomassilicoccales* was changed from 10% in young to 40% in adults (Mi et al. 2019) and also *M. luminyensis*, *Candidatus Methanomassilicoccus intestinalis* (Dittoe, Ricke, and Kiess 2018), and *M. smithii* were predominant in old ages (Biagi et al. 2016). A partnership between *Candidatus Methanobrevibacter intestinii* and butyrate producing bacteria has been detected in older ages of humans (Mohammadzadeh et al. 2024).

In poultry, reports concerned with the effects of age, the rearing period and so forth on the changes of archaea have not been found. More researches are necessary in this area.

## 3.9 | Environmental Effects on Archaea

Houshyar et al. (2021) reported that the bio-aerosol of archaea (organic dust with 0.002–100  $\mu$ m) in the cage-housed poultry operation showed a higher incidence of *M. woesei* than in the floor-housed operation. In addition, the type of ventilation (Neutral, mixed type and negative pressure) and the floor wetness may increase the number of *M. smithii* in Canada pig and cattle farms after testing by the 16S rRNA gene (Blais Lecours et al. 2011). In swine's houses, the archaea numbers in bioaerosols were more in winter than in summer (Nehmé et al. 2009).

The immunogenic spp. of the airborne archaea including M. *smithii* and M. *stadtmanae* have been found with a total number of  $10^6/\text{m}^3$  of the air in the dairy barns and  $10^4$  in the poultry houses (Kumpitsch et al. 2021). These types of archaeal bioaerosol can be a source of the respiratory diseases for workers in the farms.

The presence of archaea producing methanogen such as *M. smithii* in the large intestine of 95% of people (Dridi et al. 2012) and *M. stadtmanae* in the digestive system of 29.4% of people in a community (Baradaran Ghavami et al. 2016) was related to the presence of *M. smithii* in the drinking water in the same region or the farm (Ufnar et al. 2006), and this was indicated water contamination with human's sewage. In addition to the type of farm (house), the transporting equipment including chicken box (Salahi and Esmailizadeh 2014) baskets of slaughterhouse can also be examined for the presence of archaea.

## 4 | Conclusions

Archaea spp. exist in the ruminant and human's gut more than in no-ruminants. Archaea had many benefits for animals, humans and mono-gastric, among which we can mention prevention of metabolic and CVD, used as antibiotic and probiotic, and increased feed utilization and efficiency. The main archaea species in chickens include *Methanobrevibacter (M. woese)*. Archaeobiotics have probiotic properties and are used in the prevention of CVDs, cancer and obesity in humans. Antibiotics can reduce the number of archaea. The population and abundance of archaea increase with the age of humans. The role of archaea in human and mice immunity has been reported, but there is no report in poultry and livestock. Syntrophic interaction between archaea and microbiome and their effects on the feed efficacy and cecal fermentation are topics that need more research work.

To our knowledge, this article is the first that deals with archaea in nutrition and physiology of poultry. There is a need for more extensive researches on their role and importance in feeding of poultry and livestock. Due to the low abundance of archaea in poultry microbiome, they have not been given enough attention.

## **Author Contributions**

**Ahmad Salahi**: writing – original draft preparation, conceptualization, review, collected data, methodology, designed analysis, and editing. **Wafaa A. Abd El-Ghany**: writing – original draft preparation, conceptualization, methodology, review and editing.

## **Conflicts of Interest**

The authors declare no conflicts of interest.

#### **Institutional Review Board Statement**

Not applicable.

## **Data Availability Statement**

The authors have nothing to report.

#### Peer Review

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