Storage of poultry manure in containers – changes in physiochemical parameters and survival of intestinal bacteria

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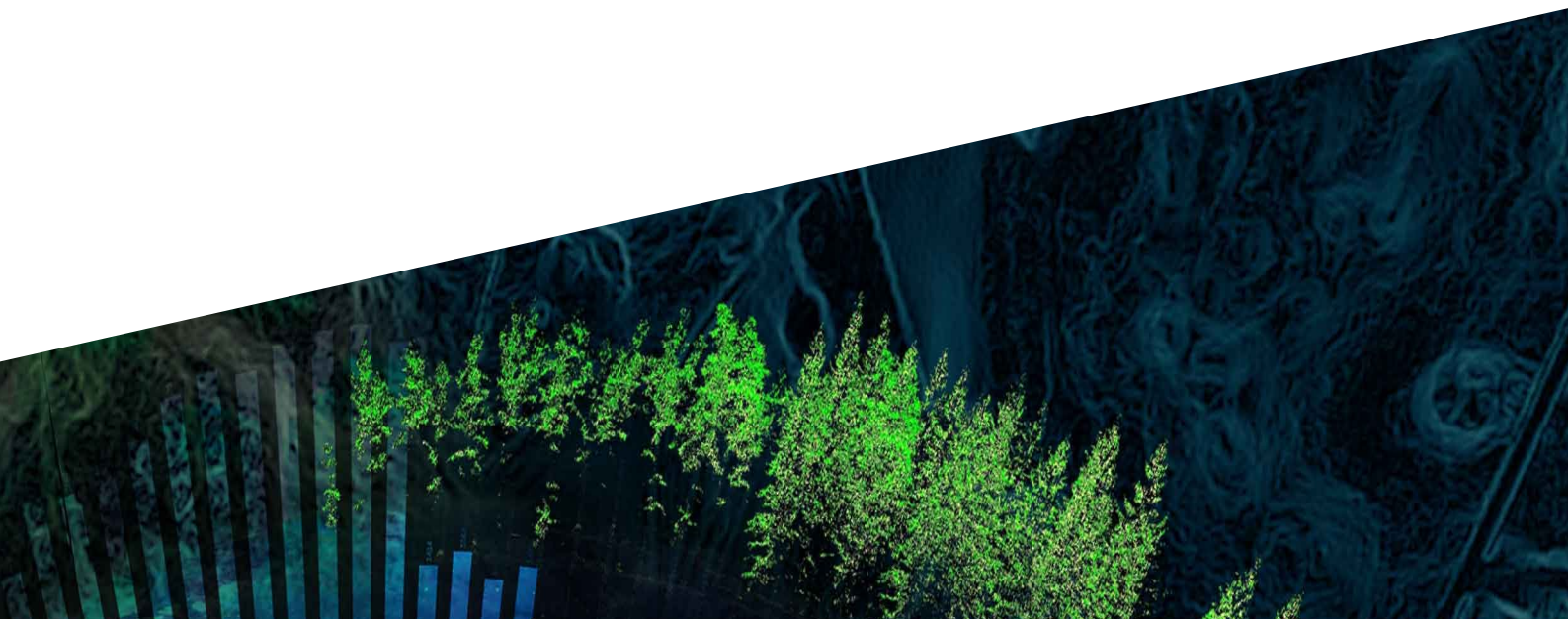

Storage of poultry manure in containers – changes in physiochemical parameters and survival of intestinal bacteria

Lagring av fjäderfägödsel i container – förändringar i fysiokemiska parametrar och överlevnad av tarmbakterier

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Abstract

High nutrient content of chicken litter has made it one of the best organic fertilizers for agricultural land. However, reusing poultry litter in agriculture can be associated with food-borne pathogens such as *E.coli, Salmonella* and *Campylobacter*. To inactivate pathogens and minimize the environmental risks, manure could be treated before spreading on the field. Composting is an effective method to reduce pathogenic bacteria counts. Stacking the manure in containers is a minimally managed method to prevent the manure particles and effluents from entering the surrounding environment and decontaminate the manure before land application. However, more knowledge about bacterial decontamination is needed to ensure complete decontamination and reduce storage time and space.

This study was conducted to estimate microbial growth or inactivation as a function of environmental and physico-chemical factors during six months of storage in containers. First, the fluctuations in the number of *Enterobacteriaceae* bacteria were investigated in two big containers with or without adding wheat straw regarding alterations in temperature, dry matter (DM), pH, and oxygen levels. Second, the survival of bacteria belonging to *Enterobacteriaceae*, *Salmonella typhimurium* and *Campylobacter jejuni* were examined in a bucket filled with the same manure. This allows for the anticipation of pathogenic bacterial elimination in the containers concerning the *Enterobacteriaceae* population.

According to bacterial analyses, the number of *Enterobacteriaceae* went below detection limit after nine weeks for the containers and four weeks for the bucket. *Salmonella* was detected after enrichment during the whole experiment period in the samples from the surface of the bucket, while it was not detected from sixth week in the middle of the bucket, except for week 12. *Campylobacter* could not be detected by enrichment one week after inoculation. According to our results, direct cultivation on selective agar media following dilution for *Enterobacteriaceae* is not a reliable indicator for complete *Salmonella* inactivation in a sample. This thesis further emphasizes the significant impact of different physico-chemical parameters on manure composting. Low pH, desiccation, and heat were some of the parameters that contributed to the bacterial inactivation. A closely monitored process with proper amounts of required elements results in faster organic decomposition and bacterial decontamination.

Keywords: Enterobacteriaceae, Salmonella, composting, straw, litter decontamination, chicken manure

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Abbreviations

AMR	Antimicrobial resistance
BG	Brilliant Green agar
BPW	Buffered peptone water
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
MALDI-TOF MS	Matrix-assisted laser desorption/ionization-time of flight mass spectrometry
mCCDA	Modified charcoal cefoperazone deoxycholate agar
MSRV	Modified semi-solid Rappaport Vassiliadis
SLU	Swedish University of Agricultural Sciences
SVA	Swedish National Veterinary Institute
VRBG	Violet red bile glucose agar
VTEC	Verotoxin-producing Escherichia coli
XLD	Xylose lysine deoxycholate agar

1. General background

The poultry industry has grown significantly over the last few decades. Between 1970 and 2005, poultry meat and egg production increased faster than that of beef and pig meat, particularly in middle income countries (Windhorst 2006). In Sweden, following an increased demand, poultry production is also increasing. In 2020, the Swedes consumed an average of 22.4 kg of chicken per year and person and approximately 110 million broiler chickens were produced, which is around a 5% increase from 2019 and 38% increase from a decade earlier at 2010 (Swedish Board of Agriculture 2021). However, poultry and its products can be linked with major human pathogens such as *Salmonella* and *Campylobacter* (Bryan & Doyle 1995), which can have a wide range of consequences for human health (Mead 2005).

The litter from a *Salmonella*-positive farm can act as a reservoir for the bacteria and pose a risk to humans, other animals, and the environment (Payne et al. 2007). Although poultry farms can contribute to the environmental load of *Campylobacter* by contaminating soil and aquatic environment (Gras et al. 2012; Schets et al. 2017), there are no specific rules in Sweden regarding manure decontamination in *Campylobacter*-positive farms. Based on Swedish manure management law, manure from a *Salmonella*-positive flock is considered infected, even though *Salmonella* has only been detected in a single draft sample (SJVFS 2014). According to SVA, infected liquid and solid manure may be spread on fields if ploughed down within hours after removal from barns. However, due to the long survival of *Salmonella* in soil (> 6 months), the soil where untreated manure has been incorporated cannot be used for grazing or forage harvest (hay, silage) during the same growing season as fertilization has taken place (Elving et al. 2018). If farmers do not possess land to spread or ploughing the manure, or during periods when spreading the manure on agricultural land is not permitted due to the high risk for nutrient leakage, the manure must be sanitized before spreading. Sanitization of the manure can be conducted by means of composting for solid manure in a pile during 6 months whereas slurry should be treated with lime, urea, ammonia, or sodium/potassium hydroxide (Elving et al. 2018). Storing the manure in a container is an alternative that can prevent the manure particles and effluents from entering the surrounding environment. There are strict regulations regarding the pile's location due to risks for spreading of *Salmonella* to the surrounding area and possible groundwater contamination. During transport and storage, litter should be well protected to prevent contamination. Therefore, storage of bedding shall be in a specific area well separated from the livestock and buildings and there must be no water flow. In addition, during storage straw should be mixed into the manure, the ground and the surface should be covered with slaked lime and the surface should be covered with a proper layer of straw (Elving et al. 2018).

1.1 Sustainability

According to the Food and Agriculture Organization of the United Nations (FAO), the world's population is estimated to reach more than 9 billion by 2050 (Alexandratos & Bruinsma 2012). Currently, livestock production captures about 30% of the ice-free surface of the Earth (Steinfeld et al. 2006). Population growth, increases in wealth and changes in the diet result in the need for about 60% increase in agricultural products from 2007 to 2050 (Alexandratos & Bruinsma 2012). This increase will put more pressure on the already over-pressured environment. To combat this immense challenge, a multifaceted and linked global strategy is needed, one of which is finding new sustainable ways to produce protein from yet inaccessible resources.

One sustainable solution is to improve the recycling of organic waste from livestock production into highly valuable fertilizer (Nordentoft et al. 2017). However, intensive livestock production is frequently located on farms that do not have enough land for proper utilisation of the manure, and this practice may lead to pollution of land, crops, water, and streams (Westerman & Bicudo 2005). Raw or fresh manure have limited uses, as they can be applied to agricultural lands just a few times during the year and are expensive to transport (Jongbloed & Lenis 1998). Composting can be used to recover degraded soils, restoring their fertility and reduce the use of chemical fertilizers and pesticides (Pergola et al. 2018). It is also an established pathogen reduction technology, which can reduce the pathogens such as *E.coli*, *Salmonella* and *Campylobacter* and improve human and animals health (Lepesteur 2022).

In addition, since poultry manure contains inorganic N, microbial available sources of C and water, greenhouse gases of N_2O and CH_4 can be emitted during composting (Chadwick et al. 2011). Manure management procedures and environment can influence the amount of GHG emission, which is of great importance for reducing emissions and improving sustainability (Møller et al. 2004; Chadwick et al. 2011; Yin et al. 2021).

1.2 Aim

There are still large knowledge gaps regarding the risk posed by manure as many of the principles are based on practical experience, and little is known regarding the survival of *Salmonella* and other coliforms in chicken manure during storage in containers as an alternative method for reducing the manure manipulation during composting. Therefore, more knowledge is needed regarding the impact of physico-chemical factors such as temperature, pH and water activity on intestinal bacteria such as *Campylobacter* and coliforms. This project aims to evaluate if storage of chicken manure in containers will provide the conditions required for eliminating *Salmonella* and *Campylobacter*, and if straw added to the manure could facilitate the composting process.

2. Literature review

2.1 Poultry manure – Risks and pathogens

The poultry industry is currently facing different environmental challenges such as manure accumulation, nitrogen (N) and phosphorus (P) leaching, and GHG emissions (Powers & Angel 2008; Berghaus et al. 2013). For centuries, manure from farm animals has been used as fertilizers to improve the structure and fertility of agricultural lands. Globally, poultry is one of the fastest-growing industries in agriculture, and the high nitrogen content of poultry manure has made it one of the best organic fertilizers compared with manure from other animals. Farmers commonly use low-cost organic materials such as wood shavings and wheat straw as litter bedding materials. However, poultry manure can contain different pathogenic bacteria such as *Salmonella* spp*., Campylobacter* spp. and *E.coli*, which can be linked to human health risks and environmental concerns (Hruby et al. 2016). According to the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), the first and second most reported zoonoses in humans are campylobacteriosis and salmonellosis, respectively. Sweden is however, one of the countries with the highest level of disease control regarding salmonellosis and campylobacteriosis (Authority & European Centre for Disease Prevention and Control 2021a). A study in USA evaluated the health burden of 168 pathogen-food combinations based on the cost of illness, loss of quality-adjusted life years and mortality in the USA. Based on the results, *Campylobacter* and *Salmonella* infections from poultry are the first and fourth pathogens regarding disease burden among 104 pathogen-food combinations, respectively (Batz et al. 2012). Although the use of poultry litter as fertilizer in agricultural lands has rarely been associated with food-borne illnesses, increased consumer awareness regarding food safety issues has increased the demand for improving on-farm manure management practices (Wilkinson et al. 2011). Therefore, before using poultry litter as fertilizer, it can be processed to destroy pathogenic organisms. Composting is an effective process for bacterial decontamination in poultry litter. During composting, the litter can be decontaminated from pathogenic bacteria through sustained high temperatures (Dumontet et al. 1999; Macklin et al. 2006).

2.2 Enterobacteriaceae

Enterobacteriaceae is a large family of Gram-negative bacteria that includes over 30 genera of bacteria, including several important pathogens such as *Escherichia*, *Salmonella*, and *Shigella*. Many of these bacteria are zoonotic pathogens that could be included in the intestinal microbiota and therefore exist in manure, with risk of

contamination of soil and agricultural products. In a long term perspective this may cause diseases in humans through direct contact with soil or food consumption (Pattison et al. 2007). *E.coli* is a well-known species in the *Enterobacteriaceae* family and is the most widely used indicator of fecal contamination in food (Pierson et al. 2007). In addition to health concerns, one of the most critical problems regarding *Enterobacteriaceae* is emerging antimicrobial resistance (AMR), which could create species resistant to most antibiotic agents (Paterson 2006). Disease outbreaks as well as sporadic cases of *E.coli* caused by contact with manure or environmental exposure have been reported (Howie et al. 2003). In 2005, there was a large outbreak of verotoxin-producing *Escherichia coli* (VTEC) in Sweden. The epidemiological investigations indicated lettuce as the source of the outbreak. The lettuce was irrigated by water from a small stream, which was contaminated by cattle grazing upstream from the irrigation point (Söderström et al. 2008).

2.3 *Salmonella*

Globally, *Salmonella* is one of the most frequent and important foodborne pathogens, and consuming poultry products such as eggs and meat are the primary sources of human infections (Kimura et al. 2004). It is a Gram-negative intracellular facultative anaerobe bacteria belonging to the family *Enterobacteriaceae* that causes major enteric disease syndromes such as gastroenteritis with vomiting and diarrhea, abdominal pain, fever and in severe cases septicaemia (Coburn et al. 2007). *Salmonella* is associated with a large number of diseases, outbreaks, deaths and huge economic losses each year. Regardless of improved health control programs and biosecurity conditions for poultry farms, the prevalence of *Salmonella*-positive flocks in the EU has been fairly high in some countries in recent years. However, the number of human cases of *Salmonella* in 2020 has decreased by 32.8% compared with 2019 (EFSA 2021b), most likely due to Covid-19 and that people seek less medical care and eating at restaurants at the same extent.

According to EFSA, *Salmonella* was accounted for 17.9% of all food-borne disease outbreaks during 2019 in the EU, with more than 91,000 human cases of salmonellosis. That means 20.0 cases per 100,000 population, which is similar to 2018 (EFSA 2021a). It is estimated that the global cost of human salmonellosis is more than 3 billion euros per year due to disease investigations and testing, health care costs, decreasing productivity, and increasing costs (Payne et al. 2007).

2.3.1 *Salmonella* in Sweden

There is zero tolerance to *Salmonella* in poultry farms as well as other livestock farms in Sweden and if any *Salmonella*, regardless of the serotype, is found in a broiler production unit, the entire flock is euthanized and cleaning and disinfection of the premises are performed (SVA 2022). In addition, according to Swedish

legislation, only heat-treated feed can be used in Swedish poultry farms (SJVFS 2006). In 1970, a *Salmonella* control and monitoring program was implemented in the entire Swedish broiler production chain with the aim to prevent *Salmonella*. In 2007, it was extended to become a more general biosecurity program. The program was developed by the Swedish Poultry Meat Association and approved by the Swedish Board of Agriculture (SJV) and aims to decrease the incidence and spread of *Salmonella* and facilitate decontamination in the event of *Salmonella*. Before approval to this program, different conditions regarding housing, barns, feed, water and manure management should be met. Besides the voluntary program, there is also a mandatory *Salmonella* control of poultry regulated by one Swedish and several EU legal acts. It involves testing every broiler flock for *Salmonella* one to two weeks prior to slaughter. In addition, once a year, an officially appointed veterinary officer visits the farm to collect the samples (SJVFS 2007). The screening program and the zero-tolerance policy in Sweden have encouraged systematic work for improved biosecurity, which in turn has led to that *Salmonella* being detected only in a limited number of poultry flocks per year (SVA 2020). According to SVA (2020), *Salmonella* were detected in only 7 Swedish poultry flocks in 2020. All of these flocks were layers and *Salmonella* was not isolated in any samples from broiler flocks.

In addition, regarding the number of human cases, the total number of confirmed cases in Sweden has decreased from 1990 cases in 2019 to 825 confirmed cases in 2020. As Sweden has the highest proportions of travel-associated cases in European countries, with 45.6% (EFSA 2021b), this reduction can be resulted from less traveling due to Covid-19 pandemic.

2.4 *Campylobacter*

Campylobacter spp. are gram-negative, oxidase-positive, and catalase-positive bacteria and the most commonly reported foodborne gastrointestinal infection in humans in the EU since 2005 (EFSA 2021b). Approximately 230,000 cases have been reported annually in Europe since 2015, but in 2020, around 120 thousand human cases of campylobacteriosis were reported to EFSA. The decrease is probably due to the Covid-19 pandemic, which had an impact on people seeking medical care and eating habits. In addition, 317 outbreaks caused by *Campylobacter* were reported in 2020 within the EU, and the most common foods responsible for these outbreaks were broiler meat and raw milk (EFSA 2021b).

Although *Campylobacter* is insignificant for poultry health, their products are the primary sources of *Campylobacter* infections in humans with different clinical signs, including diarrhea, abdominal pain, fever, headache, nausea, and vomiting (Sahin et al. 2015; Hansson et al. 2018). Consumption of undercooked poultry meat, raw milk, contaminated vegetables and drinking water together with transmission from pets and other animals can cause campylobacteriosis in humans (Adak et al. 1995; Friedman et al. 2004). *Campylobacter jejuni* and *Campylobacter coli* are the

two most important species regarding food-borne campylobacteriosis (Hansson et al. 2018). These bacteria are frequently colonizing the intestinal tract of avian hosts; therefore, their carcasses can be contaminated by intestinal content during slaughter. Nowadays, risk assessment of *Campylobacter* in broiler meat is not only used to assess the human incidence of campylobacteriosis in poultry plants but also as a tool for analyzing the effects of control measures of human zoonotic diseases (Nauta et al. 2009). Thermotolerant *Campylobacter* species such as *C. jejuni* and *C. coli* multiply at 37 to 42°C. Birds have a body temperature of 40-42°C, therefore thermotolerant *Campylobacter* spp could easily colonise the bird's intestine and be excreted in feces (SVA 2016; Umaraw et al. 2017). The bacteria can be transmitted to humans through direct contact with birds or when contaminated raw meat is consumed (Sahin et al. 2015). As a gastrointestinal bacterial pathogen, untreated poultry manure could be a source of *Campylobacter.* Spreading contaminated manure as fertilizer on agricultural lands can contaminate vegetables and fresh fruits, wild animals, and water sources through runoff and untreated wastewater (Schets et al. 2017).

2.4.1 *Campylobacter* in Sweden

Since 1991, the Swedish Poultry Meat Association has been organizing a monitoring program for *Campylobacter* to reduce the presence of *Campylobacter* spp. in Swedish chicken flocks. The program is financed by the Swedish Board of Agriculture and includes over 99% of the Swedish chickens. Over the years, the number of *Campylobacter* positive flocks in Sweden have been reduced from about 20 percent in 2002 to 4.6 percent in 2019 (Svensk Fågel 2021). Since 2005, sampling for *Campylobacter* is performed by collecting intact caeca from 10 birds of every broiler flock at the major Swedish abattoirs (Hansson et al. 2007).

2.5 Composting

Animal manure are valuable sources of nutrients and trace elements such as N, P, and K to fertilize agricultural lands (Mandal et al. 2007). However, they can contain various foodborne pathogens that pose health risks for humans and the environment.

Composting is an effective way to deal with excessive amounts of manure, eliminate odours and transfer it into safe fertilizer for soil improvement. It is an aerobic, thermophilic, and self-heating process (Kuhlman 1990) involving microbial breakdown of organic materials, which requires different ingredients such as carbon, nitrogen, oxygen, and moisture and produces various products such as carbon dioxide, water, ammonia and heat (Hadar & Mandelbaum 1992; Haarala 2012). Sustained high temperatures during composting can reduce pathogens. Therefore, a closely monitored process with proper amounts of required elements can result in faster organic decomposition and higher temperatures, which reduce the costs, decontamination time and GHG emissions.

2.5.1 Process

Under optimal conditions, composting has three distinct phases as follows:

1. **Initial Phase** (mesophilic, or moderate-temperature phase)

During the first few days, mesophilic microorganisms begin the initial decomposition phase by rapidly breaking down the easily degradable compounds such as sugars, proteins, and amino acids (Hoitink et al. 1996). The heat produced by bacterial activity causes the compost temperature to rise to around 40°C rapidly. At the end of the initial phase, the temperature reaches 40-50°C, which causes self-limitation in the mesophilic community (MacGregor et al. 1981).

2. **Peak Heating Phase** (thermophilic, or high-temperature phase)

After rising the temperature above 40-55°C, mesophilic bacteria are suppressed and a period with the highest activity by thermophilic microorganisms starts that further increase the temperature to a peak of 55-70°C. Maintaining this temperature for a more extended period is crucial for pathogenic decontamination as most of the pathogenic bacteria are destroyed at this temperature (Hoitink et al. 1996; Haarala 2012). Therefore, managing the compost pile is essential in this period. While readily metabolized compounds are slowly depleted, aeration, mixing, and adding moisture can help maintain peak temperature for a longer period.

3. **Curing Phase** (cooling and maturation phase)

In the final phase, the high-energy compounds become depleted, the core temperature gradually decreases, and mesophilic microorganisms from the outer cooler layers migrate into the compost centre, recolonize once again, and become dominant (Hadar & Mandelbaum 1992). The optimal condition for bacterial decontamination results in optimizing the operation and improving the quality of the products, which requires an understanding of microbial survival under different conditions (Wang et al. 2015).

2.5.2 Methods

There are different types of composting methods.

Windrow composting

This method involves forming organic materials or biodegradable waste into rows of long piles called windrows. It is generally used in producing large quantities of compost. Windrows sizes vary due to weather, turning equipment utilized, and initial characteristics of the waste and they normally are from 2 to 6 m in width at the base and 1 to 3 m in height (Kuhlman 1990). Windrows are aerated periodically,

either manually or mechanically, to improve porosity and oxygen content and mix cooler edges with the pile's hotter core (Hay & Kuchenrither 1990). In addition, in-house windrow composting of litter is an effective way of reducing or eliminating foodborne pathogens in farms (Macklin et al. 2008).

In-Vessel Composting

This method involves confining the composting materials within a plastic tank, silo, or concrete bunkers. In-vessel composting allows good control over the environmental conditions and essential elements during composting, such as temperature, moisture, and oxygen concentration. Organic material is mechanically turned or mixed inside the tanks, which can accelerate the composting process and reduce the time needed for pathogen decontamination (Walker et al. 2009; Sangamithirai et al. 2015).

Static Pile Composting

In a static pile, blended organic waste is placed as a large pile without physical manipulation during the composting process. It can be piled in an open or covered field or a closed container (Schaub & Leonard 1996). The pile can be placed over perforated piping, providing air circulation for controlled aeration during the composting process. The aerated static pile method enables process control for a faster microbial breakdown of organic materials, but at a higher cost.

2.6 Factors of importance during composting

While composting is a simple process, it must remain within the limits of several basic environmental conditions that affect biological activities for better performance. Although there are optimums for these factors during the composting process, the range is quite wide (Kuhlman 1990). Physico-chemical properties extensively affect the microflora and their biological activities and subsequently affect pathogenic suppressive qualities during composting. Moisture, water activity, organic material composition, oxygen, pH, and temperature are the most important factors in the composting process.

2.6.1 Moisture content

Moisture content is a critical factor in optimizing composting, as it is essential for microbial activity and microbial migration during the decomposition of organic matters (Kim et al. 2016). Low moisture content can decrease biological activity and increase heat loss, while high moisture content can result in prolonged treatment time or low degradation efficiency and nutrient loss (Li et al. 2013). Previous studies mentioned various moisture contents as optimal for manure composting based on different methods, environmental conditions, and biological

and physico-chemical properties of the materials. Fernandes et al. (1994) investigated composting of poultry manure mixed with peat or chopped straw under different initial moisture contents (73%, 76% and 80%) using static pile passive aeration method. They successfully reached high temperatures up to 70°C in all of the piles (Fernandes et al. 1994). Petric et al. (2009) mentioned initial moisture content of around 69% as the most efficient moisture content for composting poultry manure mixed with wheat straw. In the study by Li et al. (2021) the moisture content of 53% showed the highest composting temperature (61°C) and the most prolonged high-temperature period.

2.6.2 Ambient temperature

Temperature is another important factor that primarily affects the bacterial population before the start of microbial degradation, in the initial stage of composting and during self-heating. Microorganisms need to be active to decompose and hence generate heat. Fungi and bacteria responsible for self-heating composts need at least 10 to 12°C to be active and get the composting process going (Stentiford 1996).

Low ambient temperature in Sweden brings different technical challenges to the operation and management of the composting process. Colder climate increase temperature difference between compost pile and the surrounding environment, which results in higher heat transfer through conduction, convection, and radiation (Wang et al. 2013). This can delay elevated temperature period, decrease thermophilic stage and product maturity, which can result in the failure of the composting process (Larney et al. 2000; Das et al. 2002).

2.6.3 Oxygen

Composting is an aerobic biochemical process and the oxygen concentration is one of the most important influencing factors in microbial degradation of organic wastes (Haug 1993). It requires the incorporation of air, which is dependent on moisture, particle size and pore spaces (Kuhlman 1990). During composting in a pile, the porosity (pore space) is an important factor as it positively correlates to the airflow and aerobic conditions (Azim et al. 2018). Aeration is one of the most critical factors during the composting process. It can be supplied by different methods such as agitation (e.g. in windrows) or forced aeration (e.g. aerated static pile) (Stentiford 1996). Manual turning in windrows in the conventional composting method is labour-intensive, creates dust and odour, and requires additional space for the pile (Tiquia & Tam 2002).

Aeration also favours the growth of native microorganisms that will compete with pathogenic bacteria for nutrients (Ravva & Sarreal 2014; Lepesteur 2022).

A minimum of 5% Oxygen inside the pile is required for maintaining the aerobic condition, while anaerobic condition occurs with less than 1% of Oxygen (Azim et al. 2018). There are several methods to allow more air inside the manure pile and

increase the free air space during composting, such as mechanical aeration, turning the pile and using bulking materials such as wheat straw (Michel et al. 2004).
When *Salmonella* or other pathogens are detected on a farm, farmers are reluctant to manipulate the manure after piling it due to contamination risks. During turning the pile and mechanical aeration, tremendous amounts of energy are needed, and particulate matter emissions can contaminate the environment and pose a risk to human health (Kabelitz et al. 2021).
In addition, Jiang et al. (2011) showed that the aeration rate could significantly affect greenhouse gas emissions (NH_3, CH_4, and N_2O) during composting and higher aeration rates can reduce the CH_4 emission while increasing the NH_3 and N_2O losses. Therefore, other strategies to reduce costs, environmental impact, and contamination risks while maintaining or even improving composting efficiency are preferred.

2.6.4 pH

The composting process is relatively indifferent to pH; however, pH has a direct impact on disease suppression during composting (Azim et al. 2018). Different pH levels can change the sensitivity of pathogenic bacteria to high temperatures (Ugwuanyi et al. 1999). Although organic matter can be composted in a wide range of pH (from 3 to 11), optimum values are between 5.5 and 8 (De Bertoldi et al. 1983; Azim et al. 2018). Acidic and alkaline pH values may have advantages in bacterial decontamination. Acids can enter bacteria and interfere with the functions of the cell (Lepesteur 2022). Low pH is also an important factor that could increase the transition time from mesophilic to thermophilic conditions in the initial phase of composting (Sundberg et al. 2004). On the other hand, alkaline conditions favour the formation and release of the antimicrobial agent ammonia (Cronjé 2004). However, high values of pH in the initial phases of the process in association with high temperatures can cause a loss of nitrogen through volatilization of ammonia (De Bertoldi et al. 1983).

2.6.5 Organic material composition and wheat straw

The composition of organic materials is another fundamental factor that affects the outcome of the composting process. Microorganisms need carbon and nitrogen for their metabolism. Carbon bonds are rich in energy that supplies microorganisms with energy, and nitrogen is incorporated into amino acids to build proteins (Azim et al. 2018). As microbial degradation is the most important reaction during composting, the carbon to nitrogen ratio (C/N ratio) plays an essential role in improving composting process (Li et al. 2013). Previous studies suggested that maintaining C/N at the range of 25–30 is the optimum ratio for composting. High C/N ratios (larger than 30:1) usually result in slow decomposition due to nitrogen deficiency and slow population growth. On the other hand, low C/N ratios (smaller

than 15:1), i.e., when nitrogen is in excess relative to carbon, may lead to gaseous nitrogen losses (De Bertoldi et al. 1983; Azim et al. 2018).

Controlling the composting process by adjusting different variables during the process, such as moisture and oxygen, is complex and costly; however, alteration of starting conditions can be simple and less expensive (Eiland et al. 2001). One of these starting factors is the C/N ratio that can be altered by using different amounts of a bulking agent like wheat straw as carbon sources. Chicken manure is a rich source of nitrogen and if the C/N ratio is low, the amount of nitrogen losses to the atmosphere is higher (Azim et al. 2018).

Studies showed positive and significant effectiveness of carbon sources bulking agents on carbon and nitrogen preservation and mitigating gaseous emission losses (Awasthi et al. 2020; Yin et al. 2021). In addition, using a bulking agent can increase porosity and ease of compaction and is positively correlated to the airflow (Azim et al. 2018).

3. Materials and Methods

For this project, manure from a commercial Swedish broiler flock was collected at the farm the day after sending the broilers to slaughter. A thin layer of peat covered the floor of the broiler rearing facility before arrival of the day-old chickens, and no additional litter substrate was added during the rearing period. Two containers with the following approximate dimensions: length 3.8 m, width 1.1 m, and height 1.9 m, and one small bucket with a volume of 40 L were used to store manure during this project.

At the farm, one container was filled with pure manure (container M). In the other container (container S), the manure was mixed with around 1.5 m^3 (130 kg) un-chopped wheat straw. The straw was added in several layers to the manure, and manure and straw were mixed with a shovel by hand (figure 2). A 20 cm layer of straw was placed on top of the manure to increase heat preservation inside the container. No wheat straw was added to the M container. In both containers, slaked lime was spread in the bottom to mimic the recommendation when manure with *Salmonella* is stored in a pile. The volume of the content in each container was estimated to be around 8 m^3.

In each container, four temperature loggers (TG-4100 - Tinytag Aquatic 2 Datalogger, Gemini Data Loggers, Chichester, UK) were placed in the manure. Three temperature loggers were placed in the centre of each container at a depth of 20, 80, and 140 cm from the bottom, referred to as Surface (S), Middle (M) and Bottom (B) positions, respectively. The containers were about 30 cm empty and the surface loggers were covered with a 20 cm manure layer. The fourth logger was placed at a 30 cm distance from the container's lateral side in the depth of 80 cm (figure 1).

Plastic tubes with a diameter of 1.5 cm were put inside the manure with one end at the same three levels (S, M, and B) as the temperature loggers, and the other end reaching outside the containers. These tubes were used for measuring oxygen and carbon dioxide concentrations during the storage period (figure 1). The outer end of the tubes had plastic caps to prevent gas exchange and was opened only during gas measurements. The other end of the tubes inside the pile had the same plastic caps with several holes to allow gas entering.

To simulate the actual Swedish farms' condition, the containers were placed outdoor in a farmland near campus Ultuna, at the Swedish University of Agricultural Sciences (SLU). Both containers were roofed to prevent rainwater from entering the pile; however, they had bilateral openings to allow air circulation.

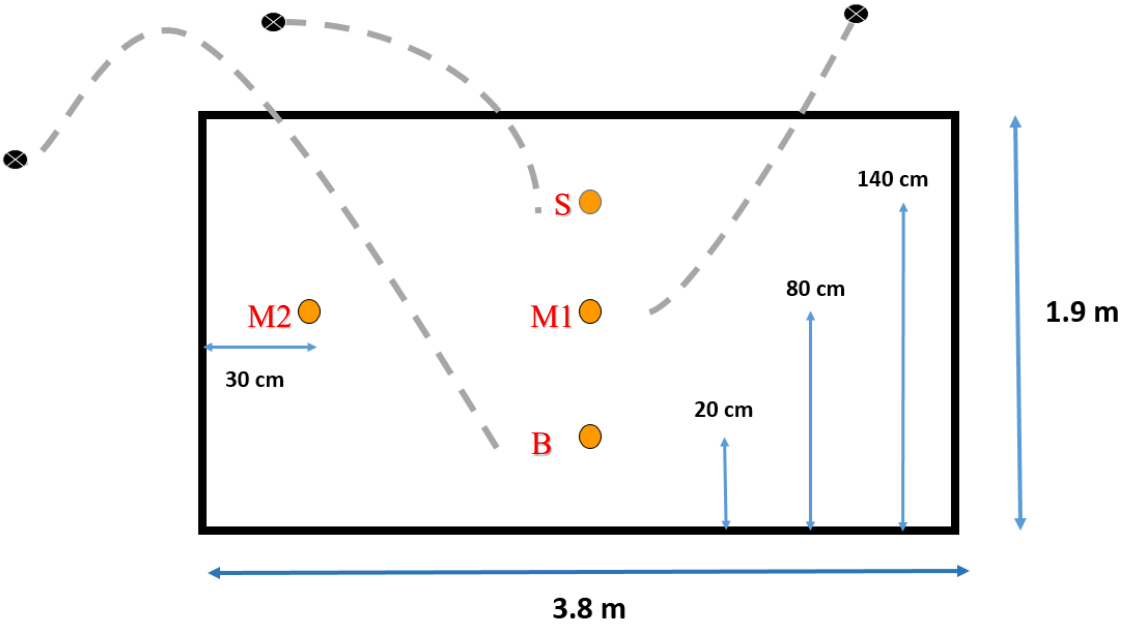

Figure 1. Schematic representation of the location of temperature loggers (orange circles) and plastic tubes (dashed lines) inside the container (black rectangular). S represents surface layer, M1 & M2 the loggers in the middle layer, and B the bottom layer.

A bucket (B) was filled with approximately 40 L of the same manure as the containers, without adding straw (figure 2). The bucket was placed in the *Salmonella* lab at the Department of Biomedical Science and Veterinary Public Health (BVF), SLU.

Manure was analyzed before storage for initial dry matter, crude protein (CP), pH, and water activity (Table 1), using the methods described below. In addition, the primary population of bacteria belonging to *Enterobacteriaceae* and the presence of *Salmonella* and *Campylobacter* in samples taken directly from manure pile at the farm were also analyzed.

Figure 2. A: Manure container (M). B: Manure container (M) with roof C: Straw container (S) before adding the extra layer of straw on the surface. D: The bucket in the Salmonella lab at BVF. E: The manual auger with a twisted rod that was used for sampling from the containers. F: Prepared samples for analysis (crucibles for measuring DM and plastic vials for pH).

The manure from the two containers and the bucket were sampled and analyzed regularly during six months. The sampling from different levels of the containers was done using a manual auger consisting of a twisted rod inside a cylinder and a handle (figure 2). To take the samples, the auger was rotated inside the manure pile by hand. The cylinder kept the manure inside the auger and prevented the manure from being mixed during removal. After removing the auger from the manure, the cylinder was removed and the samples were taken from different levels of the auger. To determine the changes in physico-chemical parameters and enumeration of bacteria, two series of parallel experiments were conducted.

3.1 Experiment 1: Storage of poultry manure in containers with or without added straw

Initial litter samples in both containers had a DM of 71.6%. We aimed for 55% moisture content in the containers for this experiment. The volume of the manure in each container was estimated to be around 8 m^3. The density of the samples was 446 and 396 g/L for M and S containers, respectively. Based on the formula below,

to reach the desired 55% moisture content, 2100 and 1900 litres of tap water were added to the pure manure and straw containers respectively.

$$Water\ amount\ (L) = Total\ fresh\ weight\ (kg)\ x \frac{(Desired - Current\ moisture\ content)}{Desired\ dry\ matter}$$

The water was added by spreading evenly on their surface for two consecutive days on the 7th and 8th day after filling the containers with manure. During water addition, several holes were made in the manure using a metal rod, to allow the water to reach lower layers.
For the next six months, samples were taken and analyzed for different parameters as follows (Table 1):

1. For bacterial analyses, samples were collected from three different levels (S, M, B) of each container and analyzed regarding the number of *Enterobacteriaceae*. The survival of *Enterobacteriaceae* was monitored with attention to the time of litter processing.
   Quantification of the number of *Enterobacteriaceae* was performed according to Nordic Committee on Food Analysis method NMKL 144. In brief, 10 g sample from each level was collected and mixed with 90 ml of sterile buffered peptone water (BPW) in a stomacher bag for 2 minutes. After that, 1 ml of mixed sample was used for 10-fold serial dilutions using 0.1% (v/v) sterile peptone water (Dilucups, LabRobot Products AB, Stenungsund, Sweden). 1 ml sample from -2 to -6 dilutions was mixed carefully with 15 ml of violet red bile glucose agar (VRBG) in a petri dish. After solidification, an over-layer of VRBG was added to the sample and incubated at 37°C for 24 h. Bacterial counts were performed on plates with less than 300 colonies. From each level, six colonies were re-cultured on blood agar, which were incubated 24 h at 37°C. These colonies were identified to species level using Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany).
2. Dry matter of samples were measured by weighing 5 g of sample from each level into a crucible and dry at 103°C for 24 h in a drying oven. To measure ash content, same sample after drying were put in a 550°C muffle furnace for 3 h and weighed after cooling in the desiccator.
3. Temperature was constantly recorded every 2 h using four temperature loggers in each container. In addition, the ambient temperature was recorded on a daily basis.
4. Oxygen (O_2) and carbon dioxide (CO_2) concentrations were measured in containers by spot sampling using a portable multi-gas detector

(GA2000 Range Gas Analysers, Geotech, Leamington Spa, UK) from different levels of the pile.

5. To measure the pH, 4 g of manure was mixed with 20 g of distilled water and put in the refrigerator for 24 h. After 24 h, the samples were taken out and left for 1 h to reach room temperature. After that, the pH was measured using an automated pH meter (Metrohm 654 pH meter, Gemini, UK).

Table 1. Performed bacterial and physico-chemical analysis of chicken manure and their frequency during 24 weeks of composting (+ represents one time sampling and – means the sampling was not done during the project)

Analysis	Prior to storage	Bucket	Containers
Enterobacteriaceae enumeration	+	Every week from 1-9 Every three weeks from 10-24	Every week from 1-9 Every three weeks from 10-24
Salmonella and *Campylobacter* presence	+	Every week from 1-9 Every three weeks from 10-24	-
Dry matter	+	Every five weeks	Every five weeks
Water activity	+	2 samples in weeks 5 & 21	2 samples in weeks 5 & 21
Gas measurement (O_2 and CO_2)	-	-	Every two weeks from 1-8 Every four weeks from 12-24
pH	-	Once every four weeks	Once every four weeks
Temperature	-	Daily from the middle	Every two hours from four different spots
Ash content	+	Once in week 21	Once in week 21
Total minerals (N, K, P, Mg, Na, S)	+	-	Once in week 24
C:N ratio	-	-	Once in week 24

Water activity analysis was conducted by sending 20 g of sample from each level to SVA, where it was analyzed by putting 5 g of sample in a water activity meter device (Aqualab TDL 2, Meter, Pullman, USA).
To measure the C/N ratio, samples from different levels of each container were taken, mixed in a plastic bag, froze in the fridge and sent to Eurofins Agro Testing Sweden for analysis.

3.2 Experiment 2: Survival of *Salmonella* and *Campylobacter* during 6 months storage in a bucket

About 10 L water was added to the bucket before the start of the experiment with the aim of 55% moisture content. Thereafter, the manure was spiked with *Salmonella Typhimurium* and *Campylobacter jejuni* to an initial concentration of 10^6-10^8 CFU/g, the manure, water and bacterial cultures were mixed thoroughly with a shovel to reach a homogenized sample in the bucket. In the sixth week of storage, the bucket was mixed thoroughly again with a shovel to allow more oxygen into the manure inside the bucket. During the first 6 weeks after inoculation, the samples for bacterial analysis were only taken from the middle part of the bucket, while from the seventh week, two separate samples were taken from both the surface and the middle.

For the next 6 months, samples from the bucket were analyzed as follows (Table 1):

1- Quantification of the number of *Enterobacteriaceae* were determined by using the same procedure described for experiment 1.

2- The presence of *Salmonella* was analyzed according to the standardized method, Salmonella NMKL 187. In this method, 25 g of sample was taken and mixed with 225ml BPW in a plastic stomacher bag to be incubated at 37°C for 18 h. After incubation, three drops of the enrichment broth and manure sample with a total volume of 100µl were added to selective modified semi-solid Rappaport Vassiliadis (MSRV) and incubated at 41.5°C for 24 h. If no suspected *Salmonella* were detected, the sample was incubated for another 24 h. Suspected *Salmonella* colonies were sub-cultured on Brilliant Green agar (BG) and xylose lysine deoxycholate agar (XLD) plates, and incubated in 37°C for 24 h. The next day, suspected *Salmonella* colonies on BG and XLD were re-cultured on blood agar, incubated at 37°C and identified the next day by MALDI-TOF MS.

3- The presence of *Campylobacter* spp. was determined using *Campylobacter* ISO 10272 method. In brief, 25 g of manure were placed in a plastic stomacher bag together with 90 ml Bolton broth (Oxoid, Basingstoke, UK) and homogenised for two minutes. In the next step, the enriched culture was first incubated for 4 h at 37°C followed by 44±4 h incubation at 41.5°C. After incubation, the enriched cultures were spread on modified charcoal cefoperazone deoxycholate agar (mCCDA) and the plates were incubated at 41.5°C for 48 h in a microaerophilic atmosphere by using CampyGen (Oxoid). Suspected *Campylobacter* colonies were re-cultured on blood agar, incubated at 41.5°C for 48 h, and identified using MALDI-TOF MS.

4- One thermometer was placed in the middle of the bucket and temperature was observed and noted every day.

5- The pH and DM of the manure were analysed using the same method as experiment 1.

All laboratory work took place in accordance with the rules of procedure and protection at SLU. The bacterial analysis was done in the BVF department, while the physico-chemical analysis was carried out in the analysis laboratory of the Department of Animal Nutrition and Management (HUV).

3.3 Result compilation and presentation

The results of *Enterobacteriaceae* enumeration and physio-chemical parameters were compiled and presented descriptively, mainly through graphs to show alterations during the project period, using Microsoft Excel version 2016. Bacteria belonging to the family *Enterobacteriaceae* were quantified, and the log10-transformed numbers presented, while the results of *Salmonella* and *Campylobacter* analyses are presented as detected or not detected.

4. Results

4.1 Bacterial analysis

The initial number of *Enterobacteriaceae* varied from 3 to 7 $\log_{10}$ CFU/g in all levels in both of the containers during the project (Figure 3). The number of *Enterobacteriaceae* fluctuated between 4 and 7 log 10 in the first 5 weeks and were constantly reduced thereafter. From the ninth week, *Enterobacteriaceae* remained below detection limit for all the samples, except for two samples in the 14th week from the surface of the pure manure container and the middle of the straw container.

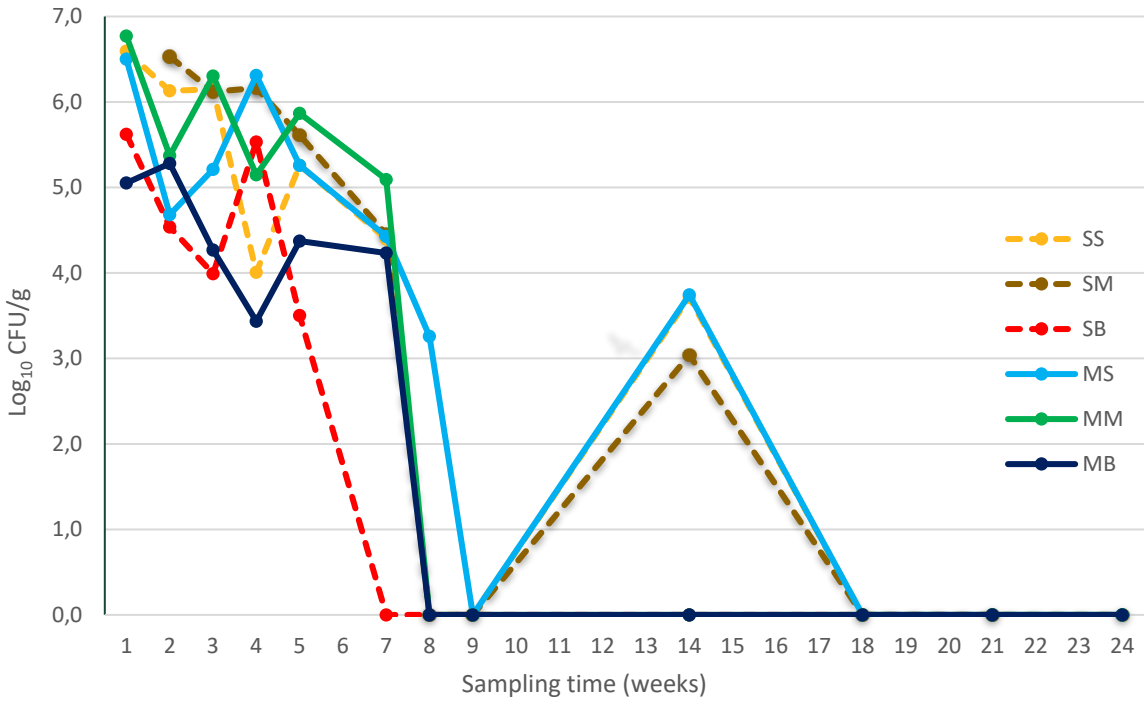

Figure 3. Number of Enterobacteriaceae in different levels of containers. The dots show the sampling weeks. Surface (SS), middle (SM) and bottom (SB) of the straw container and surface (MS), middle (MM) and bottom (MB) layer of pure manure container.

During the first three weeks of sampling, the number of *Enterobacteriaceae* in the bucket was around 4 $\log_{10}$ CFU/g. However, from the fourth week of sampling until the end of the experiment, the number of *Enterobacteriaceae* was below the detection limit (figure 4).

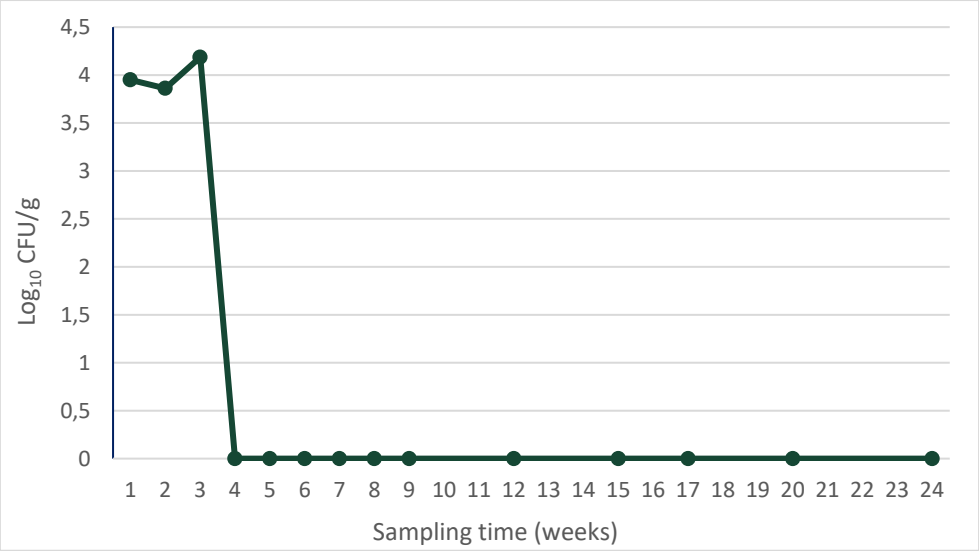

Figure 4. Number of Enterobacteriaceae in samples from the bucket. The dots show the sampling weeks.

The results of bacterial identification for the selected colonies is shown in table 2. In the bucket, *Salmonella* was the dominant bacteria belonging *to Enterobacteriaceae* during the first two weeks, while during the third week, only *Hafnia alvei* was identified. In the containers, *E.coli*, *Proteus mirabilis* and *Hafnia alvei* were the most detected bacteria during the project.

Table 2. The number of each bacterial species identified by MALDI-TOF from selected colonies. The number of colonies are presented as X/Y, where Y is the number of colonies that were analysed by MALDI-TOF and X is the number of identified colonies for each bacterial species.

Sampling time (weeks)	Bucket	Number of colonies	S container	Number of colonies	M container	Number of colonies
1	*Salmonella*	6/6	*E.coli* *Lelliottia amnigena*	4/10 6/10	*Proteus mirabilis* *E.coli*	2/14 12/14
2	*Salmonella*	6/6	*Alcaligenes faecalis* *Proteus mirabilis* *E.coli*	4/16 4/16 8/16	*Hafnia alvei* *E.coli* *Raoultella terrigena* *Proteus mirabilis*	8/18 4/18 4/18 2/16
3	*Hafnia alvei*	6/6	*E.coli* *Proteus mirabilis*	8/18 10/18	*E.coli* *Hafnia alvei* *Proteus mirabilis* *Yersinia enterocolitica*	2/16 6/16 4/16 4/16
4	-		*Paenalcaligenes suwonensis* *Proteus mirabilis*	1/7 6/7	*Proteus mirabilis* *Proteus vulgaris* *Hafnia alvei*	12/16 2/16 2/16
5	-		*Proteus mirabilis* *E.coli* *Paenalcaligens suwonensis*	10/16 4/16 2/16	*Hafnia alvei* *Citrobacter gillenii* *Proteus vulgaris*	12/16 2/16 2/16
8	-		*E.coli*	6/6	-	-
14	-		*Proteus mirabilis*	6/6	*E.coli* *Proteus mirabilis*	6/8 2/8

Campylobacter jejuni was not detected in any of the samples from the bucket (Table 3). *Salmonella* was detected during the first five weeks from all the samples, which were taken only from the middle of the bucket. In the sixth week sample taken from the middle of the bucket, *Salmonella* was not detected. From the seventh week, when two samples were taken from the bucket, *Salmonella* was only detected in the surface area, except for the 12th week that *Salmonella* was detected in both the surface and the middle.

Table 3. Results from analyses of Salmonella and Campylobacter in the bucket presented as detected (+) or not detected (-)

Sampling time	Salmonella	Campylobacter
Week 1	+	-
Week 2	+	-
Week 3	+	-
Week 4	+	-
Week 5	+	-
Week 6	-	-
Week 7 (surface)	+	-
Week 7 (middle)	-	-
Week 8 (surface)	+	-
Week 8 (middle)	-	-
Week 12 (surface)	+	-
Week 12 (middle)	+	-
Week 16 (surface)	+	-
Week 16 (middle)	-	-
Week 21 (surface)	+	-
Week 21 (middle)	-	-
Week 24 (surface)	+	-
Week 24 (middle)	-	-

Figure 5. Samples from the middle (left dish) and surface (right dish) of the bucket on selective enrichment medium for detection of Salmonella (MSRV). These samples were taken in week 16, and Salmonella was only detected on the surface area. The blue colour on the right plate has completely disappeared, because Salmonella spp. has been swarming over the surface of the agar and forms a grey continuous layer.

4.2 Temperature, dry matter and water activity

The temperature inside the bucket varied between 20 and 52.4°C during the study period (figure 6). After adding water at the beginning of the study, the temperature increased to around 45°C. After three weeks, the temperature started to decrease steadily to around 34°C at the seventh week. After mixing the manure in the sixth week, the temperature increased to around 53°C. However, after the peak, the temperature started to decrease again and until it reached the ambient temperature at 10th week and remained stable ever since.

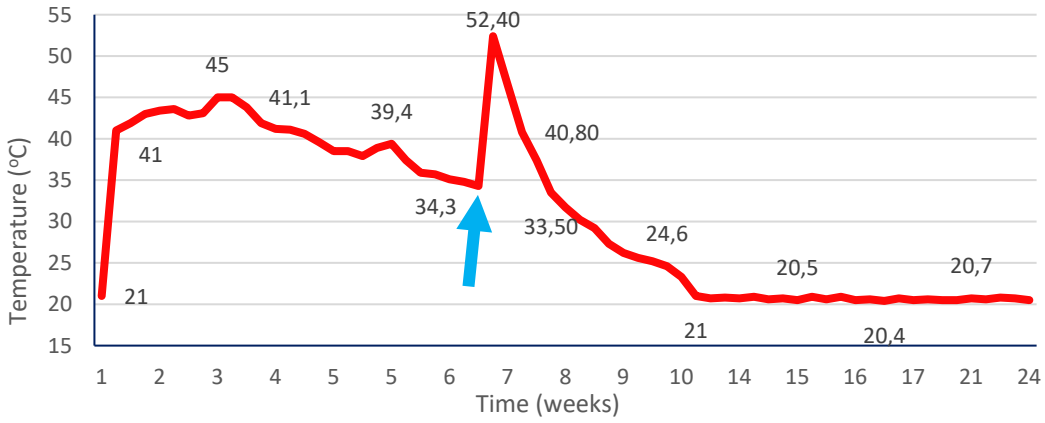

Figure 6. Bucket temperature from the middle part of the bucket. The temperature was noted every weekday during the 6 months period. The blue arrow shows the date when the manure was mixed again.

In both containers, the highest temperature was achieved at the surface level (figures 7 and 8). On the surface of the pure manure container, the temperature rose quickly, reached its maximal value (59°C) within 2–3 days, and remained over 50°C for five days. In the straw container, the temperature achieved its highest (61°C) on the 5th day on the surface and remained over 50°C for two days.
Ambient temperatures ranged from -17 to 13°C during the experiment.

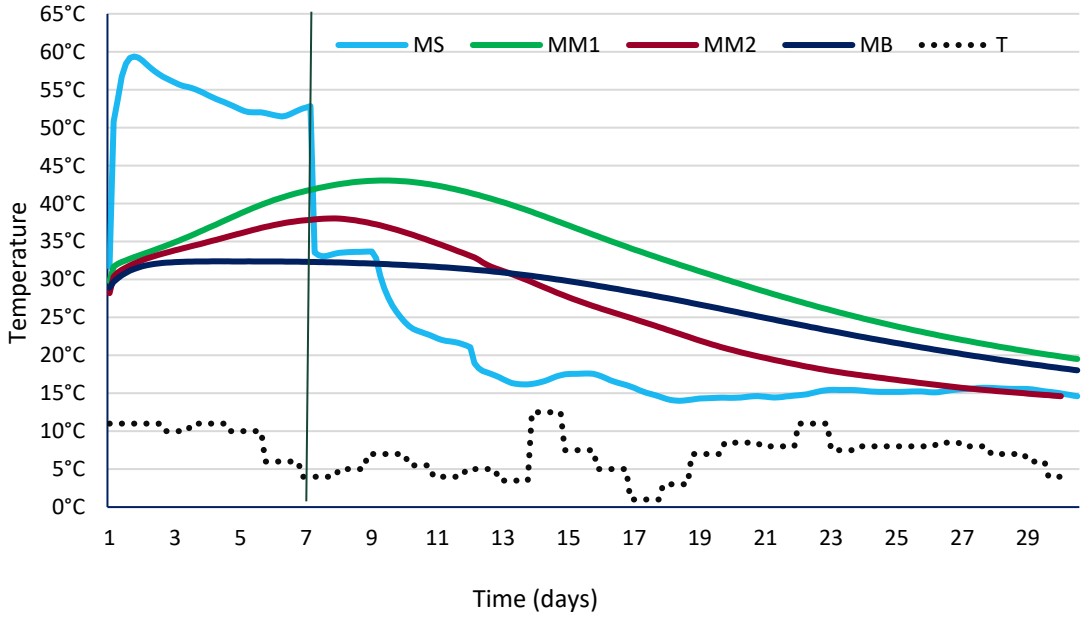

Figure 7. Temperature from different levels of the manure container during the first month of the project. Surface (MS), middle (MM1 & MM2) and bottom (MB). The T line shows the ambient temperature. The vertical line shows the time that water was added to the container on the 7th day.

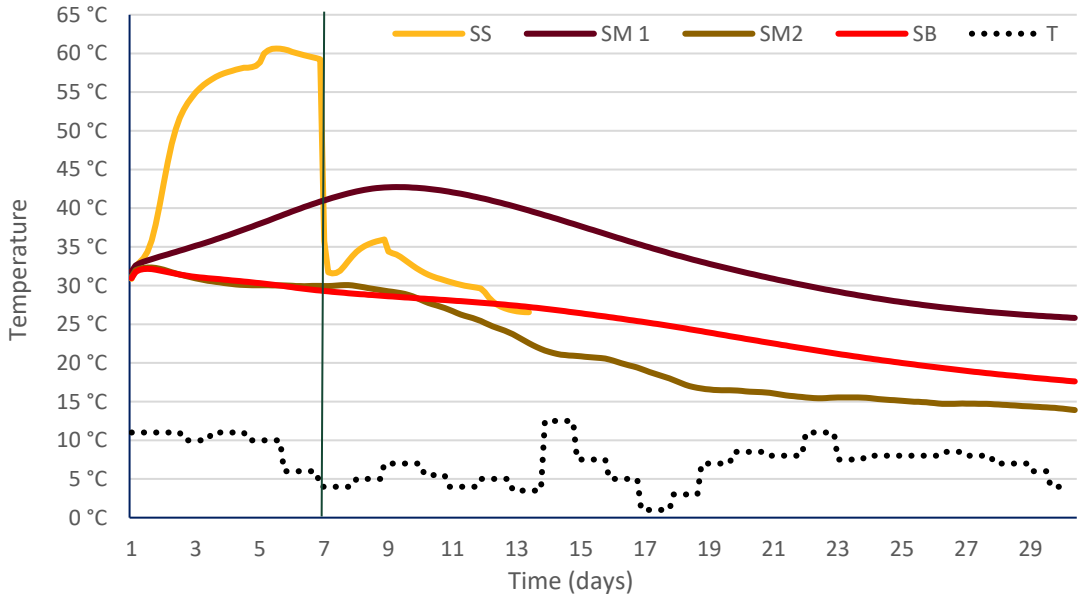

Figure 8. Temperature from different levels of the straw container during the first month of the project. Surface (SS), middle (SM1 & SM2) and bottom (SB). Due to a problem in the SS logger recordings from the second week, its results are excluded from the graph from the third week. The T line shows the ambient temperature. The vertical line shows the time that water was added to the container on 7th day.

In both containers, the middle loggers showed a slower increase in temperature than the surface, and the peak mean temperature was 44°C on the 10th day. However, the temperature in the middle part became the highest after the first week and remained highest until the 8th week compared with other levels.

The lowest peak temperatures were achieved in the bottom and loggers in the lateral side of the containers. These loggers showed a peak temperature of 32°C on the third day of the experiment.

From the second week, in all loggers, the temperature was decreasing and remained relatively stable between 0 and 10°C from the 8th week and onwards.

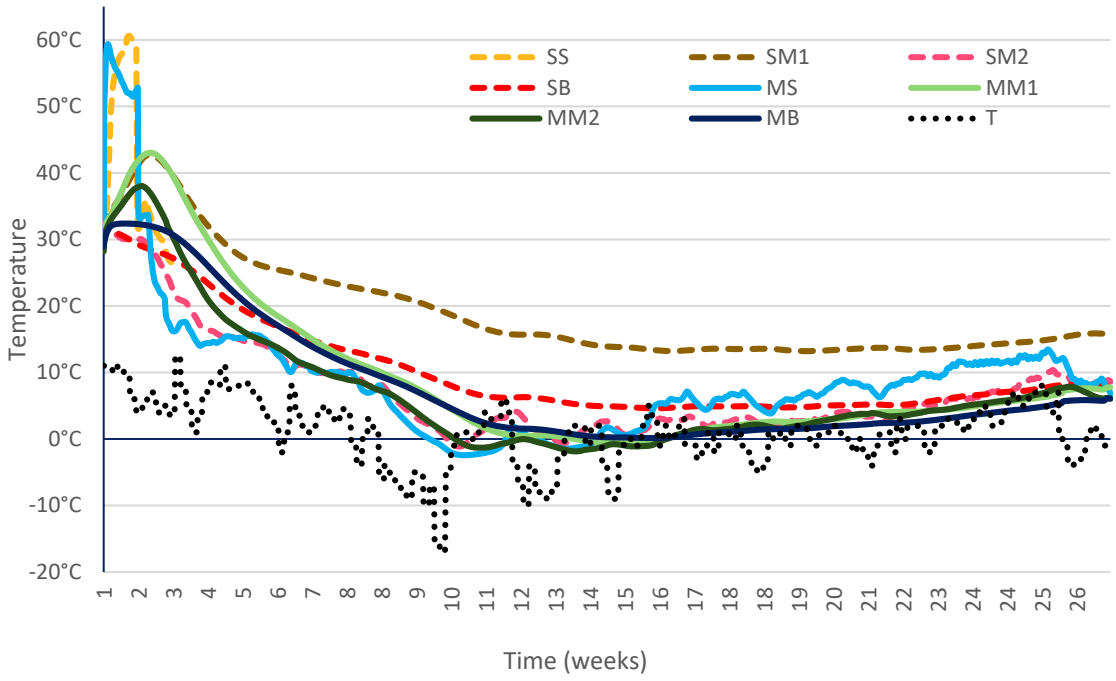

Figure 9. Temperature from different levels of both containers during six months. Surface (SS), middle (SM1 & SM2) and bottom (SB) of the straw container. Surface (MS), middle (MM1 & MM2) and bottom (MB) of the manure container. Due to a problem in the SS logger recordings from the second week, its results are excluded from the graph from the third week. The T line shows the ambient temperature.

Dry matter was lowest in the surface and middle layers and highest in the bottom layers. However, as opposed to a low amount increase in the dry matter of the containers, the dry matter concentration of the bucket nearly doubled (figure 10).

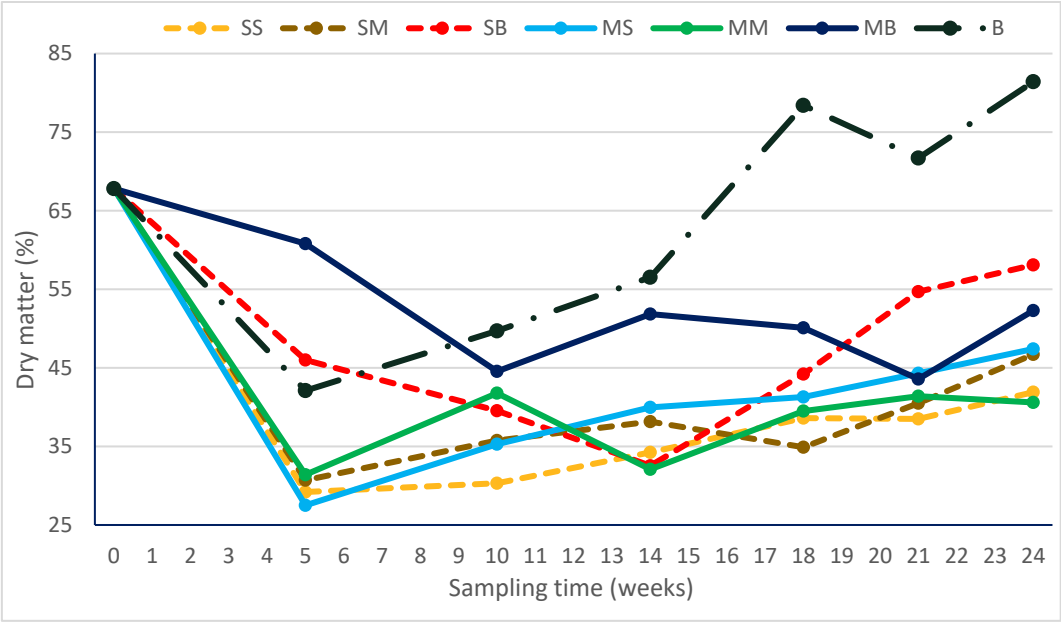

Figure 10. Dry matter content of the containers and the bucket after adding water at the first week of the experiment. The dots show the sampling weeks. The bucket (B), surface (SS), middle (SM) and bottom (SB) of the straw container and surface (MS), middle (MM) and bottom (MB) layer of pure manure container.

The water activity was 0.90 in the initial samples from the manure before adding the water (Table 4). After water addition, water activity ranged between 0.93 and 0.98 for all the levels, with the lowest amount in the bottom layers.

Table 4. Water activity in the samples from different levels of the containers. Surface (SS), middle (SM) and bottom (SB) of the straw container and surface (MS), middle (MM) and bottom (MB) layer of pure manure container.

Sampling location	Initial (before adding water)	Week 5	Week 21
SS	0.90	0.98	0.98
SM	0.90	0.98	0.97
SB	0.90	0.96	0.93
MS	0.90	0.98	0.98
MM	0.90	0.98	0.97
MB	0.90	0.93	0.97

4.3 Gas and pH analyses

The oxygen concentration was highest in the surface area. However, from the third week, the oxygen level remained below 2% in all levels for the whole project, except for the surface area of both containers, where oxygen began to increase from the 8th week and continued to rise until the end of the experiment (figure 11).
On the contrary, from the start of the experiment, the amount of CO_2 increased to more than 90% in all the levels, except for the surface area of the straw container (Figure 12). From the third week, CO_2 began to decrease and it was the lowest in the surface area of both containers at around 10%, compared with 45% for the rest of the spots.

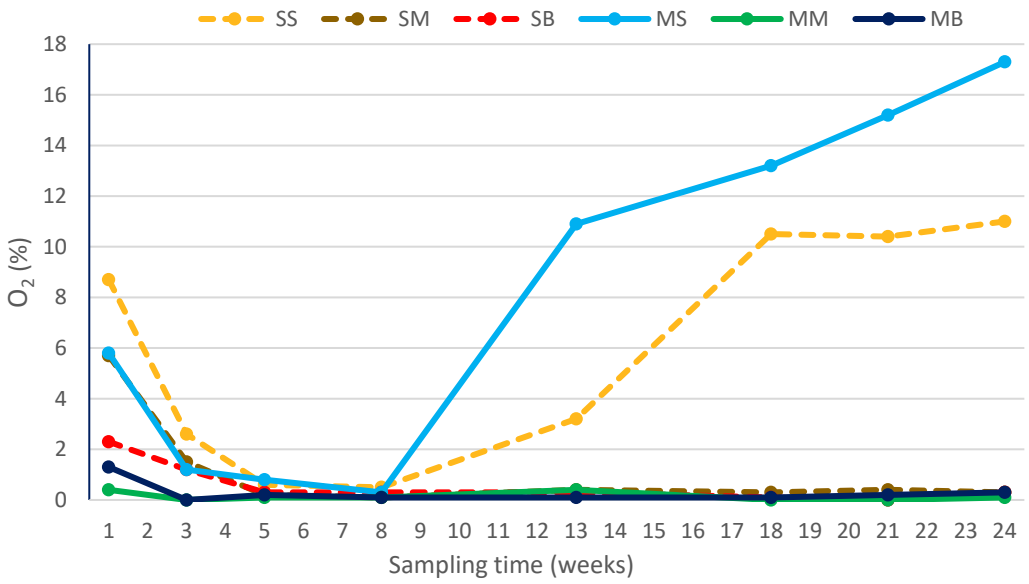

Figure 11. Percentage of oxygen in air samples from different layers of the containers. Surface (SS), middle (SM) and bottom (SB) of the straw container and surface (MS), middle (MM) and bottom (MB) layer of pure manure container. The dots show the sampling weeks.

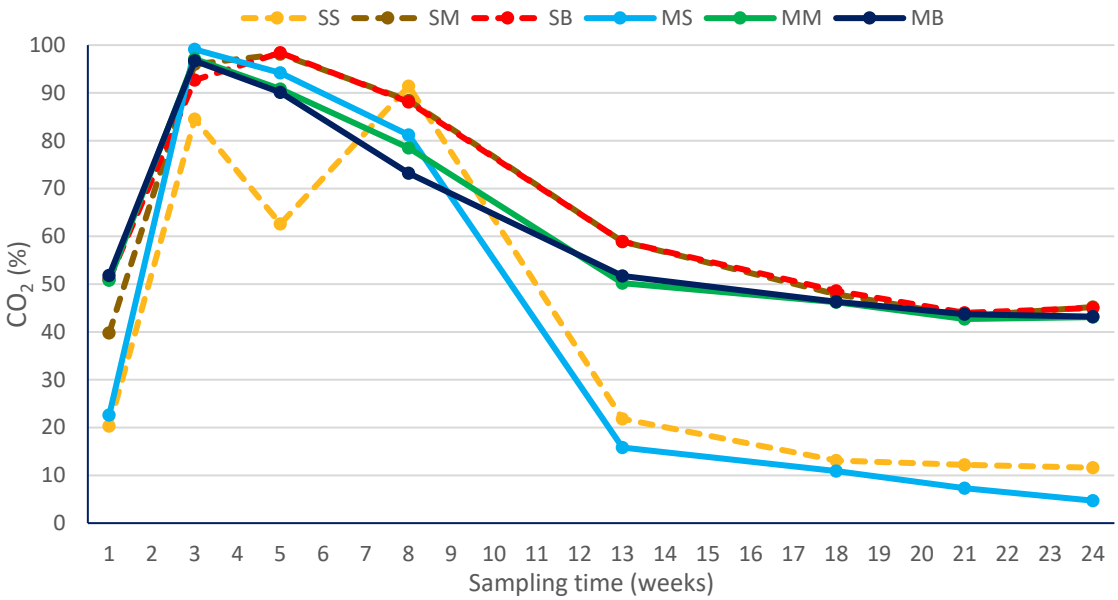

Figure 12. Percentage of carbon dioxide in air samples from different layers of the containers. The dots show the sampling weeks. Surface (SS), middle (SM) and bottom (SB) of the straw container and surface (MS), middle (MM) and bottom (MB) layer of pure manure container.

In both containers, the pH began to decrease from the start of the experiment to below 6 in all the samples, except for the surface of the straw container (figure 13). For the remaining of the experiment, the pH fluctuated between 5 and 7 for all the samples, except for the surface of the straw container, where it increased to above 8 from the 14th week. The pH remained around 9 for the bucket during the whole sampling period (figure 13).

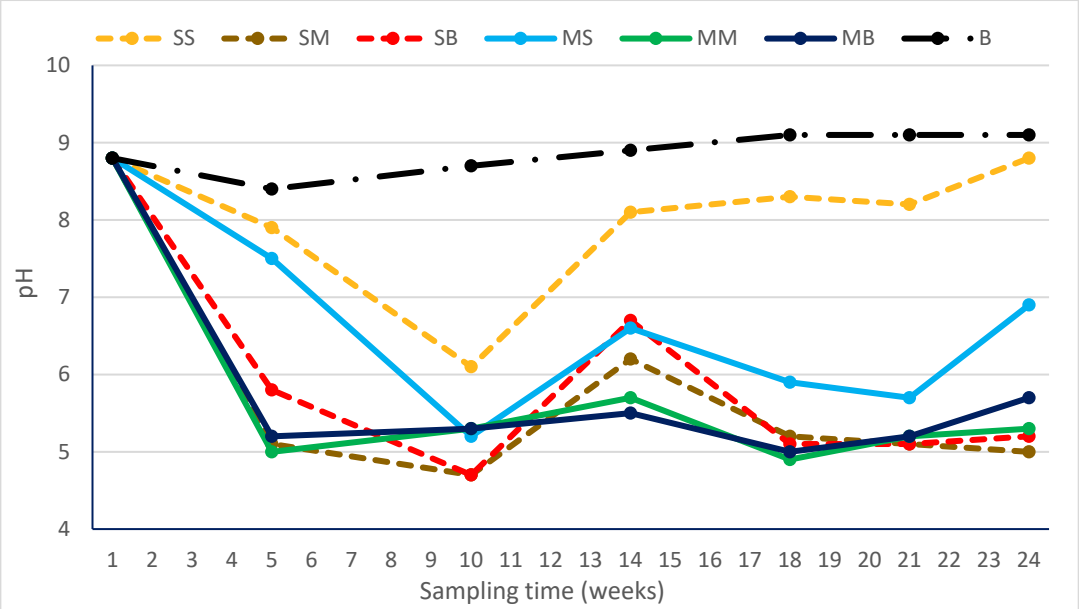

Figure 13. Average pH of the samples from different levels of the containers and the bucket during composting. The bucket (B), surface (SS), middle (SM) and bottom (SB) of the straw container and surface (MS), middle (MM) and bottom (MB) layer of pure manure container. The dots show the sampling weeks.

4.4 Ash, minerals and C/N ratio

The concentration of all of the analysed parameters, ash, crude protein and minerals in pre-dried samples decreased from the start of the experiment (Table 5). The ratio of C/N at the end of the experiment was 9 for the pure manure container, which was a little higher than the straw container at 8.5.

Table 5. The concentrations of ash, minerals and C/N ratio in pre-dried samples from two series of analyses performed prior and at the end of the experiment on broiler manure during composting.

Analysis	Prior to storage	M container	S container
Ash (g/kg)	69.6	49.3	50.7
Crude protein (g/kg)	27.4	18.2	18
NH_3-N (g/kg)	12.95	5.5	7
K (g/kg)	23.91	11	8.4
P (g/kg)	6.37	3.4	3.6
Mg (g/kg)	5.51	2.9	3.2
Na (g/kg)	1.06	0.46	0.35
S (g/kg)	4.97	2.1	2.1
C/N ratio	-	9	8.5

5. Discussion

The objective of this thesis was to evaluate if storage of chicken manure in containers will provide the conditions required for elimination of *Enterobacteriaceae, Salmonella* and *Campylobacter*, and if straw added to the manure will facilitate the composting process. Therefore, the pathogenic bacterial decontamination in relation to changes in physico-chemical parameters was investigated during six months of manure storage in two containers and a bucket.

Despite the differences in physico-chemical parameters between both containers and the bucket, the *Enterobacteriaceae* decreased below the detection limit in all of them during the experiment. In the containers, the number of *Enterobacteriaceae* fluctuated between 4 and 7 $\log_{10}$ during the first eight weeks. After this period, the number of bacteria decreased rapidly, which resulted in *Enterobacteriaceae* disappearance in the samples taken in the ninth week. After the ninth week, *Enterobacteriaceae* were only detected once in the 14th week from the samples of the middle of the straw container and the surface of the pure manure container. According to Elving et al. (2010) pathogenic bacteria can persist or even regrow in the insufficiently self-heated areas of the piles. However, in our study, there were no specific changes in any other parameter during the 14th week and *Enterobacteriaceae* remained below the detection limit for the rest of the project period for these two layers. Therefore, it cannot be excluded that the one-time re-grow of *Enterobacteriaceae* in the middle of the S container and on the surface of the M container was a result from contamination during sampling in the field or analyses in the lab.

Based on their methods, studies reported different survival times of pathogenic bacteria in manure ranging from a few weeks to more than six months (Kudva et al. 1998; Jiang et al. 2003; Hutchison et al. 2004; Kwak et al. 2005; Nicholson et al. 2005; Chroni et al. 2009; Nyberg et al. 2010; Wilkinson et al. 2011; Millner et al. 2014; Thomas et al. 2020). Chroni et al. (2009) found that the population of *E. coli* declined to below the detection limit first after the 57th day of composting, despite that temperature had reached 67°C by the 25th day. Results of Nicholson et al. (2005) study showed three months survival time for *E. coli* , *Salmonella* and *Campylobacter* in stored slurries. At the same time, all these pathogens survived for less than one month in solid manure heaps where temperatures greater than 55°C were obtained (Nicholson et al. 2005).

In our study, there was no notable difference between *Enterobacteriaceae* survival time between S and M containers, which is different from the results of the study by Millner et al. (2014). According to their results, *E. coli* and *Salmonell*a in manure reduced from 8 to 9 $\log_{10}$ CFU/g to below detection limits at 25–30 cm depths within 7 days in the static piles mixed with straw, while for the pile with

pure manure only 3–4 logs of reduction were obtained during the same period (Millner et al. 2014).

In both containers, the number of *Enterobacteriaceae* was lowest in the bottom layer (SB and MB) during the entire sampling period. This can be resulted from higher DM in the bottom layer, as the water was added from the surface. When analyzing bacterial growth, studies have found that moisture content directly impacts the composting process, and low moisture content results in low bacterial growth (Petric et al. 2009; Li et al. 2021). Thomas et al. (2020) investigated the effect of moisture content on the survival of *E.coli* during chicken manure composting. The results showed the fastest decrease of *E. coli* in mixtures with lower moisture content, compared with the moist mixtures, despite having lower maximum temperatures in the drier mixtures (Thomas et al. 2020).

In the bucket, during the first three weeks *Enterobacteriaceae* numbers were around 4 $\log_{10}$, thereafter the amount decreased below the detection limit in the fourth week and was never detected again during the whole experiment. The faster bacterial decontamination in the bucket can be resulted from higher oxygen content in the bucket, as it was mixed a lot more at the beginning of the experiment compared with the containers. In addition, the surface/volume ratio was higher in the bucket compared with the containers, and it had less compaction due to lower manure depth, resulting in higher free air space and aeration. According to other studies, in the presence of oxygen, the time required for the inactivation of bacterial pathogens such as *Salmonella* and *E.coli* can be reduced by more than half (Munch et al. 1987; Pandey et al. 2015; Lepesteur 2022).

Campylobacter was not detected at any time point after inoculation of the manure in the bucket. However, the first sample was taken 6 days after inoculation, which means that the survival time for *Campylobacter* in the manure in this study was less than 6 days. In an Irish study *C. jejuni* survival time was 48 h in broiler's feces and 4 h in used litter. The results also showed a higher *C. jejuni* survival at 20°C compared with 25°C and 30°C (Smith et al. 2016). Ahmed et al. (2013) showed that the survival times of *C. jejuni* ranged from 72 to 96 h in artificially inoculated feces and varied from 120 to 144 h in the manure from naturally colonized birds. *C. jejuni* is a microaerophilic bacterium and cannot grow under normal atmospheric oxygen tension conditions. However, they can increase their survival by growing in biofilms or interacting with other microorganisms (Hilbert et al. 2010).

Salmonella was detected in all samples taken from the middle part of the bucket during the first six weeks. After mixing the bucket in the sixth week, the highest temperature was achieved (52.4°C), which resulted in *Salmonella* disappearing during the next week's samples taken from the middle part of the bucket. According to previous studies, multiple cycles of higher temperatures may be more effective against pathogenic bacteria decontamination at lower temperatures than a single high temperature cycle (Hess et al. 2004). After the 6th week, in samples taken from both the surface and the middle of the bucket, *Salmonella* was only detected in the surface area, except for the 12th week when *Salmonella* was also detected in the

middle part. The reason behind the survival of *Salmonella* in the surface part could be the lower temperature on the surface compared with the middle part of the bucket. According to Briancesco et al. (2008), some pathogenic bacteria appear to survive the composting process and composting facilities may produce compost with viable *Salmonella.* It is possible that *Salmonella* detection in the 12th week could be either the sampling error or recontamination of the middle part. As the bucket size was small, there was a possibility for the samples to be mixed during sampling from the middle part. However, according to previous studies, survived bacteria in cooler outer layers can multiply and recontaminate other portions of the manure pile (Elving et al. 2010; Wilkinson et al. 2011).

This study aimed to investigate if *Enterobacteriaceae* is a good indicator to predict *Salmonella* survival and decontamination during manure storage. In the bucket, the *Enterobacteriaceae* was only detected in the samples from the first three weeks by direct plating, while *Salmonella* was detected during the whole project in the surface of the bucket using enrichment method. Based on our results, direct plating of *Enterobacteriaceae* cannot predict complete decontamination of *Salmonella* during storage. However, the results showed marked decrease in the number of *Salmonella* in the bucket. Firstly because during the first 2 weeks, *Salmonella* was the dominant bacteria identified by MALDI-TOF MS in randomly selected colonies (6 from 6), while in the third week, only *Hafnia alvei* was detected. Secondly, from the third week, *Salmonella* could only be detected by enrichment method. It should be mentioned that in enrichment method, even a few number of bacteria can multiply and be detected, while in direct cultivation on selective agar media, there is a detection limit and it does not mean complete eradication of the bacteria. Therefore, in Sweden with zero-tolerance policy for *Salmonella*, enrichment methods should be considered above direct plating to ensure complete *Salmonella* decontamination inside manure. In addition, as we had added *Salmonella* Typhimurium and *Campylobacter jejuni* to an initial concentration of 10^6-10^8 CFU/g, it is also important to investigate the reduction in lower concentrations and not overestimate the reduction through high bacterial concentrations.

Regarding environmental and physico-chemical parameters, studies identified different factors affecting the survival of pathogenic microorganisms in piled manure, such as temperature, pH, moisture, manure's chemical composition, and stacking method (Kwak et al. 2005; Bush et al. 2007; Payne et al. 2007; Petric et al. 2009; Wilkinson et al. 2011; Thomas et al. 2020). However, their results show a variance in the effect of each factor on the bacterial decontamination during composting.

Straw can be used in static piles to increase the porosity and C/N ratio of the manure and as an insulative barrier (by covering the pile). In this study, adding the straw didn't make substantial effect on physico-chemical parameters, temperature and bacterial decontamination. This result is different from previous studies, which showed that using straw increases peak temperature, prolong the thermophilic stage and pathogen die-off rate during composting (Millner et al. 2014; Wei et al. 2014).

Millner et al. (2014) found increased aeration, self-heating, and heat retention and shorter *Salmonella* and *E.coli* survival time by adding straw to the manure during manure composting in a static pile. Similarly, Wei et al. (2014) saw a shorter time to enter thermophilic phase, higher temperature and longer duration of thermophilic phase during composting chicken manure mixed with tomato stalk, compared with pure manure.

The required time-temperature exposure for pathogen inactivation in animal waste varies. However, studies suggested that for decontaminating large populations of *E. coli*, the manure should be composted for 1 week, and preferably 2 weeks at a minimum temperature of 50°C and lower temperatures increases the duration (Jiang et al. 2003; Chroni et al. 2009). According to Millner et al. (2014) decline of bacterial populations corresponds to exposure to temperatures above 45°C for more than 3 days. Hess et al. (2004) investigated heat inactivation of *E. coli* during manure composting in three temperature ranges: 40°C to 50°C, 50°C to 60°C, and greater than 60°C. The results showed that laboratory-grown *E. coli* needed approximately 300 degree days of heating, while *E.coli* from infected cattle went below detection limit after approximately 180 degree days of heating (Hess et al. 2004).

In our study, only the surface of both containers reached the temperatures above 50°C during the first week. In the pure manure container, peak temperature of 59.4°C was achieved in the second day of composting, and the temperature was above 50°C for 5 days. In the straw container, the peak temperature was a little higher (60.6°C) while remained above 50°C for 4 days. However, adding the water on 7^{th} day seems to be the reason for sharp decrease in the temperature on the surface of the both containers. Therefore, it is not possible to predict the duration of peak heating phase for the surface layers of the containers. The temperature did not reach above 45°C in the middle and the bottom layers, probably due to low oxygen concentration (below 5%) and low moisture content (32.2%) during the first week of storage. Oxygen and moisture are two of the most important parameters needed to promote microbial growth and reaching and sustaining high temperatures during composting (Petric et al. 2009; Vinnerås et al. 2010; Lepesteur 2022). Wang et al. (2007) studied the effect of oxygen concentration on the composting process and maturity in manure mixed with straw. According to their results, the duration of the thermophilic phase above 50°C under microaerobic treatment (manual turning and O_2 <1.5%) was longer than the aerobic treatment (forced air plus turning and O_2 >5%), however, the composting temperature at the later phases declined more slowly under aerobic conditions (Wang et al. 2007). During the first week of our experiment, the concentration of oxygen was above the 5% limit for aerobic treatment in only three spots (surfaces of both containers and the middle of the straw container). However, in the third-week samples, all spots had oxygen content below the 5%. The oxygen remained under 2% for the remaining project period, except for the surface spots of both containers that increased from the 8^{th} week to around 17 and 11 percent in the 24^{th} week for manure and straw containers,

respectively. The fast increase of oxygen concentration in the surface area could be resulted from changes in the manure structure due to composting, which allows more air circulation in the surface area. Several factors could be the reason behind low oxygen content in the containers. The moisture content has a significant impact on physical parameters during composting, such as free air space, air permeability, and thermal conductivity (Jiang et al. 2011; Huet et al. 2012). High moisture levels and small particle sizes can result in compaction and reducing the air-filled porosity (free air space) (Das & Keener 1997; Azim et al. 2018). As we added the water from the surface of the containers, the moisture content was higher in the surface and the middle part than in the bottom layer. This could result in compaction of the upper layers and less free air space and airflow, resulting in lower oxygen concentration and anaerobic conditions. This is in agreement with the results from the study by Huet et al. (2012). They investigated the impact of compaction and moisture content on free air space and air permeability. According to the results, depth and moisture content had a significant impact on free air space, air permeability and thermal conductivity of composting materials (Huet et al. 2012).
Although timing, peak and duration of the thermophilic stage are important factors determining the microbial survival during composting process, a combination of factors affect the bacterial growth and survival. In the study by Droffner & Brinton (1995) *Salmonella* and *E. coli* survived for 59 days at about 60°C in an industrial compost, while they were inactivated when the temperature decreased to 40°C in the compost curing. They concluded that it is hard to correlate peak temperature and the duration of high temperature to the destruction of pathogens and removal of these microorganisms during composting is complex and not simply the result of a thermal physical environment (Droffner & Brinton 1995).
Another important factor in manure's composting is C/N ratio (Li et al. 2013; Thomas et al. 2020). In this study 2 m^3 of un-chopped wheat straw was added to the S container. The C/N ratio was 8.5 for straw container and 9 for manure container at the end of the study. However, in our study C/N ratio was only measured once at the end of the project. According to studies, during the composting process, there is a decrease in the C/N ratio, and a lower C/N ratio at the end of the process is an indicator of compost maturation (Huang et al. 2004; Jiang et al. 2011). The similar C/N ratios in both containers might also be due to the method of analyses. As un-chopped wheat straw was mixed inside the containers, the samples for measuring C/N ratio might only include the manure portion and not the straw part. According to previous studies, the optimal amount of straw to be added during the composting process is around 5:1 (manure to straw dry weight) and the optimal C/N ratio is between 20-30 (Petric & Selimbašić 2008; Z et al. 2013; Zhang et al. 2018). Huang et al. (2004) investigated two different C/N ratios of 30 and 15 in two aerobic static piles. C/N ratio of 30 resulted in a faster rise in temperature, higher maximum temperature, and more prolonged thermophilic phase (Huang et al. 2004).

The pH in the containers decreased from 8.8 in the first week to below 6 in the 5th week, except for in the surface of both containers. The decrease in pH is in-line with the decrease in O_2 and increase in CO_2 and was foreseeable. Several factors such as high biological activity, anaerobic conditions, and ammonia volatilization could affect pH levels during composting (Azim et al. 2018). Generally, in the initial phases of the composting process, the pH begins to decrease due to the activity of acid-forming bacteria that break down organic substrates into organic acid intermediates and produce a high amount of CO_2 (Atchley & Clark 1979). After the first stage and the disappearance of easily degradable organic substrates, the pH increases, and bacterial hydrolysis of protein and organic nitrogen produces ammonia (Azim et al. 2018). In this study, the pH remained low for the entire project period, which is probably due to low ventilation and oxygen concentration. According to studies, effective ventilation and higher oxygen concentrations result in a higher final pH during maturation and if the available oxygen is low or lacking, the initial tendency for the pH to decrease is enforced due to anaerobic degradation (Ferrer et al. 2001; Vinnerås et al. 2010). However, at the final stage, the pH can drop again due to the release of H+ ions during nitrification (Atchley & Clark 1979; Azim et al. 2018). Changes in pH contribute to pathogen inactivation during the composting process. When pH is low, acids enter bacteria cells and interfere with the functions of the cell (Lepesteur 2022). Erickson et al. (2014) investigated the thermal and non-thermal factors affecting the survival of *salmonella* and *listeria monocytogenes* in animal manure-based compost mixtures. Results showed pathogens inactivation in swine manure compost mixture, regardless of very little generated heat, which were characterized by significantly higher levels of volatile acids compared with the other two compost mixtures (Erickson et al. 2014). They concluded that volatile acids could result in pathogen inactivation when temperatures are too low at the surface of the static compost piles or during winter composting when heat is lost too quickly (Erickson et al. 2014). Similarly, in our study, the *Enterobacteriaceae* number went below the detection limit regardless of the little heat generated in the containers' middle and bottom layers, which could be resulted from low pH and volatile acids. During the experiment period, pH was constantly higher in the bucket compared with both containers. Higher pH in the bucket can be resulted from lower number of bacteria and higher aeration due to manure mixing and a higher surface/volume ratio.

Reducing pathogenic bacteria during composting is a multifactorial process, and a combination of factors contribute to bacterial decontamination. High temperature, pH variation, and desiccation in our project could contribute to bacterial decontamination. There are however other contributing factors to pathogen inactivation that were not included in this study. Some of these factors are the activity of beneficial microorganisms that produce antimicrobial compounds and compete for nutrients or prey on pathogens, ammonia, and volatile acids (Lepesteur 2022).

Investigating other microorganisms, adding the water before or during piling the manure inside the containers and adding more wheat straw should be considered for future studies.

6. Conclusion

This study presents the effects of adding straw and changes in physico-chemical parameters of manure on bacterial decontamination over six months. Storing the poultry manure in containers resulted in the *Enterobacteriaceae* to decrease below detection limit from the 9th week, while the corresponding time in the bucket was only 4 weeks. Although the *Campylobacter* disappeared during the first week of the experiment, six months of storage did not eliminate the presence of the *Salmonella* on the surface of the bucket. This study showed that direct cultivation methods for *Enterobacteriaceae* is not a reliable indicator for complete *Salmonella* inactivation in a sample, and to ensure that a complete decontamination has been achieved, enrichment methods should still be considered. Adding the straw did not result in a huge difference in the physico-chemical parameters and the amount and duration of the peak temperature were fairly similar between the two containers. According to this study, storage of manure in a container is an effective method for reducing the number of pathogenic bacteria, however to prevent the risk of *Salmonella* survival during the composting period, achieving optimal composting conditions is of utmost importance.

References

Adak, G.K., Cowden, J.M., Nicholas, S. & Evans, H.S. (1995). The Public Health Laboratory Service national case-control study of primary indigenous sporadic cases of campylobacter infection. *Epidemiology & Infection*, 115 (1), 15–22. https://doi.org/10.1017/S0950268800058076

Ahmed, M.F.M., Schulz, J. & Hartung, J. (2013). Survival of Campylobacter jejuni in naturally and artificially contaminated laying hen feces. *Poultry Science*, 92 (2), 364–369. https://doi.org/10.3382/ps.2012-02496

Alexandratos, N. & Bruinsma, J. (eds.) (2012). *World agriculture towards 2030/2050: the 2012 revision*. (ESA Working Papers 12-03). https://doi.org/10.22004/ag.econ.288998

Atchley, S.H. & Clark, J.B. (1979). Variability of Temperature, pH, and Moisture in an Aerobic Composting Process. *Applied and Environmental Microbiology*, 38 (6), 1040–1044. https://doi.org/10.1128/aem.38.6.1040-1044.1979

Authority, E.F.S. & European Centre for Disease Prevention and Control (2021a). The European Union One Health 2019 Zoonoses Report. *EFSA Journal*, 19 (2), e06406. https://doi.org/10.2903/j.efsa.2021.6406

Authority, E.F.S. & European Centre for Disease Prevention and Control (2021b). The European Union One Health 2020 Zoonoses Report. *EFSA Journal*, 19 (12), e06971. https://doi.org/10.2903/j.efsa.2021.6971

Awasthi, M.K., Duan, Y., Awasthi, S.K., Liu, T. & Zhang, Z. (2020). Influence of bamboo biochar on mitigating greenhouse gas emissions and nitrogen loss during poultry manure composting. *Bioresource Technology*, 303, 122952. https://doi.org/10.1016/j.biortech.2020.122952

Azim, K., Soudi, B., Boukhari, S., Perissol, C., Roussos, S. & Thami Alami, I. (2018). Composting parameters and compost quality: a literature review. *Organic Agriculture*, 8 (2), 141–158. https://doi.org/10.1007/s13165-017-0180-z

Batz, M.B., Hoffman, S. & Morris, J.G., JR. (2012). Ranking the Disease Burden of 14 Pathogens in Food Sources in the United States Using Attribution Data from Outbreak Investigations and Expert Elicitation†. *Journal of Food Protection*, 75 (7), 1278–1291. https://doi.org/10.4315/0362-028X.JFP-11-418

Berghaus, R.D., Thayer, S.G., Law, B.F., Mild, R.M., Hofacre, C.L. & Singer, R.S. (2013). Enumeration of Salmonella and Campylobacter spp. in Environmental Farm Samples and Processing Plant Carcass Rinses from Commercial Broiler Chicken Flocks. *Applied and Environmental Microbiology*, 79 (13), 4106–4114. https://doi.org/10.1128/AEM.00836-13

De Bertoldi, M., Vallini, G. & Pera, A. (1983). The biology of composting: A review. *Waste Management & Research*, 1 (2), 157–176. https://doi.org/10.1016/0734-242X(83)90055-1

Briancesco, R., Coccia, A.M., Chiaretti, G., Della Libera, S., Semproni, M. & Bonadonna, L. (2008). Assessment of microbiological and parasitological quality of composted wastes: health implications and hygienic measures. *Waste Management & Research*, 26 (2), 196–202. https://doi.org/10.1177/0734242X07085064

Bryan, F.L. & Doyle, M.P. (1995). Health Risks and Consequences of Salmonella and Campylobacter jejuni in Raw Poultry. *Journal of Food Protection*, 58 (3), 326–344. https://doi.org/10.4315/0362-028X-58.3.326

Bush, D.J., Poore, M.H., Rogers, G.M. & Altier, C. (2007). Effect of stacking method on Salmonella elimination from recycled poultry bedding. *Bioresource Technology*, 98 (3), 571–578. https://doi.org/10.1016/j.biortech.2006.02.017

Chadwick, D., Sommer, S., Thorman, R., Fangueiro, D., Cardenas, L., Amon, B. & Misselbrook, T. (2011). Manure management: Implications for greenhouse gas emissions. *Animal Feed Science and Technology*, 166–167, 514–531. https://doi.org/10.1016/j.anifeedsci.2011.04.036

Chroni, C., Kyriacou, A., Georgaki, I., Manios, T., Kotsou, M. & Lasaridi, K. (2009a). Microbial characterization during composting of biowaste. *Waste Management*, 29 (5), 1520–1525. https://doi.org/10.1016/j.wasman.2008.12.012

Coburn, B., Grassl, G.A. & Finlay, B.B. (2007). Salmonella, the host and disease: a brief review. *Immunology & Cell Biology*, 85 (2), 112–118. https://doi.org/10.1038/sj.icb.7100007

Cronjé, A.L. (2004). *Ammonia emissions and pathogen inactivation during controlled composting of pig manure*. (d_ph). University of Birmingham. https://etheses.bham.ac.uk/id/eprint/155/ [2022-03-15]

Das, K.C., Tollner, E.W. & Tornabene, T.G. (2002). Windrow Composting of Paper Mill By-Products: Scale-Up and Seasonal Effects. *Compost Science & Utilization*, 10 (4), 347–355. https://doi.org/10.1080/1065657X.2002.10702097

Droffner, M.L. & Brinton, W.F. (1995). Survival of E. coli and Salmonella populations in aerobic thermophilic composts as measured with DNA gene probes. *Zentralblatt fur Hygiene und Umweltmedizin = International journal of hygiene and environmental medicine*, 197 (5), 387–397

Dumontet, S., Dinel, H. & Baloda, S.B. (1999). Pathogen Reduction in Sewage Sludge by Composting and Other Biological Treatments: A Review. *Biological Agriculture & Horticulture*, 16 (4), 409–430. https://doi.org/10.1080/01448765.1999.9755243

Eiland, F., Klamer, M., Lind, A.-M., Leth, M. & Bååth, E. (2001). Influence of Initial C/N Ratio on Chemical and Microbial Composition during Long Term Composting of Straw. *Microbial Ecology*, 41 (3), 272–280

Elving, J., Högberg, A., Ågren, E. & vesterlund-carlson, catrin (2018). *Underlag för riskbaserad hantering av gödsel från salmonella och VTEC-smittade besättningar*.

Elving, J., Ottoson, J. r., Vinnerås, B. & Albihn, A. (2010). Growth potential of faecal bacteria in simulated psychrophilic/mesophilic zones during composting of organic waste. *Journal of Applied Microbiology*, 108 (6), 1974–1981. https://doi.org/10.1111/j.1365-2672.2009.04593.x

Erickson, M.C., Liao, J., Ma, L., Jiang, X. & Doyle, M.P. (2014). Thermal and Nonthermal Factors Affecting Survival of Salmonella and Listeria monocytogenes in Animal Manure–Based Compost Mixtures. *Journal of Food Protection*, 77 (9), 1512–1518. https://doi.org/10.4315/0362-028X.JFP-14-111

Fernandes, L., Zhan, W., Patni, N.K. & Jui, P.Y. (1994). Temperature distribution and variation in passively aerated static compost piles. *Bioresource Technology*, 48 (3), 257–263. https://doi.org/10.1016/0960-8524(94)90155-4

Ferrer, J., Páez, G., Mármol, Z., Ramones, E., Chandler, C., Marín, M. & Ferrer, A. (2001). Agronomic use of biotechnologically processed grape wastes. *Bioresource Technology*, 76 (1), 39–44. https://doi.org/10.1016/S0960-8524(00)00076-6

Friedman, C.R., Hoekstra, R.M., Samuel, M., Marcus, R., Bender, J., Shiferaw, B., Reddy, S., Ahuja, S.D., Helfrick, D.L., Hardnett, F., Carter, M., Anderson,

B., Tauxe, R.V., & for the Emerging Infections Program FoodNet Working Group (2004). Risk Factors for Sporadic Campylobacter Infection in the United States: A Case-Control Study in FoodNet Sites. *Clinical Infectious Diseases*, 38 (Supplement_3), S285–S296. https://doi.org/10.1086/381598

Gras, L.M., Smid, J.H., Wagenaar, J.A., Boer, A.G. de, Havelaar, A.H., Friesema, I.H.M., French, N.P., Busani, L. & Pelt, W. van (2012). Risk Factors for Campylobacteriosis of Chicken, Ruminant, and Environmental Origin: A Combined Case-Control and Source Attribution Analysis. *PLOS ONE*, 7 (8), e42599. https://doi.org/10.1371/journal.pone.0042599

Haarala, C. (2012). *Compost and its effects on soilborne plant pathogens*. [Second cycle, A1E]. https://stud.epsilon.slu.se/4261/ [2022-05-01]

Hadar, Y. & Mandelbaum, R. (1992). Suppressive compost for biocontrol of soilborne plant pathogens. *Phytoparasitica*, 20 (1), S113–S116. https://doi.org/10.1007/BF02980420

Hansson, I., Forshell, L.P., Gustafsson, P., Boqvist, S., Lindblad, J., Engvall, E.O., Andersson, Y. & Vågsholm, I. (2007). Summary of the Swedish Campylobacter Program in Broilers, 2001 through 2005. *Journal of Food Protection*, 70 (9), 2008–2014. https://doi.org/10.4315/0362-028X-70.9.2008

Hansson, I., Sandberg, M., Habib, I., Lowman, R. & Engvall, E.O. (2018). Knowledge gaps in control of Campylobacter for prevention of campylobacteriosis. *Transboundary and Emerging Diseases*, 65 Suppl 1, 30–48. https://doi.org/10.1111/tbed.12870

Haug, R.T. (1993). Aeration Requirements. *The Practical Handbook of Compost Engineering*. Routledge

Hay, J.C. & Kuchenrither, R.D. (1990). Fundamentals and Application of Windrow Composting. *Journal of Environmental Engineering*, 116 (4), 746–763. https://doi.org/10.1061/(ASCE)0733-9372(1990)116:4(746)

Hess, T.F., Grdzelishvili, I., Sheng, H. & Hovde, C.J. (2004). Heat Inactivation of E. coli During Manure Composting. *Compost Science & Utilization*, 12 (4), 314–322. https://doi.org/10.1080/1065657X.2004.10702200

Hilbert, F., Scherwitzel, M., Paulsen, P. & Szostak, M.P. (2010). Survival of Campylobacter jejuni under Conditions of Atmospheric Oxygen Tension with the Support of Pseudomonas spp. *Applied and Environmental Microbiology*, 76 (17), 5911–5917. https://doi.org/10.1128/AEM.01532-10

Hoitink, H. a. J., Stone, A.G. & Grebus, M.E. (1996). Suppression of Plant Diseases by Composts. *The Science of Composting*, 373–381. https://doi.org/10.1007/978-94-009-1569-5_35

Howie, H., Mukerjee, A., Cowden, J., Leith, J. & Reid, T. (2003). Investigation of an outbreak of Escherichia coli O157 infection caused by environmental exposure at a scout camp. *Epidemiology & Infection*, 131 (3), 1063–1069. https://doi.org/10.1017/S0950268803001250

Hruby, C.E., Soupir, M.L., Moorman, T.B., Shelley, M. & Kanwar, R.S. (2016). Effects of tillage and poultry manure application rates on Salmonella and fecal indicator bacteria concentrations in tiles draining Des Moines Lobe soils. *Journal of Environmental Management*, 171, 60–69. https://doi.org/10.1016/j.jenvman.2016.01.040

Huang, G.F., Wong, J.W.C., Wu, Q.T. & Nagar, B.B. (2004). Effect of C/N on composting of pig manure with sawdust. *Waste Management*, 24 (8), 805–813. https://doi.org/10.1016/j.wasman.2004.03.011

Huet, J., Druilhe, C., Trémier, A., Benoist, J.C. & Debenest, G. (2012a). The impact of compaction, moisture content, particle size and type of bulking agent on initial physical properties of sludge-bulking agent mixtures before composting. *Bioresource Technology*, 114, 428–436. https://doi.org/10.1016/j.biortech.2012.03.031

Hutchison, M.L., Walters, L.D., Moore, A., Crookes, K.M. & Avery, S.M. (2004). Effect of Length of Time before Incorporation on Survival of Pathogenic Bacteria Present in Livestock Wastes Applied to Agricultural Soil. *Applied*

and Environmental Microbiology,. https://doi.org/10.1128/AEM.70.9.5111-5118.2004
Jiang, T., Schuchardt, F., Li, G., Guo, R. & Zhao, Y. (2011). Effect of C/N ratio, aeration rate and moisture content on ammonia and greenhouse gas emission during the composting. *Journal of Environmental Sciences*, 23 (10), 1754–1760. https://doi.org/10.1016/S1001-0742(10)60591-8
Jiang, X., Morgan, J. & Doyle, M.P. (2003). Fate of Escherichia coli O157:H7 during Composting of Bovine Manure in a Laboratory-Scale Bioreactor. *Journal of Food Protection*, 66 (1), 25–30. https://doi.org/10.4315/0362-028X-66.1.25
Jongbloed, A.W. & Lenis, N.P. (1998). Environmental concerns about animal manure. *Journal of Animal Science*, 76 (10), 2641–2648. https://doi.org/10.2527/1998.76102641x
Kabelitz, T., Biniasch, O., Ammon, C., Nübel, U., Thiel, N., Janke, D., Swaminathan, S., Funk, R., Münch, S., Rösler, U., Siller, P., Amon, B., Aarnink, A.J.A. & Amon, T. (2021). Particulate matter emissions during field application of poultry manure - The influence of moisture content and treatment. *Science of The Total Environment*, 780, 146652. https://doi.org/10.1016/j.scitotenv.2021.146652
Kim, E., Lee, D.-H., Won, S. & Ahn, H. (2016). Evaluation of Optimum Moisture Content for Composting of Beef Manure and Bedding Material Mixtures Using Oxygen Uptake Measurement. *Asian-Australasian Journal of Animal Sciences*, 29 (5), 753–758. https://doi.org/10.5713/ajas.15.0875
Kimura, A.C., Reddy, V., Marcus, R., Cieslak, P.R., Mohle-Boetani, J.C., Kassenborg, H.D., Segler, S.D., Hardnett, F.P., Barrett, T., Swerdlow, D.L., & for the Emerging Infections Program FoodNet Working Group (2004). Chicken Consumption Is a Newly Identified Risk Factor for Sporadic Salmonella enterica Serotype Enteritidis Infections in the United States: A Case-Control Study in FoodNet Sites. *Clinical Infectious Diseases*, 38 (Supplement_3), S244–S252. https://doi.org/10.1086/381576
Kudva, I.T., Blanch, K. & Hovde, C.J. (1998). Analysis of Escherichia coli O157:H7 Survival in Ovine or Bovine Manure and Manure Slurry. *Applied and Environmental Microbiology*,. https://doi.org/10.1128/AEM.64.9.3166-3174.1998
Kuhlman, L.R. (1990). Windrow composting of agricultural and municipal wastes. *Resources, Conservation and Recycling*, 4 (1), 151–160. https://doi.org/10.1016/0921-3449(90)90039-7
Kwak, W.S., Huh, J.W. & McCaskey, T.A. (2005a). Effect of processing time on enteric bacteria survival and on temperature and chemical composition of broiler poultry litter processed by two methods. *Bioresource Technology*, 96 (14), 1529–1536. https://doi.org/10.1016/j.biortech.2004.12.018
Larney, F.J., Olson, A.F., Carcamo, A.A. & Chang, C. (2000). Physical changes during active and passive composting of beef feedlot manure in winter and summer. *Bioresource Technology*, 75 (2), 139–148. https://doi.org/10.1016/S0960-8524(00)00040-7
Lepesteur, M. (2022). Human and livestock pathogens and their control during composting. *Critical Reviews in Environmental Science and Technology*, 52 (10), 1639–1683. https://doi.org/10.1080/10643389.2020.1862550
Li, M.-X., He, X.-S., Tang, J., Li, X., Zhao, R., Tao, Y.-Q., Wang, C. & Qiu, Z.-P. (2021). Influence of moisture content on chicken manure stabilization during microbial agent-enhanced composting. *Chemosphere*, 264, 128549. https://doi.org/10.1016/j.chemosphere.2020.128549
Li, Z., Lu, H., Ren, L. & He, L. (2013a). Experimental and modeling approaches for food waste composting: A review. *Chemosphere*, 93 (7), 1247–1257. https://doi.org/10.1016/j.chemosphere.2013.06.064
MacGregor, S.T., Miller, F.C., Psarianos, K.M. & Finstein, M.S. (1981). Composting Process Control Based on Interaction Between Microbial Heat

Output and Temperature. *Applied and Environmental Microbiology*, 41 (6), 1321–1330. https://doi.org/10.1128/aem.41.6.1321-1330.1981
Macklin, K.S., Hess, J.B. & Bilgili, S.F. (2008). In-House Windrow Composting and Its Effects on Foodborne Pathogens. *Journal of Applied Poultry Research*, 17 (1), 121–127. https://doi.org/10.3382/japr.2007-00051
Macklin, K.S., Hess, J.B., Bilgili, S.F. & Norton, R.A. (2006). Effects of In-House Composting of Litter on Bacterial Levels. *Journal of Applied Poultry Research*, 15 (4), 531–537. https://doi.org/10.1093/japr/15.4.531
Mandal, A., Patra, A.K., Singh, D., Swarup, A. & Ebhin Masto, R. (2007). Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. *Bioresource Technology*, 98 (18), 3585–3592. https://doi.org/10.1016/j.biortech.2006.11.027
Mead, G.C. (2005). Introduction. In: Mead, G.C. (ed.) *Food Safety Control in the Poultry Industry*. Woodhead Publishing, xvii–xviii. https://doi.org/10.1016/B978-1-85573-954-3.50026-0
Michel, F.C., Pecchia, J.A., Rigot, J. & Keener, H.M. (2004). Mass and Nutrient Losses During the Composting Of Dairy Manure Amended with Sawdust or Straw. *Compost Science & Utilization*, 12 (4), 323–334. https://doi.org/10.1080/1065657X.2004.10702201
Millner, P., Ingram, D., Mulbry, W. & Arikan, O.A. (2014). Pathogen reduction in minimally managed composting of bovine manure. *Waste Management*, 34 (11), 1992–1999. https://doi.org/10.1016/j.wasman.2014.07.021
Møller, H.B., Sommer, S.G. & Ahring, B.K. (2004). Methane productivity of manure, straw and solid fractions of manure. *Biomass and Bioenergy*, 26 (5), 485–495. https://doi.org/10.1016/j.biombioe.2003.08.008
Munch, B., Errebo Larsen, H. & Aalbæck, B. (1987). Experimental studies on the survival of pathogenic and indicator bacteria in aerated and non-aerated cattle and pig slurry. *Biological Wastes*, 22 (1), 49–65. https://doi.org/10.1016/0269-7483(87)90099-1
Nauta, M., Hill, A., Rosenquist, H., Brynestad, S., Fetsch, A., van der Logt, P., Fazil, A., Christensen, B., Katsma, E., Borck, B. & Havelaar, A. (2009). A comparison of risk assessments on Campylobacter in broiler meat. *International Journal of Food Microbiology*, 129 (2), 107–123. https://doi.org/10.1016/j.ijfoodmicro.2008.12.001
Nicholson, F.A., Groves, S.J. & Chambers, B.J. (2005). Pathogen survival during livestock manure storage and following land application. *Bioresource Technology*, 96 (2), 135–143. https://doi.org/10.1016/j.biortech.2004.02.030
Nordentoft, S., Fischer, C., Bjerrum, L., Heckmann, L. h. & Hald, B. (2017). Reduction of Escherichia coli, Salmonella Enteritidis and Campylobacter jejuni in poultry manure by rearing of Musca domestica fly larvae. *Journal of Insects as Food and Feed*, 3 (2), 145–153. https://doi.org/10.3920/JIFF2016.0058
Nyberg, K.A., Vinnerås, B., Ottoson, J.R., Aronsson, P. & Albihn, A. (2010). Inactivation of Escherichia coli O157:H7 and Salmonella Typhimurium in manure-amended soils studied in outdoor lysimeters. *Applied Soil Ecology*, 46 (3), 398–404. https://doi.org/10.1016/j.apsoil.2010.10.004
Pandey, P.K., Biswas, S., Vaddella, V.K. & Soupir, M.L. (2015). Escherichia coli persistence kinetics in dairy manure at moderate, mesophilic, and thermophilic temperatures under aerobic and anaerobic environments. *Bioprocess and Biosystems Engineering*, 38 (3), 457–467. https://doi.org/10.1007/s00449-014-1285-3
Paterson, D.L. (2006). Resistance in gram-negative bacteria: Enterobacteriaceae. *American Journal of Infection Control*, 34 (5, Supplement), S20–S28. https://doi.org/10.1016/j.ajic.2006.05.238
Pattison, M., McMullin, P., Bradbury, J.M. & Alexander, D. (2007). *Poultry Diseases*. Elsevier Health Sciences.

Payne, J.B., Osborne, J.A., Jenkins, P.K. & Sheldon, B.W. (2007). Modeling the Growth and Death Kinetics of Salmonella in Poultry Litter as a Function of pH and Water Activity. *Poultry Science*, 86 (1), 191–201. https://doi.org/10.1093/ps/86.1.191

Pergola, M., Persiani, A., Palese, A.M., Di Meo, V., Pastore, V., D'Adamo, C. & Celano, G. (2018). Composting: The way for a sustainable agriculture. *Applied Soil Ecology*, 123, 744–750. https://doi.org/10.1016/j.apsoil.2017.10.016

Petric, I. & Selimbašić, V. (2008). Composting of poultry manure and wheat straw in a closed reactor: optimum mixture ratio and evolution of parameters. *Biodegradation*, 19 (1), 53–63. https://doi.org/10.1007/s10532-007-9114-x

Petric, I., Šestan, A. & Šestan, I. (2009a). Influence of initial moisture content on the composting of poultry manure with wheat straw. *Biosystems Engineering*, 104 (1), 125–134. https://doi.org/10.1016/j.biosystemseng.2009.06.007

Pierson, M.D., Zink, D.L. & Smoot, L.M. (2007). Indicator microorganisms and microbiological criteria. *Food microbiology: fundamentals and frontiers*, (Ed.3), 69–85

Powers, W. & Angel, R. (2008). A Review of the Capacity for Nutritional Strategies to Address Environmental Challenges in Poultry Production. *Poultry Science*, 87 (10), 1929–1938. https://doi.org/10.3382/ps.2008-00090

Ravva, S.V. & Sarreal, C.Z. (2014). Survival of Salmonella enterica in Aerated and Nonaerated Wastewaters from Dairy Lagoons. *International Journal of Environmental Research and Public Health*, 11 (11), 11249–11260. https://doi.org/10.3390/ijerph111111249

Sahin, O., Kassem, I.I., Shen, Z., Lin, J., Rajashekara, G. & Zhang, Q. (2015). Campylobacter in Poultry: Ecology and Potential Interventions. *Avian Diseases*, 59 (2), 185–200. https://doi.org/10.1637/11072-032315-Review

Sangamithirai, K.M., Jayapriya, J., Hema, J. & Manoj, R. (2015). Evaluation of in-vessel co-composting of yard waste and development of kinetic models for co-composting. *International Journal of Recycling of Organic Waste in Agriculture*, 4 (3), 157–165. https://doi.org/10.1007/s40093-015-0095-1

Schaub, S.M. & Leonard, J.J. (1996). Composting: An alternative waste management option for food processing industries. *Trends in Food Science & Technology*, 7 (8), 263–268. https://doi.org/10.1016/0924-2244(96)10029-7

Schets, F.M., Jacobs-Reitsma, W.F., van der Plaats, R.Q.J., Heer, L.K.-D., van Hoek, A.H.A.M., Hamidjaja, R.A., de Roda Husman, A.M. & Blaak, H. (2017). Prevalence and types of Campylobacter on poultry farms and in their direct environment. *Journal of Water and Health*, 15 (6), 849–862. https://doi.org/10.2166/wh.2017.119

SJVFS (2006). SJVFS 2006:81 Saknr M 39. Statens jordbruksverks föreskrifter och allmänna råd om foder; ISSN 1102-0970

SJVFS (2007). SJVFS 2007:19 Saknr K 104. Statens jordbruksverks föreskrifterom obligatorisk salmonellakontroll av fjäderfän. ISSN 1102-0970

SJVFS (n.d.). SJVFS 2014: 34 Saknr K 102 Föreskrifter om ändring i Statens jordbruksverks föreskrifter (SJVFS 2004:2) om bekämpande av salmonella hos djur; ISSN 1102-0970

Smith, S., Meade, J., Gibbons, J., McGill, K., Bolton, D. & Whyte, P. (2016). The impact of environmental conditions on Campylobacter jejuni survival in broiler faeces and litter. *Infection Ecology & Epidemiology*, 6 (1), 31685. https://doi.org/10.3402/iee.v6.31685

Söderström, A., Österberg, P., Lindqvist, A., Jönsson, B., Lindberg, A., Blide Ulander, S., Welinder-Olsson, C., Löfdahl, S., Kaijser, B., De Jong, B., Kühlmann-Berenzon, S., Boqvist, S., Eriksson, E., Szanto, E., Andersson, S., Allestam, G., Hedenström, I., Ledet Muller, L. & Andersson, Y. (2008). A Large Escherichia coli O157 Outbreak in Sweden Associated with

Locally Produced Lettuce. *Foodborne Pathogens and Disease*, 5 (3), 339–349. https://doi.org/10.1089/fpd.2007.0065
Steinfeld, H., Gerber, P., Wassenaar, T.D., Nations, F. and A.O. of the U., Castel, V., Rosales, M., M, M.R. & Haan, C. de (2006). *Livestock's Long Shadow: Environmental Issues and Options*. Food & Agriculture Org.
Stentiford, E.I. (1996). Composting Control: Principles and Practice. In: De Bertoldi, M., Sequi, P., Lemmes, B., & Papi, T. (eds.) *The Science of Composting*. Dordrecht: Springer Netherlands, 49–59. https://doi.org/10.1007/978-94-009-1569-5_6
Sundberg, C., Smårs, S. & Jönsson, H. (2004). Low pH as an inhibiting factor in the transition from mesophilic to thermophilic phase in composting. *Bioresource Technology*, 95 (2), 145–150. https://doi.org/10.1016/j.biortech.2004.01.016
SVA (2016). Surveillance of infectious diseases in animals and humans in Sweden 2016, National Veterinary Institute (SVA), Uppsala, Sweden. SVA:s rapportserie 45 ISSN 1654-7098
SVA (2020). Surveillance of infectious diseases in animals and humans in Sweden 2020, National Veterinary Institute (SVA), Uppsala, Sweden. SVA:s rapportserie 68 1654-7098
SVA (2022). SVA (National Veterinary Institute). Salmonella control/surveillance. Available at: http://www.sva.se. Accessed 03 February 2022.
Svensk Fågel (2021). Monitoring program for campylobacter. 2021. https://svenskfagel.se/program/campylobacter. Accessed 03 February 2022.
Swedish Board of Agriculture (2021). Slaughter of poultry at slaughterhouse by Animal species. https://jordbruksverket.se/om-jordbruksverket/jordbruksverkets-officiella-statistik
Thomas, C., Idler, C., Ammon, C. & Amon, T. (2020). Effects of the C/N ratio and moisture content on the survival of ESBL-producing Escherichia coli during chicken manure composting. *Waste Management*, 105, 110–118. https://doi.org/10.1016/j.wasman.2020.01.031
Tiquia, S.M. & Tam, N.F.Y. (2002). Characterization and composting of poultry litter in forced-aeration piles. *Process Biochemistry*, 37 (8), 869–880. https://doi.org/10.1016/S0032-9592(01)00274-6
Ugwuanyi, J.O., Harvey, L.M. & McNeil, B. (1999). Effect of process temperature, pH and suspended solids content upon pasteurization of a model agricultural waste during thermophilic aerobic digestion. *Journal of Applied Microbiology*, 87 (3), 387–395. https://doi.org/10.1046/j.1365-2672.1999.00831.x
Umaraw, P., Prajapati, A., Verma, A.K., Pathak, V. & Singh, V.P. (2017). Control of campylobacter in poultry industry from farm to poultry processing unit: A review. *Critical Reviews in Food Science and Nutrition*, 57 (4), 659–665. https://doi.org/10.1080/10408398.2014.935847
Vinnerås, B., Agostini, F. & Jönsson, H. (2010). Sanitation by Composting. In: Insam, H., Franke-Whittle, I., & Goberna, M. (eds.) *Microbes at Work: From Wastes to Resources*. Berlin, Heidelberg: Springer, 171–191. https://doi.org/10.1007/978-3-642-04043-6_9
Walker, L., Charles, W. & Cord-Ruwisch, R. (2009). Comparison of static, in-vessel composting of MSW with thermophilic anaerobic digestion and combinations of the two processes. *Bioresource Technology*, 100 (16), 3799–3807. https://doi.org/10.1016/j.biortech.2009.02.015
Wang, K., He, C., You, S., Liu, W., Wang, W., Zhang, R., Qi, H. & Ren, N. (2015). Transformation of organic matters in animal wastes during composting. *Journal of Hazardous Materials*, 300, 745–753. https://doi.org/10.1016/j.jhazmat.2015.08.016
Wang, K., Li, W., Li, Y., Gong, X., Wu, C. & Ren, N. (2013). The modelling of combined strategies to achieve thermophilic composting of sludge in cold

region. *International Biodeterioration & Biodegradation*, 85, 608–616. https://doi.org/10.1016/j.ibiod.2013.03.005

Wang, W., Wang, X., Liu, J., Ishii, M., Igarashi, Y. & Cui, Z. (2007). Effect of Oxygen Concentration on the Composting Process and Maturity. *Compost Science & Utilization*, 15 (3), 184–190. https://doi.org/10.1080/1065657X.2007.10702331

Wei, L., Shutao, W., Jin, Z. & Tong, X. (2014). Biochar influences the microbial community structure during tomato stalk composting with chicken manure. *Bioresource Technology*, 154, 148–154. https://doi.org/10.1016/j.biortech.2013.12.022

Westerman, P.W. & Bicudo, J.R. (2005). Management considerations for organic waste use in agriculture. *Bioresource Technology*, 96 (2), 215–221. https://doi.org/10.1016/j.biortech.2004.05.011

Wilkinson, K.G., Tee, E., Tomkins, R.B., Hepworth, G. & Premier, R. (2011a). Effect of heating and aging of poultry litter on the persistence of enteric bacteria. *Poultry Science*, 90 (1), 10–18. https://doi.org/10.3382/ps.2010-01023

Windhorst, H.-W. (2006). Changes in poultry production and trade worldwide. *World's Poultry Science Journal*, 62 (4), 585–602. https://doi.org/10.1017/S0043933906001140

Yin, Y., Yang, C., Li, M., Zheng, Y., Ge, C., Gu, J., Li, H., Duan, M., Wang, X. & Chen, R. (2021). Research progress and prospects for using biochar to mitigate greenhouse gas emissions during composting: A review. *Science of The Total Environment*, 798, 149294. https://doi.org/10.1016/j.scitotenv.2021.149294

Z, L., H, L., L, R. & L, H. (2013). Experimental and modeling approaches for food waste composting: a review. *Chemosphere*, 93 (7). https://doi.org/10.1016/j.chemosphere.2013.06.064

Zhang, L., Li, L., Pan, X., Shi, Z., Feng, X., Gong, B., Li, J. & Wang, L. (2018). Enhanced Growth and Activities of the Dominant Functional Microbiota of Chicken Manure Composts in the Presence of Maize Straw. *Frontiers in Microbiology*, 9. https://www.frontiersin.org/article/10.3389/fmicb.2018.01131 [2022-04-20]

Popular science summary

How long can infective bacteria survive in poultry manure during storage in containers?

Farmers and consumers are increasingly concerned about minimizing the environmental risks and the safety of the food. Chicken litter is a rich source of different nutrients, therefore it can be used as fertilizer for agricultural lands. Sometimes poultry flocks are infected with *Salmonella* and in this case, the manure will also be contaminated with the bacteria. The majority of Swedish poultry producers have their own fields and can either compost the manure in a pile outdoors for 6 months or plough it down immediately, to minimize the risk for spreading of *Salmonella*. However, some farms lack this possibility as they do not have land of their own. For them composting the manure in a container could be a solution. By this method, there is no need for the farmer to manipulate the manure during these 6 months and the container can prevent the manure particles and effluents from entering and contaminating the surrounding environment. However, it is then important that the conditions in the container and the heat produced during composting ensures complete *Salmonella* elimination during storage.

In this study, we tried to investigate, how long we need to store the poultry manure in containers, to make sure that it is safe to be used as fertilizer. We focused on the most important bacteria related to the poultry industry, which are *E.coli*, *Salmonella* and *Campylobacter*. Previous studies have suggested that mixing manure with straw may reduce the time needed to kill these bacteria. Therefore, we used two big containers filled with fresh chicken manure and added wheat straw in only one of them and placed them outside on a farmland for 6 months. We were not allowed to add *Salmonella* and *Campylobacter* to the stored manure in the outside containers, therefore we also used a small bucket in a *Salmonella* lab at SLU. We filled the bucket with the same manure as outdoor containers and added *Salmonella* and *Campylobacter* to it. By using the small bucket, we were able to compare the results and anticipate the bacterial elimination for *Salmonella* in farm conditions. *E.coli* and *Salmonella* are both from the same bacterial family (*Enterobacteriaceae*), and the manure always contains *E.coli*. These characteristics led us to use the *E.coli* count as an indicator of *Salmonella* survival in the outdoor containers, where we were unable to add *Salmonella* due to safety regulations.

We know from other studies, that different environmental and chemical factors can influence bacterial survival and die-off rate during composting. Some of these factors are the temperature inside and outside the piles, manure's moisture content and the amounts of oxygen and pH. In order to measure these factors and see their impact on infective bacteria numbers, we put several loggers inside the manure piles and took the weekly manure samples from three different levels of the piles (surface, middle and bottom). We counted the number of bacteria from these samples each week.

According to our results, there was no more *E.coli* to be detected in our samples after nine weeks for the containers and four weeks for the bucket. *Salmonella* was detected during the whole experiment period of six months in the samples from the surface of the bucket, while it was not detected from the sixth week in the middle of the bucket, except for week 12. This was probably due to lower temperature in the surface area compared with the middle part of the small bucket. On the other hand, *Campylobacter* was more sensitive and it disappeared from the bucket samples after the first week of the experiment. Our experiment, however, didn't find any obvious difference in the container with straw, compared with the pure manure container. Further research is needed to explore if adding more wheat straw, or other environmental and chemical conditions could result in a faster bacterial die-off rate during poultry manure composting.

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Appendix 1

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