Lactic Acid Fermentation

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This chapter addresses lactic acid fermentation, a process that provides a way to store carcasses for at least 25 weeks and produce an end product that may be both pathogen-free and nutrient-rich. Lactic acid fermentation should be viewed as a means to preserve carcasses until they can be rendered. The low pH prevents undesirable degradation processes.

The process of lactic acid fermentation is simple and requires little equipment. Indeed, the process needs only a tank and a grinder. Fermentation is an anaerobic process that can proceed in any sized non-corrosive container provided it is sealed and vented for carbon dioxide release. During this process, carcasses can be decontaminated and there is a possibility of recycling the final products into feedstuff. Fermentation products can be stored until they are transported to a disposal site.

Carcasses are ground to fine particles, mixed with a fermentable carbohydrate source and culture inoculant, and then added to a fermentation container. Grinding aids in homogenizing the ingredients. For lactic acid fermentation, lactose, glucose, sucrose, whey, whey permeates, and molasses are all suitable carbohydrate sources. The carbohydrate source is fermented to lactic acid by Lactobacillus acidophilus.

Under optimal conditions, including a fermentation temperature of about 35°C (95°F), the pH of fresh carcasses is reduced to less than 4.5 within 2 days. Fermentation with L. acidophilus destroys many bacteria including Salmonella spp. There may be some microorganisms that can survive lactic acid fermentation, but these can be destroyed by heat treatment through rendering.

Biogenic amines produced during putrefaction are present in broiler carcasses. Tamim and Doerr (2000) argue that the presence of a single amine (tyramine) at a concentration above 550 ppm indicates a real risk of toxicity to animals being fed. This concentration is higher in the final product after rendering because the rendered product has less moisture than the fermentation broth. Thus, efforts should be made to reduce putrefaction. Properly prepared products will remain biologically stable until they are accepted for other processes such as rendering.

Taking into account the value of fermentation by-products, Crews et al. (1995) estimate the cost of fermentation of poultry carcasses to be $68–171 per ton. Other calculations that exclude the value of fermentation by-products suggest the costs of fermentation of cattle carcasses to be about $650 per ton. The challenges with lactic acid fermentation are complete pathogen containment, fermentation tank contamination, and corrosion problems.

An intriguing idea is to plan for fermentation during the actual transportation of carcasses to the rendering sites; in such a scenario, railroad tank cars could be used for fermentation. This might prove useful, even in the case of an emergency carcass disposal situation. Fermentation could likely be carried out easily in these tank cars, perhaps in less time and with lower costs than other techniques requiring the actual construction of a fermentation tank. Of course, research is needed to ascertain the commercial feasibility of this idea.

In 1984, Dobbins of the University of Georgia proposed lactic acid fermentation as a biosecure method for recycling carcasses (Blake & Donald, 1992 and 1995a). At Auburn University in 1990, initial investigations into the fermentation of poultry carcasses were carried out with the goal of developing an on-farm fermentation system suitable for broiler production operations. In March 1992, the first disposal facility was constructed to demonstrate the feasibility of on-farm fermentation of poultry carcasses.
carcasses; the Agricultural Engineering Department at Auburn University designed the prototype.

Lactic acid fermentation is commonly referred to as pickling because microorganisms are inactivated and the decomposition process ceases when the pH is reduced to approximately 4.5 (Cai et al., 1994). Given its capacity to inactivate microorganisms and decompose biological material, lactic acid fermentation is used for decontamination and storage of carcasses in poultry production. Significantly, rendering companies will generally accept products produced by lactic acid fermentation (Damron, 2002). For poultry producers, the utilization of lactic fermentation to store carcasses reduces the cost of transportation to rendering facilities by 90%; it is much more expensive to pay renderers to pick up fresh carcasses (Blake & Donald, 1992 and 1995b).

Section 3 – Principles of Operation

3.1 – Introduction

For millennia, people have used lactic acid fermentation, which is a natural process, to preserve food and feeds (Campbell-Platt & Cook, 1995; Wood, 1985). Fermentation is an anaerobic process in which lactic acid bacteria transform sugar into lactic acid (see Figure 1). Lactic acid is a natural, low-pH, effective preservative. This process has been used by Blake and Donald (1995a) to manage poultry carcasses and by Kherrati et al. (1998) for slaughterhouse wastes.

A fermentable carbohydrate source such as sucrose, molasses, whey, or ground corn is added to the ground carcasses. The ratio between fermentable carbohydrate and carcasses is 20:100 by weight (Blake & Donald, 1995a). The sugar is fermented to lactic acid by indigenous bacteria such as Lactobacillus acidophilus. This bacterial species is naturally present in the intestine of poultry; but for all animal species, including poultry, it is desirable to

FIGURE 1. Lactic acid fermentation.

3.2 – Process Description

Carcasses are ground into smaller pieces to facilitate fermentation (Johnston et al., 1998). These smaller particles absorb lactic acid better than do whole carcasses. Furthermore, the mixture of ground carcasses permits better homogenization of the fermented material. The recommended particle size is 2.5 cm (1.0 inch) in diameter, or lower.
provide an additional inoculation of *Lactobacillus acidophilus* culture. The production of lactic acid creates acidification, which decreases the pH of the carcass material. Under optimal conditions, fermentation reduces the pH from 6.5 to 4.5 within 48 hours (Morrow & Ferket, 2002). This decrease in pH preserves the nutrients and permits the carcasses to be stored for several months before rendering or use for other purposes (Sander et al., 1995).

The ground carcasses are put in a nearly closed tank. Fermentation is a natural process that takes place in the absence of oxygen, but small amounts of oxygen are in fact helpful in starting the process. Fermentation is often conducted in a tank with a gate to vent the carbon dioxide produced.

### 3.3 – Process Requirements

#### Equipment

The process of lactic acid fermentation is simple and requires little equipment. Indeed, the process needs only a tank and a grinder. According to Tibbetts et al. (1987), the size of the container does not influence fermentation, but the use of a non-corrosive container is desirable to avoid corrosion. Fermentation tanks could be closed with a gate to vent the carbon dioxide produced, or have a small opening to allow carbon dioxide to flow out of the tank. The grinder used must be able to produce bits of carcasses sized 2.5 cm (1.0 inch) in diameter, or smaller. This size is recommended for better homogenization between different substances and better penetration of lactic acid into the carcass material.

#### Supplies and chemicals

Fresh carcasses contain few carbohydrate sources capable of being used by *Lactobacillus*. Consequently, a carbohydrate source such as sugar, whey, molasses, or ground corn should be added to the carcasses. Carbohydrates should be added in proportion to the carcass weight; for example, it is necessary to add 20 kg (44 lb) of molasses for every 100 kg (220 lb) of broiler carcasses (Blake & Donald, 1995a).

Unfortunately, sugar does not guarantee a good fermentation. It is also necessary to check two other factors—time of putrefaction and temperature of fermentation. After the death of an animal, putrefaction of tissue begins and produces some biogenic amines (see toxic risk below). The putrefaction process slows fermentation and may result in an end pH above 4.5. This is problematic because the fermentation process is imperfect above pH 4.5 and is good below pH 4.5. To avoid the complications arising from putrefaction, fermentation should be initiated promptly and an active inoculum of lactic acid cultures should be used.

According to Tamim and Doerr (2000), the temperature for fermentation should be above 30°C (86°F) to obtain a biologically safe final product with a pH of less than 4.5. If lactic acid fermentation incompletely acidifies the carcasses, a mineral or organic acid should be directly added.

#### Utility requirements

Utility requirements include water and electricity. After each use, the interior of the grinder and tank should be rinsed with water and disinfected. The grinder must be dismantled for complete washing and disinfection.

#### Construction and start-up time

The start-up time depends on the time required to transport all the equipment and supplies to the site. It is necessary to bring the equipment and material to the site before slaughter as the time lapse between slaughter and initiation of lactic fermentation should be minimized. Preparations prior to slaughter include the following:

1. The grinder and carbohydrate source can be easily moved on-site with trucks.
2. Fermentation can be carried out in several milk trucks, tank trailers, or railroad cars, which are easy to move and are generally resistant to corrosion (Hermel, 1992).
3. Lactic acid bacteria are procured and cultured to produce an inoculum, which is then added to the slurry of ground carcasses and carbohydrates.
Capacity

There is no maximum or minimum fermentation capacity, according to Tibbetts et al. (1987); the size of the fermentation container does not influence fermentation. Any closeable, corrosion-resistant container may be used for lactic acid fermentation.

The number of vessels (containers) required for a carcass disposal event can be calculated easily. The mass fraction of water should be at least 70% by weight for lactic acid fermentation. For 100 kg (220 lb) of carcasses, 20 kg (44 lb) of sugar is needed. If the carcass material is 70% water, the total dry mass is 50 kg (110 lb) and the total mass is 167 kg (367 lb) for a 70% moisture mixture. A reasonable tank volume is 200 liters, or 2 liters per kg of carcass. For 1000 animals and 500 kg (1100 lbs) live weight each, the required tank volume would be one million liters or 1,000 m³ (35,315 ft³). Eight railroad tank cars of 130 m³ (4,590 ft³) each could supply this fermentation volume. For tank trucks with a capacity of 20 m³ (706 ft³), 50 trucks would be needed.

3.4 – End Products

The aim of lactic fermentation is preservation and decontamination of the carcass material. Once carcass material is decontaminated, it can be sent to rendering plants. Other potential uses of fermented carcasses include mink and fox feed, aquaculture feeds, or other animal feeds. For example, up to 20% of fermented meat could be added to growing-finishing pigs’ rations; this neither decreases nor increases the pigs’ feed-to-gain ratios (Tibbetts et al., 1987). Most importantly, any use of fermentation end products must be considered carefully in order to avoid the transmission of pathogenic agents to other animals. Heat processes (cooking) can be used to ensure the destruction of any pathogens present.

3.5 – Economics

There are certain costs involved with the lactic fermentation process. The initial investment cost for setting up a tank is usually high. The net cost of fermentation, which includes variable costs and the value of by-products, is modest.

Taking into account the value of fermentation by-products, researchers have estimated the cost of fermentation of poultry carcasses to be $68–171 per ton (Crews et al., 1995; Blake & Donald, 1995b). The cost of molasses is about $40 per metric ton ($36 per US ton) and a polyethylene tank, which holds 500 gallons (1,890 liters) costs $640. For 1,000 animals with a weight of 500 kg (1100 lbs) each, the costs would be as shown in Table 1.

The cost would be much less if one uses available mobile tanks, such as tank trucks or railroad tank cars, because fermentation could occur during transit and it would therefore not be necessary to purchase tanks.

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost / kg</th>
<th>Cost for 1000 cattle a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanks</td>
<td>$0.678</td>
<td>$339,000</td>
</tr>
<tr>
<td>Molasses</td>
<td>$0.008</td>
<td>$4,000</td>
</tr>
<tr>
<td>Expenses</td>
<td>$0.028</td>
<td>$14,000</td>
</tr>
<tr>
<td><strong>Total cost</strong></td>
<td><strong>$0.714</strong></td>
<td><strong>$357,000</strong></td>
</tr>
</tbody>
</table>

aThe cattle are assumed to weigh 500 kg or 1100 lbs. each.

An estimation of the cost during an emergency is therefore $714 per metric ton of carcasses (~$650 per US ton). This price does not include the sale of by-products to rendering companies or resale of used equipment. The type of tank used for estimation is a 500-gallon (1.895 m³) horizontal leg tank with an estimated cost of $640; an example tank is shown in Figure 2 below.

**FIGURE 2.** Type of tank used for estimation (United States Plastic Corporation, 2004).
Section 4 – Disease Agent Considerations

4.1 – Pathogen Containment

*Lactobacillus acidophilus* produces lactic acid and an antimicrobial agent called lactocidin, which has a broad antibacterial spectrum (Vincent et al., 1959; Coconnier et al., 1997). Together, low pH and temperature contribute to the destruction of bacterial pathogens and inactivation of viruses.

Bacteria

The survival period of *Salmonella* and its resistance to temperature is important while destroying bacteria (Shotts et al., 1984). Fermentation with *Lactobacillus acidophilus* destroys many bacteria such as *Salmonella typhimurium* within five days at 30°C (86°F) and 40°C (104°F). Significantly, citric, lactic, phosphoric, acetic, and propionic acid are all inhibitors of *Salmonella*. Table 2 shows the inhibition of the acids produced in the lactic acid fermentation compared to citric acid and phosphoric acid.

Most bacteria are destroyed within two days except the group E *Streptococcus*, which is similar to *Lactobacillus* (Dobbins, 1987). Germination of spores is also inhibited by low pH and lactic acid. Germination of *Bacillus subtilis* spores is strongly inhibited at pH 4.5 with 0.5% lactic acid, as shown in Figure 3.

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Concentration of antimicrobial agent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1%</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>18.3</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>19.1</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>15.4</td>
</tr>
<tr>
<td>Citric acid</td>
<td>15.3</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>16.4</td>
</tr>
</tbody>
</table>

Most bacteria are destroyed within two days except the group E *Streptococcus*, which is similar to *Lactobacillus* (Dobbins, 1987). Germination of spores is also inhibited by low pH and lactic acid. Germination of *Bacillus subtilis* spores is strongly inhibited at pH 4.5 with 0.5% lactic acid, as shown in Figure 3.

![Figure 3](image)
Viruses

Both temperature and pH affect the viability of viruses. The adenovirus group (canine hepatitis), which is the most difficult virus to destroy, is destroyed within five days at 30°C (86°F) and 40°C (104°F). Viruses of the myxo virus group (e.g., Newcastle disease) are destroyed in just two days (Dobbins, 1987). However, Wooley et al. (1981) report that Newcastle disease and infectious canine hepatitis have survived for 96 hours at 30°C (86°F) in fermented, edible waste material. In the same study, pseudorabies virus and the viral agent of avian infectious bronchitis were inactivated in 24 hr at 30°C (86°F), measles virus and vesicular stomatitis virus in a few hours, and porcine picornavirus in 72 hours. Foot and mouth disease virus disassembles below pH 7, and rhinovirus loses its infectivity at about pH 5 (Twomey et al., 1995). Some viruses—like enterovirus, cardiovirus, and hepatovirus—are actually stable at pH 3 or lower, and poliovirus, an enterovirus, retains its infectivity even at pH 1.5 (Twomey et al., 1995). While acid-resistant, these viruses can be destroyed by heat. Thus mild heat treatment is needed to make sure that all viruses are destroyed.

4.2 – Risk of Contamination

There is a risk of toxic products that may be present following lactic acid fermentation and rendering. If the rendered product is used as an animal feed, it is important to realize that certain toxic agents can survive this treatment. If carcasses are sterilized after particle size reduction and prior to inoculation, the risk of contamination is reduced significantly.

4.3 – Toxic Risk

During the fermentation of broiler carcasses, certain amino acids have been shown to undergo decarboxylation and become biogenic amines (see Table 3). Necrotic cellular debris in the intestines of carcasses has been associated with biogenic amines in animal protein products. The level of biogenic amines depends on the state of decomposition of carcasses that are used in lactic acid fermentation.

<table>
<thead>
<tr>
<th>Biogenic Amine</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadaverine</td>
<td>Lysine</td>
</tr>
<tr>
<td>Histamine</td>
<td>Histidine</td>
</tr>
<tr>
<td>Phenylethylamine</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Putrescine</td>
<td>Arginine, Methionine</td>
</tr>
<tr>
<td>Spermine, Spermidine</td>
<td>Arginine, Methionine</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>Tyramine</td>
<td>Tyrosine</td>
</tr>
</tbody>
</table>

Only spermidine and spermine are reduced during fermentation. All biogenic amines produced during putrefaction are present in broiler carcasses (Table 4), and Tamim and Doerr (2000) argue that the presence of a single amine (tyramine) at a concentration above 550 ppm indicates a real risk of toxicity to animals being fed. This concentration is higher in the final product after rendering because the rendered product has less moisture than the fermentation broth. Thus, efforts should be made to reduce putrefaction.

<table>
<thead>
<tr>
<th>Amine</th>
<th>Putrefaction</th>
<th>Fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadaverine</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Histamine</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Phenylethylamine</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Putrescine</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Spermidine</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>Spermine</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Tyramine</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Key: (++) indicates produced; (--) indicates reduced.
Section 5 - Implications to the Environment

Lactic acid fermentation does not have any significant environmental effects if the products of fermentation are rendered and/or processed into marketable products. The process allows the carcasses to be stored until they can be processed.

Section 6 – Advantages and Disadvantages

The advantages and disadvantages associated with lactic acid fermentation are presented in Table 5.

TABLE 5. Advantages and disadvantages of lactic acid fermentation of carcasses.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decontamination of carcasses</td>
<td>All pathogens are not destroyed</td>
</tr>
<tr>
<td>Possibility of recycling into a feedstuff</td>
<td>Risk of contamination</td>
</tr>
<tr>
<td>Possibility of storage</td>
<td>Problem of corrosion</td>
</tr>
<tr>
<td>Potentially mobile process</td>
<td>Need carbohydrate source and culture of Lactobacillus acidophilus</td>
</tr>
</tbody>
</table>

Section 7 – Critical Research Needs

1. Investigate combining lactic acid fermentation and transportation processes to minimize the risk of pathogen spread during transportation.

   One intriguing idea regarding lactic acid fermentation is to carry it out during transportation in railroad tank cars. These tank cars are available in almost all locations and can be made non-corrosive. The number of tank cars can vary based on the amount of carcasses involved. These tanks might prove particularly useful in emergency situations. The advantages of using these tanks include the following: they are available in large numbers, they can be reused with proper cleaning, and they take less time to assemble as compared to traditional equipment used currently in other processes.

2. Investigate additional treatments such as lactic acid addition, thermal processing, and radio frequency heating for their economic and technical feasibility and for their ability to kill pathogens in carcass material.

   Research should focus on other methods that might be used to kill pathogens in conjunction with lactic acid fermentation processes. Lactic acid fermentation will not destroy all pathogens. There may be some harmful microorganisms that require additional treatment for complete destruction/inactivation. Additional
treatments such as lactic acid addition may be appropriate and should be investigated. Thermal processing may also be beneficial in some cases. There is a need to conduct experiments on the fate of pathogens in carcasses that are ground and subjected to lactic acid fermentation. For each pathogen, there is a need to know the pH level and lactic acid concentration that are sufficient to destroy it. If ground carcasses are fermented at ambient conditions, further research is needed to understand the effect of inoculum size and temperature on the competition of lactic acid fermentation with putrefaction.

Radio frequency heating is another process that may be used to kill pathogens (Wang et al., 2003). A thorough study is necessary to determine its economic and technical feasibility. It is now widely used in industrial applications and also for heating fruits, vegetables, and fish on a large scale. However, non-uniform temperatures and difficulty in application of overpressure can be problems of concern in this process. Using this process, better quality end products can be obtained in a shorter time and with less energy. Radio frequency heating should be investigated for its economic and technical suitability for carcass disposal.

References


