Preservation of Hatchery Waste by Lactic Acid Fermentation.
1. Laboratory Scale Fermentation

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ABSTRACT In Experiment 1, two types of hatchery wastes, including cockerel chicks (CC), and shell waste (SW) blended with CC (60:40 CC:SW), were ground and mixed with a by-product carbohydrate (5, 10, and 15% wt/wt) with or without a bacterial culture and fermented for a period of 21 d. Cockerel chicks fermented with 15% carbohydrate and the culture had a pleasant smell and little H₂S production. Elevated H₂S concentrations were recorded for CC:SW samples at all carbohydrate levels when the culture was not added. No NH₃ was detected from any treatments during fermentation. The addition of culture to the CC and CC:SW by-products resulted in pH values lower (P < 0.05) than those without culture on Day 21, and the 15% carbohydrate treatment significantly reduced pH beyond the 5% carbohydrate. Final proximate composition of CC and CC:SW samples with culture were not significantly different from those without culture added.

In Experiment 2, carbohydrate was added at 10.0, 13.3, 16.7, and 20.0% wt/wt to CC and CC:SW in the presence of the bacterial culture. Shell waste alone was fermented with 15, 20, and 25% carbohydrate and the culture. Moisture level in this experiment was adjusted to approximately 70% for all treatments. The lowest pH for the CC and CC:SW treatments was observed at the 16.7% carbohydrate level. Shell waste pH was better maintained at the 20 and 25% carbohydrate levels. After fermentation for 21 d CC, CC:SW and SW treatments from Experiments 1 and 2 contained negligible Escherichia coli, and no Salmonella were detected.

(Key words: fermentation, Leghorn hatchery waste, Lactobacillus, Streptococcus)

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INTRODUCTION

Fermentation has been used for many years as a method of preserving a variety of products. Numerous bacterial species have preservation capabilities manifested by their organic acid production. These organisms are relatively harmless and are involved in the production of yogurt, cheese, and silage. Wooley et al. (1981) stated that Lactobacillus fermentation may be used as a safe, convenient, farm-operated process in which edible wastes can be recycled into feed ingredients. With an active culture, anaerobic environment and available sugar, fermentation can generate the lactic acid and low pH necessary to inhibit the growth of harmful bacteria and preserve most by-products. Fermentation with lactic acid bacteria has been demonstrated with fish and slaughterhouse by-products as reported by Echenne et al. (1984) and Skrede and Nes (1988), respectively. Murphy and Silbert (1990), evaluated a variety of carbohydrates for the preservation of poultry carcasses using acid generation, low pH, and destruction of enterobacteria as indicators of successful fermentation. More recently, Patterson et al. (1994) ensiled poultry processing condemnations and offal as an initial preservation step for the tropics. The authors later co-extruded the fermented by-products with cereals to generate new feed ingredients. The fermented ingredients were evaluated with broiler chickens and determined to be equal to or better than unfermented control ingredients for live performance to 6 wk, and carcass and parts yield.

Wisman (1964) and Vandepopuliere et al. (1977) demonstrated that quickly processed hatchery by-products were palatable, nutrient-dense dietary ingredients for both broilers and laying hens. However, one limitation to successfully recycling the rich nutrients in hatchery by-products is their highly perishable nature. Therefore, the objectives of this study were to: 1) attempt preservation without cold storage, or freezing, 2) evaluate the ability of lactic acid fermentation to stabilize hatchery by-products using different levels of a by-product carbohydrate with or without a commercial microbial culture, and 3) determine the effects of the fermentation process on nutrient and microbial quality.

MATERIALS AND METHODS

Experiment 1

Two waste streams, including cockerel chicks (CC) and shell waste (SW) consisting of infertile eggs, dead
The dried samples were then ground and analyzed for percentage ether extract (EE), ash (AOAC, 1990), Ca (atomic absorption spectrophotometry, AOAC, 1990), and P (colorimetry, Fiske and SubbaRow, 1925). The fermented samples were also evaluated for the presence of *Escherichia coli*, *Streptococcus*, *Salmonella* (AOAC, 1990), and *Lactobacillus* (Vanderzant and Splittstoesser, 1992) microorganisms. All analyses and determinations were done in triplicate.

**Experiment 2**

In this experiment CC and a 60:40 ratio of CC:SW were mixed with the carbohydrate at the rate of 10.0, 13.3, 16.7, and 20.0% wt/wt and shell waste (SW) alone at 15, 20, and 25% wt/wt. Liquid culture was added at 0.2% to all treatments and the products were fermented for a period of 21 d. Water was added to equalize the final moisture content to approximately 70% and to facilitate mixing. On Days 0, 1, 3, 5, 13, and 21, pH determinations were made using a pH meter. All other measurements and analyses were similar to the previous experiment.

**Statistical Analysis.** Data from Experiments 1 and 2 were subjected to statistical analysis using the General Linear Models (GLM) procedures of SAS® (SAS Institute, 1990). Experiment 1 treatments were arranged as a 3 × 2 factorial with three levels of carbohydrate and the absence or presence of the culture at 0 and 0.2%. Experiment 2 data were analyzed by one-way analysis of variance procedures for a completely randomized design. Differences among treatment means (P < 0.05) were assessed using Tukey’s test (Steel and Torrie, 1980).

**RESULTS AND DISCUSSION**

**Experiment 1**

When the buckets were opened on Day 21 to make observations, fermented CC samples with 10 or 15% carbohydrate in the presence or absence of culture were semi-solid, dark brown to pink in color, and had a pleasant fermented smell. Cockerel chick samples with 5% carbohydrate with or without culture had a greenish brown surface, with visible specks of fungus and an unpleasant odor. The lower carbohydrate level (5%) was also responsible for generating more H2S gas (≥100 ppm) than treatments with 10 or 15%. Adding bacterial culture to the 5% carbohydrate treatments reduced H2S production more than 200 ppm on Days 5 and 13, whereas similar H2S levels (approximately 150 ppm) were observed on Days 3 and 21 among treatments with or without culture added. Ammonia gas was not detected from any of the CC treatments during the 21-d study. Fermentation of CC with either 10 or 15% carbohydrate produced little or no H2S and NH3, indicating better protein preservation. Long-term fermentation (Days 13 and 21) was further improved when the bacterial culture was present, as indicated by the lower H2S levels.
Fermentation of CC:SW at the 15% carbohydrate level in the presence of culture resulted in a dark brown product, with a semi-solid consistency, and a sweet fermented smell. Treatments at the lower carbohydrate levels (5 and 10%) with or without culture were greenish brown in color, had some surface fungus and an offensive smell. Adding the bacterial culture to all treatments resulted in lower H2S levels (≤ 50 ppm) through Day 13, yet on Day 21 H2S concentration measured 250, 220, and 25 ppm for the 5, 10, and 15% carbohydrate treatments, respectively. When no culture was added, higher H2S levels were produced (≥ 50 ppm) by the 5 and 10% carbohydrate treatments throughout the 3-wk study. On Day 21 even the 15% carbohydrate treatment generated measurable H2S (150 ppm) when no culture was added. No NH3 gas was detected during fermentation of the CC:SW treatments. Clearly the best treatment combination for maintaining CC:SW was to include the bacterial culture with the highest (15%) level of carbohydrate.

Initial pH of CC treatments averaged 6.73 before the fermentation process began on Day 0; however, within 1 d, sufficient organic acids had accumulated reducing average pH to 5.5. The fermentation process maintained an acid environment and pH of 4.0 and 4.5 for treatments with and without culture from Days 3 through 13. Between Days 13 and 21, pH of the treatment buckets increased to higher levels. Final pH and chemical composition of the CC treatments on Day 21 are reported in Table 1. The 15% carbohydrate level generated a significantly lower pH than the 5% level. Adding liquid culture resulted in a lower average pH (5.29, P < 0.05) than buckets without culture added (5.58). The best treatment combination in terms of generating organic acids and maintaining the lowest pH at 3 wk (P < 0.05) was the 15% carbohydrate level with culture added (pH = 5.17, data not shown). The temperature of the treatments during the fermentation period was 18 to 24 C. Hamm and Whitehead (1982) reported that a temperature range of 18 to 22 C, was satisfactory for hatchery waste preserved for 1 wk without affecting proximate composition. Other reports have documented successful preservation of broiler carcasses and offal with temperatures ranging from 18 to 25 C in the presence of adequate carbohydrate. Adding liquid culture had no significant effect on the DM, CP, EE, or ash contents of the fermented CC samples (Table 1). Increasing the percentage of carbohydrate diluted the concentration of CP, ash, Ca and P, yet increased the DM percentage significantly (P < 0.01). Carbohydrate level had no significant effect on the EE concentration.

Initial pH of the CC:SW treatments before fermentation averaged 7.0. During the 21-d fermentation period, a strikingly similar pattern of pH change to the CC was observed with the CC:SW treatments. By Day 3, average pH had dropped to 4.0 and 4.5 for treatments with and without the microbial culture (P < 0.01); these levels were maintained through Day 13. Between Day 13 and Day 21, pH increased to higher levels, especially for treatments with lower levels of carbohydrate and no added culture. Final pH and chemical composition of the CC:SW treatments on d 21 are summarized in Table 2. Greater amounts of fermentable carbohydrate (10 and 15%) generated significantly lower pH results at the end of the 3-wk study. Adding liquid culture was again responsible for maintaining a lower average pH (5.38, P < 0.05) than buckets without the culture added (5.73). The greatest acid production, and lowest pH was produced by the treatment with 15% carbohydrate plus the culture inoculum (pH = 5.24).

The addition of culture had no impact on the nutrient composition of the fermented CC:SW samples at 21 d, whereas increasing carbohydrate levels significantly increased DM and decreased CP, ash, Ca, and P percentages. As with the CC treatments, increasing carbohydrate level had no influence on the EE concentration of the CC:SW treatments.

### Table 1. Percentage chemical composition and pH of cockerel chicks after 21 d fermentation, Experiment 1 (DM basis)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>pH</th>
<th>DM (g/100 g)</th>
<th>CP (g/100 g)</th>
<th>EE (g/100 g)</th>
<th>Ash (g/100 g)</th>
<th>Ca (g/100 g)</th>
<th>P (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (CHO)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>5.55 ± 0.17^a</td>
<td>20.95 ± 1.09^a</td>
<td>54.80 ± 1.16^a</td>
<td>33.17 ± 0.97</td>
<td>6.40 ± 0.04^a</td>
<td>1.25 ± 0.04^a</td>
<td>0.90 ± 0.03^a</td>
</tr>
<tr>
<td>10%</td>
<td>5.44 ± 0.17^ab</td>
<td>23.00 ± 0.51^ab</td>
<td>48.34 ± 0.69^ab</td>
<td>30.35 ± 0.93</td>
<td>5.67 ± 0.22^ab</td>
<td>1.04 ± 0.04^ab</td>
<td>0.78 ± 0.05^ab</td>
</tr>
<tr>
<td>15%</td>
<td>5.31 ± 0.20^bc</td>
<td>24.26 ± 0.43^bc</td>
<td>43.93 ± 0.51^bc</td>
<td>30.17 ± 0.66</td>
<td>5.38 ± 0.09^bc</td>
<td>0.92 ± 0.04^bc</td>
<td>0.69 ± 0.04^bc</td>
</tr>
<tr>
<td>Culture^2 (C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5.29 ± 0.14^c</td>
<td>22.68 ± 2.12</td>
<td>49.27 ± 5.11</td>
<td>30.56 ± 0.58</td>
<td>5.89 ± 0.45</td>
<td>1.08 ± 0.14</td>
<td>0.80 ± 0.10</td>
</tr>
<tr>
<td>No</td>
<td>5.58 ± 0.11^a</td>
<td>22.79 ± 0.97</td>
<td>48.77 ± 1.30</td>
<td>30.70 ± 1.30</td>
<td>5.75 ± 0.51</td>
<td>1.06 ± 0.16</td>
<td>0.79 ± 0.11</td>
</tr>
</tbody>
</table>

*^aMeans within a column with no common superscript differ significantly (P < 0.05).

Values represent the means of four replicates ± SD.

^2Culture contained Lactobacillus plantarum, Pediococcus acidlactici, and Streptococcus faecium. Values represent the means of six replicates.
TABLE 2. Percentage chemical composition and pH of cockerel chicks:shell wastes after 21 d fermentation, Experiment 1 (DM basis)\(^1\)

<table>
<thead>
<tr>
<th>Factorial</th>
<th>pH</th>
<th>DM</th>
<th>CP</th>
<th>EE</th>
<th>Ash</th>
<th>Ca</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (CHO)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>5.72 ± 0.23(^a)</td>
<td>24.20 ± 0.23(^a)</td>
<td>36.37 ± 0.61(^a)</td>
<td>16.99 ± 0.78</td>
<td>39.45 ± 1.28(^a)</td>
<td>20.53 ± 1.48(^a)</td>
<td>0.66 ± 0.03(^a)</td>
</tr>
<tr>
<td>10%</td>
<td>5.52 ± 0.17(^b)</td>
<td>27.84 ± 1.90(^b)</td>
<td>30.81 ± 1.40(^b)</td>
<td>16.88 ± 1.16</td>
<td>35.46 ± 0.52(^b)</td>
<td>18.47 ± 1.02(^b)</td>
<td>0.54 ± 0.02(^b)</td>
</tr>
<tr>
<td>15%</td>
<td>5.42 ± 0.21(^c)</td>
<td>30.76 ± 1.23(^c)</td>
<td>28.25 ± 0.84(^c)</td>
<td>16.53 ± 0.88</td>
<td>33.50 ± 2.05(^b)</td>
<td>17.21 ± 1.60(^b)</td>
<td>0.49 ± 0.03(^b)</td>
</tr>
<tr>
<td>Culture(^2) (C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5.38 ± 0.13(^b)</td>
<td>27.77 ± 2.89</td>
<td>31.54 ± 3.72</td>
<td>16.45 ± 0.76</td>
<td>35.49 ± 3.53</td>
<td>18.14 ± 1.86</td>
<td>0.57 ± 0.09</td>
</tr>
<tr>
<td>No</td>
<td>5.73 ± 0.15(^c)</td>
<td>27.44 ± 3.49</td>
<td>32.08 ± 3.92</td>
<td>17.15 ± 0.93</td>
<td>36.76 ± 2.23</td>
<td>19.34 ± 1.91</td>
<td>0.55 ± 0.08</td>
</tr>
</tbody>
</table>

\(^{a-c}\)Means within a column with no common superscript differ significantly (\(P < 0.05\)).

\(^1\)Values represent the means of four replicates ± SD.

**Microbial analysis of the CC and CC:SW treatments after 21 d of fermentation are presented in Table 3. The use of culture did not significantly affect (\(P > 0.05\)) the Lactobacillus or Streptococcus counts when compared to samples without culture added. Concentrations of E. coli were negligible, and no Salmonella species were recovered from either by-product with or without the added culture. Fermentation experiments involving poultry offal and carcasses (Cai et al., 1994b) have shown that this process eliminated or reduced Salmonella species, fecal coliforms, and fecal Streptococci to insignificant levels after 7 d.**

Carbohydrate at the 15% level appears sufficient to produce enough lactic acid to preserve the CC for up to 3 wk. Cai et al. (1994a) reported that male chicks fermented with 15% brewers solubles, had a pH of 4.3 or less when stored for 8 d. The addition of culture to the CC and CC: SW samples was intended to promote rapid lactic acid generation. A measurable reduction in pH was observed as a result of adding the culture. Although adding bacterial culture had a negligible effect on the final microbial analysis, the practice aided acid formation, resulting in a lower pH and improved the appearance and odor of the products. Urlings et al. (1993) similarly reported that inoculation of poultry by-products with L. plantarum and E. faecium with 10% sugar beet pulp and 2% dextrose had a greater potential for maintaining a lower pH for 3 wk.

**Experiment 2**

The physical characteristics of all CC and CC:SW treatments at the conclusion of the 21-d fermentation period were dark brown to pink in color, with no offensive odor. The material in these treatments had separated into an upper solid and lower liquid layers. The SW treatments had a greenish color and the presence of colonies on the surface. Negligible H\(_2\)S (25 ppm) production was observed from CC and CC:SW samples, whereas the SW at the lowest carbohydrate level (15%) generated high concentrations of H\(_2\)S by Day 21 (250 to 500 ppm). No

**Table 3. Microbiological analysis following fermentation**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Culture(^3)</th>
<th>Lactobacillus (log cfu/g)</th>
<th>Streptococci (log cfu/g)</th>
<th>Escherichia coli (log cfu/g)</th>
<th>Salmonella (log cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC(^4)</td>
<td>Yes</td>
<td>8.44</td>
<td>6.28</td>
<td>ng(^2)</td>
<td>none</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>8.45</td>
<td>6.84</td>
<td>ng</td>
<td>none</td>
</tr>
<tr>
<td>CC:SW(^4)</td>
<td>Yes</td>
<td>7.79</td>
<td>8.05</td>
<td>ng</td>
<td>none</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>7.78</td>
<td>7.96</td>
<td>ng</td>
<td>none</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC(^5)</td>
<td>Yes</td>
<td>7.25</td>
<td>5.95</td>
<td>ng</td>
<td>none</td>
</tr>
<tr>
<td>CC:SW(^5)</td>
<td>Yes</td>
<td>8.29</td>
<td>7.05</td>
<td>ng</td>
<td>none</td>
</tr>
<tr>
<td>SW(^5)</td>
<td>Yes</td>
<td>7.82</td>
<td>6.97</td>
<td>ng</td>
<td>none</td>
</tr>
</tbody>
</table>

\(^{a-c}\)Means within a column with no common superscript differ significantly (\(P < 0.05\)).

\(^1\)Values represent the means of six replicates.

\(^2\)ng = negligible.

\(^3\)Culture contained Lactobacillus plantarum, Pediococcus acidlactici, and Streptococcus faecium.

\(^4\)CC = cockerel chicks.

\(^5\)CC:SW = cockerel chicks:shell waste.

\(^6\)SW = shell waste.
ammonia production was recorded from any of the treatments. Fermentation temperature ranged from 18 to 24 C during the 21-d period.

The changes in treatment pH during the 21-d study are presented in Figure 1 for the CC samples. The 10% carbohydrate treatment had a pH value significantly (P < 0.05) higher than the 20.0% treatment on Day 3. The pH decreased further by Day 5, 13, and 21 for all fermented CC samples, whereas the lowest levels were recorded on 21 d for the 16.7 and 20.0% treatments. Final pH and chemical composition are reported after 21 d of fermentation in Table 4. No significant differences in pH, DM, EE, ash, or minerals occurred among the carbohydrate levels tested; however, as observed in the previous experiment, increasing the proportions of carbohydrate reduced the CP content of the CC samples (P < 0.01).

Small but significant treatment differences due to carbohydrate level were observed in pH on Days 0, 1, and 5 (Figure 2). The pH of all the fermented samples was stable until Day 13. By 21 d, the pH of all treatments had risen to more than 5.0, indicating fermentable carbohydrate was limiting. No significant differences in DM, EE,
Experiment 1. Whereas adding water did aid the effect of adding carbohydrate that resulted in higher DM levels in CC, CC:SW, and SW (Table 4) and offset the loss of moisture, it did not appear to enhance the fermentation process; however, it did equalize the level of moisture (70 to 74%) in all products, CC, CC:SW, and SW (Table 4) and offset the effect of adding carbohydrate that resulted in higher DM content in Experiment 1. Whereas adding water did aid mixing the by-products with the dry carbohydrate initially, the separation of the treatments into an upper solid, and lower liquid layers would reduce homogeneity of the nutrients and complicate mechanical handling. Furthermore, removing the water before feed fabrication would be a considerable expense. For the reasons mentioned, adding water can not be justified.

Successful preservation of hatchery by-products occurred at the higher levels of carbohydrate for CC (15%), CC:SW (16.7%), and SW (20%), when a Lactobacillus, Streptococcus, and Pediococcus culture was added. Apparently, indigenous lactic acid bacteria in the immature chicks and hatchery by-products were either less efficient or were present at lower levels in these studies than in fermentation studies using fresh by-products from more mature birds (Cai et al., 1994a; and Patterson et al., 1994). These authors previously demonstrated successful preservation of poultry carcasses and offal relying completely on indigenous lactic acid generating organisms.

Cockerel chicks and CC:SW in this study fermented with the culture and ≥ 16.7% carbohydrate maintained a low pH (≤ 5.0) and adequate acid level to preserve for up to 21 d. Shell waste was equally maintained at pH < 4.7 with ≥ 20% carbohydrate plus culture for the same period of time. No evidence of spoilage was observed at these carbohydrate and culture levels, e.g., H2S, NH3, odor, or spoiled appearance.

The fermentation treatments employed in Experiments 1 and 2 had measurable effects upon the microbial quality and nutritional constituents of hatchery by-products. Results summarized in Table 3 would suggest that both Salmonella species and E. coli are not a concern at the 21-d sampling time. Other investigators have demonstrated the reduction or elimination of Salmonella, fecal coliforms, and other pathogenic organisms from poultry by-products with fermentation processing (Talkington et al., 1981; Dobbins, 1988; Cai et al., 1994b; and Patterson et al., 1994). The elimination of pathogenic organisms is an important benefit at the hatchery for the biosecurity of breeding stock as well as commercial birds. Additionally, pathogen reduction is an important first step in recycling hatchery by-products into feed ingredients. Small but measurable changes in nutrient concentrations were observed from treatments taken before and after fermentation processing (Table 5). Dry matter was reduced in Experiments 1 and 2 for CC and CC:SW treatments (P < 0.05), but SW levels remained the same after fermentation. Adding water during the initial treatment mixing in Experiment 2 to equalize moisture levels did lessen the degree of DM change during fermentation. The conversion of supplemental carbohydrate to organic acids may explain some of the loss of DM over time. Ohyama et al. (1975) reported that aerobic fermentation with low pH and high lactic acid levels during normal fermentation can be more susceptible to some DM losses. Crude protein levels of the CC treatments were increased approximately 3% (P < 0.05) by fermentation, whereas CP in CC:SW samples was either

![Figure 3](https://academic.oup.com/ps/article-abstract/76/9/1212/1507959)
not influenced or reduced in Experiments 1 and 2, respectively. Concentrations of EE were increased in the CC treatments and Ca in the CC:SW in both Experiments (P < 0.05). Other results for Ca, P, and ash concentration were either not significantly affected or variable. Other investigators have reported similar changes, i.e., increases in DM and CP (P < 0.05) and no change in fat and ash concentrations in poultry carcasses and offal as a result of fermentation processing (Cai et al., 1994b; Cai and Sander, 1995).

The results from the present study indicate that fermentation of hatchery waste may be a viable alternative to current hatchery waste handling practices. Such a process may be less costly than landfilling and generate a stable and biosecure product suitable for rendering or direct feed fabrication. These combined results suggest the nutrients in hatchery by-products can be successfully preserved with fermentation for up to 3 wk with little or no change in nutrient concentration.

REFERENCES


