Evaluation of different selective media and culturing techniques for the quantification of Campylobacter ssp. from broiler litter

A. S. Kiess, H. M. Parker, and C. D. McDaniel

Poultry Science Department, Mississippi State University, Mississippi State, MS 39762

ABSTRACT Poultry is a major reservoir for Campylobacter, the leading cause of foodborne illness in the United States, but how broilers become initially colonized is still under debate. Broiler litter is a potential source, but the best technique for quantifying Campylobacter from litter is still unknown. Therefore, our objectives were to determine if certain media are more selective for quantifying Campylobacter and if enrichment allows for the detection of stressed or viable but nonculturable cells from broiler litter samples. In this trial, 5 media and 2 culturing techniques were used to enumerate Campylobacter from broiler litter. The media used were campy-Line agar (CLA), campy-cefex agar (CCA), modified CCA, Campylobacter agar plates (CAP), and modified charcoal cefoperazone deoxycholate agar. Litter samples were obtained from a commercial broiler house. Each sample was equally divided and diluted 10-fold into peptone, for direct plating, or 4-fold into Campylobacter enrichment broth. Samples diluted in peptone were direct-plated onto each media and incubated under microaerophilic conditions for 48 h at 42°C. Samples diluted in enrichment broth were incubated under the same conditions for 24 h, then further diluted to 10-fold before plating. Plates from enriched samples were incubated for an additional 24 h after plating. After incubation, all plates (direct and enriched) were counted and presumptive positive colonies were confirmed using a Campylobacter latex agglutination kit. Results indicated that there was no difference in the ability of any of the selective media tested to grow Campylobacter. Direct-plated samples had a higher Campylobacter isolation rate compared with enriched samples. The CLA and CAP were able to suppress total bacterial growth better than modified charcoal cefoperazone deoxycholate, modified CCA, and CCA. The CLA and CAP were the only media able to detect total bacterial population shifts over time. In conclusion, it is important before making a final decision on a selective medium to consider the medium’s ability to suppress total bacterial growth as well as isolate Campylobacter.

Key words: broiler litter, selective media, enrichment, Campylobacter, food safety

INTRODUCTION

Campylobacter is the leading cause of bacterial gastroenteritis among individuals in the United States (Newell and Fearnley, 2003). It is associated with livestock (Gregory et al., 1997), wild birds (Craven et al., 2000), domesticated pets (Damborg et al., 2004), as well as humans (Adak et al., 1995; Linton et al., 1997). Although the potential for a Campylobacter outbreak by any one of these sources is high, the major reservoir of this pathogen is considered to be poultry (Oyofo et al., 1989; Gast, 1997). How broilers become initially colonized by Campylobacter is still under debate, but it is known that approximately 83.3% of fresh poultry meat products are contaminated by this organism (Kramer et al., 2000).

Several studies have tried to determine the initial source of Campylobacter colonization in poultry but none have been successful. It has been suggested that vertical transmission may be the route by which broiler chicks initially become colonized (Shanker et al., 1986; Buhr et al., 2002; Hiett et al., 2002, 2003; Sahin et al., 2003; Cox et al., 2005; Vizzier-Thaxton et al., 2006). Other research does not agree with these theories, stating that parent and grandparent flocks could not be identified as the source of offspring colonization (Callcott et al., 2006).

Colonization of broilers has been linked to many differ-
ent on-farm sources such as farm animals (Gregory et al., 1997), rodents (Meerbng and Kijlstra, 2007), wild birds (Craven et al., 2000), hatchery tray liners (Byrd et al., 2007), beetles (Skov et al., 2004), insects (Ekdahl et al., 2005), water (Pearson et al., 1993; Stern et al., 2001), RH (Line, 2006), and subsequent flocks (Van de Giessen et al., 1992; Achen et al., 1998). All of these studies suggest a variety of ways that Campylobacter can be introduced to the broiler flock, but none of them can be confirmed as the initial source of infection.

Litter is a material that broilers are in constant contact with, making this material a likely source for initial colonization. Even though the potential for infection by litter could be high, research investigating this material is sparse. This may be due to the fact that Campylobacter is thought of as a fragile organism that most likely cannot survive in the harsh environment of broiler litter, but evidence of its survival in litter is available (Montrose et al., 1984). The amount of Campylobacter needed to survive and colonize a broiler does not have to be large because as few as 40 viable colony-forming units are enough to colonize a broiler (Cawthraw et al., 1996). Investigations on the survival of Campylobacter in broiler litter biofilms provides evidence to support broiler litter as an initial source of broiler flock colonization (Trachoo et al., 2002; Hanning et al., 2009). It has also been demonstrated that 3 to 4 infected birds have the ability to spread Campylobacter throughout 95% of the flock within 11 d (Van Gerwe et al., 2005). These findings make it reasonable to speculate that 3 to 4 birds out of 20,000 to 25,000 in a flock have the opportunity to come in contact with Campylobacter located within the broiler litter, which can then lead to colonization of the entire flock.

It is of utmost importance to prove that litter is an initial source of colonization as well as to determine the most efficient selective media and culturing technique for isolating low numbers of Campylobacter from broiler litter samples. Several studies have evaluated the effectiveness of different media and culturing techniques on their ability to isolate Campylobacter from various sources. Bolton and Robertson (1982) demonstrated the effectiveness of Skirrow-Preston media in isolating Campylobacter from human feces. Siragusa et al. (2004) evaluated the effectiveness of campy-Line agar (CLA) and campy-cefex agar (CCA) to isolate Campylobacter from broiler carcasses. Potturi-Venkata et al. (2007) demonstrated the effectiveness of 5 different media in the isolation of Campylobacter from live broilers. To our knowledge, there is no information available that describes the best selective media or culturing technique to use when isolating Campylobacter from broiler litter samples. Therefore, the objective of this study was to evaluate 5 different media and 2 culturing techniques to determine the best media for quantifying Campylobacter from broiler litter samples and determine if enrichment allows for the detection of stressed or viable but nonculturable cells.

**Materials and Methods**

**Sample Collection**

Broiler litter samples were collected from a commercial broiler house during 3 separate days of grow-out, d 22, 24, and 36, respectively. A total of 6 random samples were collected on d 22 and 24, but only 3 samples were collected on d 36. Each litter sample consisted of at least 20 g of litter material, which was collected from the top 3.8 cm of the litter bed. The litter samples were collected in sterilized 60-mL Nalgene screw-cap containers (Fisher Scientific, Marietta, GA). Each litter sample was placed on ice immediately after collection and remained on ice until samples were processed. Processing occurred within 1 h of collection.

**Media**

The 5 selective media evaluated to determine their ability to grow Campylobacter from broiler litter were CLA, Campylobacter agar plates (CAP), CCA, modified CCA (mCCA), and modified charcoal cefopazone deoxycholate agar (mCCDA). The composition of each medium is provided in Table 1. All media were prepared and stored in the dark at 4°C no longer than 1 wk before use. All basal media components were autoclaved at 121°C for 15 min. The sterilized basal media were tempered in a Precision Model 2872 digital circulating water bath (Thermo Fisher Scientific, Marietta, GA) at 50 to 55°C before the lysed horse blood and filter-sterilized antibiotics were added. The supplements were allowed to gently mix with the basal media before being poured into 100 × 15 mm sterile polystyrene Petri dishes (VWR International LLC, Suwanee, GA). Campylobacter enrichment broth (CEB) was made following the manufacturer’s instructions.

**Microbial Analysis**

Broiler litter samples were analyzed according to the following procedure. Ten grams of each litter sample was diluted 10-fold in peptone. The diluted samples were then stomached for 30 s at 130 rpm in a Brinkmann/Seward 440C Stomacher (Thermo Fisher Scientific). After stomaching, samples were serially diluted, and 100 μL of each dilution was directly streaked onto each selective media plate in duplicate. Streaked plates were then placed into Mart anaerobic chambers (Mart Microbiology B.V., Drachten, the Netherlands) and flushed with a microaerophilic gas mixture (80% N2, 10% CO2, 5% H2, and 5% O2) using the Mart Anoxomat AN2CTS Mark II System (Mart Microbiology B.V.). Chambers were then placed into a 0.57-m3 Precision Model 815 low-temperature incubator (Thermo Fisher Scientific) for 48 h at 42°C.

In addition to the direct-plate method of samples, enrichment was also performed in an attempt to re-
cover stressed or viable but nonculturable cells. For this procedure, an additional 10 g of litter was diluted 4-fold in CEB. The composition of CEB can be found in Table 1. The diluted CEB samples were also placed into Mart anaerobic chambers and flushed with the same microaerophilic gas mixture as described above. However, CEB samples were only incubated for 24 h at 42°C. After the initial incubation period was complete, CEB samples were removed from the incubator and further diluted to 10-fold using peptone. The CEB samples were then serially diluted and 100 μL of each dilution was streaked onto each selective media plate in duplicate. The streaked plates were then flushed once again with the microaerophilic gas mixture and placed back into the incubator for an additional 24 h.

**Confirmation**

At the end of the 48-h incubation period, both the direct-plated and CEB samples were removed from the incubator. These plates were then counted to enumerate the total amount of bacterial colonies on each plate. These same plates were then reexamined for presumptive positive *Campylobacter* colonies with typical and characteristic colony morphology. Plates that demonstrated the typical and characteristic colony morphology had no more than 5 colonies selected for testing, using a campy-latex agglutination kit for *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari* by Panbio (Panbio Inc., Columbia, MD; Miller et al., 2008). Plates that were confirmed positive for *Campylobacter*

---

**Table 1. Composition of the media used to isolate *Campylobacter* from broiler litter**

<table>
<thead>
<tr>
<th>Medium(^1)</th>
<th>Base</th>
<th>Supplement(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAP(^3)</td>
<td><em>Brucella</em> agar (43 g/L) Ferrous sulfate (25 mg/L) Sodium bisulfite (25 mg/L) Sodium pyruvate (25 mg/L) Deionized water (1 L) Lysed horse blood (70 mL/L) Novobiocin (5 mg/L) Polymyxin B sulfate (0.35 mg/L) Trimethoprim (5 mg/L) Vancomycin (10 mg/L) Cefoperazone (33 mg/L)</td>
<td></td>
</tr>
<tr>
<td>CCA(^3)</td>
<td><em>Brucella</em> agar (43 g/L) Ferrous sulfate (500 mg/L) Sodium bisulfite (200 mg/L) Sodium pyruvate (500 mg/L) Deionized water (950 mL) Lysed horse blood (50 mL/L) Amphotericin B (10 mg/L) Cefoperazone (33 mg/L)</td>
<td></td>
</tr>
<tr>
<td>mCCA(^3)</td>
<td><em>Brucella</em> agar (43 g/L) Ferrous sulfate (500 mg/L) Sodium bisulfite (200 mg/L) Sodium pyruvate (500 mg/L) Deionized water (950 mL) Amphotericin B (10 mg/L) Cefoperazone (33 mg/L)</td>
<td></td>
</tr>
<tr>
<td>mCCDA(^4)</td>
<td>Nutrient broth no. 2 (25 g/L) Bacteriological charcoal (4 g/L) Casein hydrolysate (3 g/L) Sodium deoxycholate (1 g/L) Ferrous sulfate (250 mg/L) Sodium pyruvate (250 mg/L) Agar (12 g/L) Deionized water (1 L) Hemin (10 mg/L) Polymyxin B sulfate (0.35 mg/L) Trimethoprim (5 mg/L) Vancomycin (10 mg/L) Cyclohexamide (100 mg/L) Cefoperazone (33 mg/L)</td>
<td></td>
</tr>
<tr>
<td>CLA(^5)</td>
<td><em>Brucella</em> agar (43 g/L) Ferrous sulfate (500 mg/L) Sodium bisulfite (200 mg/L) Sodium pyruvate (500 mg/L) α-Ketoglutaric acid (1 g/L) Sodium carbonate (600 mg/L) Deionized water (1 L) Lysed horse blood (50 mL/L) Cefoperazone (20 mg/L) Vancomycin (20 mg/L)</td>
<td></td>
</tr>
<tr>
<td>CEB(^6)</td>
<td>Enzymatic digest of animal tissue (10 g/L) Lactalbumin (5 g/L) Yeast extract (5 g/L) Sodium chloride (5 g/L) Hemin (10 mg/L) Sodium pyruvate (500 mg/L) α-Ketoglutaric acid (1 g/L) Sodium metabisulfite (500 mg/L) Sodium carbonate (600 mg/L) Deionized water (1 L) Triphenyltetrazolium chloride (200 mg/L) Polymyxin B sulfate (250 mg/L) Cefoperazone (33 mg/L)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)CAP = *Campylobacter* agar plates; CCA = campy-cefex agar; mCCA = modified campy-cefex agar; mCCDA = modified charcoal cefoperazone deoxycholate agar; CLA = campy-Line agar; CEB = *Campylobacter* enrichment broth.

\(^2\)All supplements were purchased from Sigma-Aldrich (St. Louis, MO) except for the lysed horse blood, which was obtained from Lampire Biological Laboratories (Pipersville, PA). Vancomycin [Thermo Fisher Scientific Inc. (Acros Organics product), Fair Lawn, NJ] and CEB supplements were purchased from Oxoid Ltd. (New York, NY) as Bolton Broth Selective Supplement (SR018E).

\(^3\)All base ingredients were purchased from Fisher Scientific Inc. (Fair Lawn, NJ) except for sodium pyruvate, which was purchased from Sigma-Aldrich.

\(^4\)Campylobacter blood-free selective agar (CM0739) was purchased from Oxoid Ltd.

\(^5\)All base ingredients were purchased from Fisher Scientific Inc. except for sodium pyruvate and α-ketoglutaric acid, which were purchased from Sigma-Aldrich.

\(^6\)Campylobacter enrichment broth (7526) was purchased from Acumedia (Lansing, MI).
were set aside until all plates were confirmed, and then they were recounted to determine the total number of *Campylobacter*-positive colonies on the plate.

**Statistical Analysis**

A randomized complete block design was used for the analysis with a split plot in time (age). Bacterial counts were converted to base-10 logarithm colony-forming units per gram of litter material. Because every litter sample was examined on each medium, litter sample represented blocks. A $2 \times 5$ factorial arrangement of treatments with 2 plating techniques and 5 media was used. The GLM procedure of SAS was used with Fisher’s protected least significant difference to separate treatment means at $\alpha = 0.05$ (Steel and Torrie, 1980).

**RESULTS AND DISCUSSION**

It is unclear whether or not enriching broiler litter samples with CEB is a useful culturing technique for isolating *Campylobacter*. Certain studies have indicated that enrichment does not enhance the recovery of *Campylobacter* from poultry fecal, cecal, crop, and broiler carcasses (Martin et al., 1983; Musgrove et al., 2001). Others have determined that when enrichment broth is used, the isolation rate of *Campylobacter* from human feces can be increased (Bolton and Robertson, 1982). Although enrichment of samples is debatable, due to the harsh nature of broiler litter, it was decided that an enrichment procedure would be used in this study. The results of this study indicated that direct plating significantly increased the chance of isolating *Campylobacter* when compared with using CEB, 2.1 compared with 0.15 mean log cfu/g of litter, respectively (Figure 1). These findings are in agreement with previous poultry research, in which fecal, cecal, crop, and carcasses were enriched but the enrichment did not provide an advantage in the detection of *Campylobacter* (Martin et al., 1983; Musgrove et al., 2001).

In an attempt to better understand the magnitude by which direct plating improved the isolation rate when compared with enrichment, the ratio of *Campylobacter* to total bacterial growth was calculated and analyzed. The results of this analysis demonstrated that 37% of the total bacterial growth on samples directly plated was *Campylobacter*. However, for enriched samples, *Campylobacter* only accounted for 2% of the total bacterial growth (Figure 2). The differences in the isolation rate of *Campylobacter* between these 2 culturing techniques indicate that enrichment of broiler litter samples using CEB does not enhance the growth of stressed or viable but nonculturable cells.

Even though these findings suggest that enrichment with CEB does not enhance the isolation rate, a different enrichment broth may be able to increase the isolation rate of *Campylobacter* from litter samples. Bolton and Robertson (1982) demonstrated that when human fecal samples were enriched in Preston broth (283/630) the isolation rate was much higher than fecal samples that were direct-plated on Skirrow (85/630) or Preston (183/630) media alone. Martin et al. (1983) stated that the reason they may not have seen an increase in the isolation rate of *Campylobacter* from the enriched samples in their study could have been due to the fact that the quantity of *Campylobacter* in their poultry fecal samples may not have been large enough to allow for the recovery by direct plating. If this were the case, then enrichment should increase the isolation rate of *Campylobacter* from broiler litter because broiler litter is a harsh environment for *Campylobacter* and low quantities of the organism are likely to be recovered. Perhaps another enrichment broth may be more successful at isolating *Campylobacter* from broiler litter.

**Figure 1.** Total bacterial and *Campylobacter* growth (mean log cfu/g of litter) from broiler litter samples that were either direct-plated onto 5 different selective media or enriched in *Campylobacter* enrichment broth for 24 h before plating on the 5 different selective media. Black columns represent total bacterial growth. White columns represent *Campylobacter* growth. Columns with different letters indicate differences in *Campylobacter* growth over the different culturing techniques ($P < 0.05$).

**Figure 2.** The ratio of *Campylobacter* colonies to total bacterial colonies (expressed as a percentage) from broiler litter samples that were either direct-plated onto 5 different selective media or enriched in *Campylobacter* enrichment broth for 24 h before plating onto the 5 different selective media. The black column represents samples that were direct-plated. The white column represents samples that were enriched. Columns with different letters indicate differences in culturing techniques ($P < 0.0001$).
SELECTIVE MEDIA FOR ISOLATING CAMPYLOBACTER

Several selective media are available for the isolation of Campylobacter from clinical (Gun-Munro et al., 1987; Endtz et al., 1991) as well as poultry sources (Stern et al., 1992; Oyarzabal et al., 2005; Potturi-Venkata et al., 2007). These different media have been tested on a wide range of samples including human feces as well as poultry feces, cecal, crop, carcass, and carcass rinses, but the most efficient medium for growing Campylobacter from broiler litter has not been determined. Therefore, in this study, 5 different selective media for isolating Campylobacter were compared.

No differences were detected among any of the 5 selective media for their ability to grow Campylobacter (Figure 3). This finding was surprising because others have found different selective media to be better suited for the growth of Campylobacter from different clinical and poultry sources. When isolating Campylobacter from poultry rinse samples, it has been suggested that CCA and mCCA are able to grow more Campylobacter than CLA and mCCDA (Oyarzabal et al., 2005). Campy-cefex agar has been determined to be a better medium for growing Campylobacter from carcass samples when compared with mCCDA and campy-blood agar (Stern et al., 1992). It has also been determined that mCCA is able to grow Campylobacter better from poultry cecal and fecal samples than mCCDA, CLA, CAP, or Campylobacter agar base (Potturi-Venkata et al., 2007).

Even though there was no difference in the growth of Campylobacter using all 5 media in this study, certain media were able to suppress the growth of bacterial contaminants better than others, indicating that they were a better isolation medium. This is illustrated in Figure 3. The CLA agar was determined to isolate Campylobacter the best out of all of the media tested. It was able to suppress the total bacterial count (3.04 mean log cfu/g of litter) better compared with all of the other media tested and was still able to grow Campylobacter at a level that was not significantly different from the other media tested. The CAP agar also suppressed the total bacterial count (4.24) compared with CCA (6.32), mCCA (7.05), and mCCDA (7.36) while providing a level of Campylobacter detection that was not different from the other media tested. The addition of different as well as more selective supplements to CLA and CAP is most likely responsible for suppressing the growth of contaminating bacteria on the plates. Even though CCA, mCCA, and mCCDA were able to grow Campylobacter from the broiler litter samples, they also allowed for the growth of approximately 2 to 3 mean log cfu/g more total bacteria than the CLA or CAP. This level of bacterial contaminants on the plate made isolation and identification of suspect Campylobacter colonies more difficult, therefore allowing for the opportunity to overlook those Campylobacter colonies that were present.

An interaction between media and culture technique was also detected for total bacterial growth. The CLA and CAP indicated that enrichment of broiler litter yielded significantly fewer total bacteria than broiler litter that had been direct-plated. This result was the exact opposite than what was found for mCCDA, mCCA, and CCA, in which the enriched broiler litter had significantly higher total bacterial counts than broiler litter that was direct-plated (Figure 4). A possible explanation for this interaction may be due to the selective supplements used in the enrichment broth and the selective media. Campylobacter enrichment broth, mCCDA, CCA, and mCCA all use the same set of selective supplements to suppress the growth of contaminating bacteria. If the contaminating bacteria formed a resistance to the supplements in the CEB, it may then be possible for those bacteria to overgrow mCCDA, CCA, and mCCA once plated. In the case of CLA and CAP, a different set as well as additional selective supplements are used. These different and additional supplements may allow CLA and CAP to suppress the contaminating bacteria better than mCCDA, CCA, and mCCA due to the fact the contaminating bacteria did not have the opportunity to form a resistance to their selective supplements. This demonstrates the importance of carefully choosing a media that supports the culture technique being used when sampling broiler litter. If an enrichment broth is identified that increases the growth of Campylobacter from broiler litter, then using a selective medium with different selective supplements will most likely provide the best opportunity for isolating the Campylobacter.

There was also a media × time interaction for total bacterial growth. The results of this interaction indicated that CAP and CLA are capable of detecting changes in total bacterial populations over time, whereas mCCDA, mCCA, or CCA were not. This is apparent due to the reduction of total bacteria growth detected on d
36 of production compared with that detected on d 24 by CLA and CAP media but not by mCCDA, mCCA, or CCA media (Figure 5). Although literature defining the microbial populations in poultry litter as well as the microbial growth curve is limiting, it is speculated that shifts do occur over time. In the literature that is available, changes in the microbial populations within a house have been attributed to litter pH, moisture, and temperature (Lovanh et al., 2007). As broilers age, changes in house environment are inevitable even though management practices are implemented to try and reduce their effect. Due to these slight changes in the house environment over time, the possibility for microbial populations to change, increase, or decrease is possible. Kiess et al. (2007) demonstrated that Campylobacter colonization in commercial turkeys changed over time. Peak colonization was determined to occur at 3 wk of age, and as the flock aged, the quantity of recoverable Campylobacter decreased.

In the case of broiler litter, CLA and CAP media are the most effective at suppressing contaminating bacterial growth when using either culture technique. This is important because all 5 media are capable of growing the same amount of Campylobacter but direct plating provides a significantly higher number of Campylobacter colonies when compared with enrichment. If mCCDA, mCCA, or CCA are chosen as the media to use for isolating Campylobacter from broiler litter, one would not want to enrich the broiler litter sample with CEB due to the fact it will increase the total number of bacteria on the plate, possibly making it more difficult to identify suspect Campylobacter colonies.

In conclusion, selecting the appropriate selective media and culturing technique for isolating Campylobacter is important. In the case of broiler litter, CLA or CAP appear to be 2 selective media that provide the best opportunity for isolating Campylobacter. This was not due to their ability to increase Campylobacter’s growth rate but rather due to their ability to reduce the amount of contaminating bacterial growth on the selective media plate. The opportunity broiler litter provides for bacteria other than Campylobacter to survive can be attributed to the broiler litter environment, which is very different than the environment Campylobacter comes in contact with, in or on the bird. It is also important to consider the appropriate selective media for isolating Campylobacter when enrichment is determined necessary for isolation. Although our results demonstrated that direct plating significantly increased the isolation rate of Campylobacter, if an enrichment procedure is used, CLA or CAP would provide the most efficient isolation rate of Campylobacter. This is demonstrated by the low amount of contaminating bacterial growth detected on CLA and CAP after broiler litter had been enriched with CEB. Selecting the right selective media also allows for the detection of bacterial population shifts over time. The CLA and CAP were again 2 selective media that provided this opportunity, whereas other selective media tested were not. Overall, the results of this study demonstrate that for a selective medium and culture technique to be efficient at isolating Campylobacter from broiler litter, its ability to suppress the amount of total bacterial growth must be taken into account before making a final decision.

REFERENCES


Figure 4. Interaction between selective media and culturing technique for total bacterial growth (mean log cfu/g of litter). Black columns represent the total bacterial growth for broiler litter samples that were direct-plated. White columns represent the total bacterial growth for broiler litter samples that were enriched in Campylobacter enrichment broth (CEB) before plating. Selective media include modified charcoal cefoperazone deoxycholate agar (mCCDA), modified campy-cefex agar (mCCA), campy-cefex agar (CCA), Campylobacter agar plates (CAP), and campy-Line agar (CLA). Columns with different letters represent differences in plating method and selective media (P < 0.0001).

Figure 5. Interaction between selective media and day, bird age at time of collection, for total bacterial growth (mean log cfu/g of litter). Black columns represent the total bacterial growth on d 22 of grow-out. Gray columns represent the total bacterial growth on d 24 of grow-out. White columns represent total bacterial growth on d 36 of grow-out. Selective media include modified charcoal cefoperazone deoxycholate agar (mCCDA), modified campy-cefex agar (mCCA), campy-cefex agar (CCA), Campylobacter agar plates (CAP), and campy-Line agar (CLA). There was an interaction between selective media and day of grow-out for CAP and CLA. Columns with different letters represent differences in selective media over bird age at collection (P < 0.05).


