INTRODUCTION

Campylobacter jejuni is one of the leading causes of human bacterial foodborne gastroenteritis worldwide including the United States (Scallan et al., 2011). An estimated 400 to 500 million cases of human campylobacteriosis occur globally and approximately 2 million cases are estimated to occur annually in the United States (Ruiz-Palacios, 2007). Handling and consumption of raw or undercooked poultry meat is considered one of the main risk factors for Campylobacter-associated gastroenteritis in humans (Humphrey et al., 2007), which manifests clinically as self-limiting diarrhea, generally lasting for 2 to 5 d. Campylobacter jejuni is a commensal microorganism in chickens and mainly resides in the lower intestine with cecum and cloacal crypts being the major site of colonization (Beery et al., 1988; Achen et al., 1998). Campylobacter jejuni is excreted via feces and spreads rapidly within whole flock once infection is established (Sahin et al., 2002). About 90% commercial broilers are naturally colonized with $10^5$ to $10^9$ cfu of C. jejuni per gram of intestinal contents during slaughter (Stern et al., 2001). Carcasses originating from C. jejuni-free flocks can be cross-contaminated with C. jejuni during poultry processing if they are processed together with C. jejuni-infected flocks (Genigeorgis et al., 1986). Therefore, preslaughter strategies for reducing C. jejuni colonization in chicken intestine may provide a useful tool for the postslaughter reduction of C. jejuni contamination in chicken carcasses.
Various on-farm intervention methods have been implemented to reduce *C. jejuni* colonization in chickens. These include introduction of competing microbial populations into newly hatched chicks (Mead et al., 1996; Hakkinen and Schneitz, 1999), chlorination of drinking water (Stern et al., 2002), vaccination (Wyszyńska et al., 2004; Buckley et al., 2010), feed withdrawal immediately before slaughter (Byrd et al., 1998), or selective breeding of *C. jejuni*-free poultry (Skirrow, 1982). Most of these approaches have varying efficacy and as a result these have not been implemented widely in reducing *C. jejuni* colonization in poultry. In the last decade, the research on *C. jejuni* has focused on identifying genetic factors or proteins underlying colonization of this commensal organism in chickens. Several in vitro and in vivo studies have revealed that *C. jejuni* expresses proteins that play an important role in intestinal colonization in chickens. For instance, studies using genetically defined mutants of *C. jejuni* or purified proteins have revealed that FlmA (flagellar proteins), CadF (*Campylobacter* adhesion to fibronectin), FlpA (fibronectin binding protein A), and Peb1A (*Campylobacter* putative adhesion) proteins contribute to the intestinal colonization of *C. jejuni* in chickens and mouse (Nachamkin et al., 1993; Flanagan et al., 2009). Additionally, disruption of jlpA, encoding a surface exposed lipoprotein JlpA (*Jejuni* lipoprotein A), was shown to reduce adherence of *C. jejuni* to human HEp-2 epithelial cells (Jin et al., 2001). Finally, membrane-associated proteins such as CmC (Campylobacter multidrug efflux C) and PorA (major outer membrane protein or MOMP) are immunogenic and have been shown to reduce *C. jejuni* colonization in chicken and mouse (Lin et al., 2003; Huang et al., 2007; Islam et al., 2010). In addition to being involved in colonization, all of the above proteins are highly conserved among different *C. jejuni* strains and are exposed on the bacterial surface, potentially making them amenable to binding and neutralizing effects of antibodies. For the purpose of this study, we refer these proteins as *C. jejuni* colonization-associated proteins (CAP). Most importantly, it has been reported that the maternal antibodies collected from chickens born from *C. jejuni*-infected parent stocks contain antibodies reactive to the majority of the above CAP (Shaof-Sweeney et al., 2008). It is believed that these maternal antibodies play an important role in protection against *C. jejuni* colonization until chickens reach 2 wk of age (Sahin et al., 2003; Cawthraw and Newell, 2010).

It is known that immunization of hens with bacterial whole-cell lysate or purified proteins results in the production of egg yolks enriched with antibodies (IgY) against proteins of interest. Several studies have evaluated the usefulness of such chicken egg-yolk-derived IgY to prevent or eliminate a variety of foodborne and other avian pathogens such as *Salmonella* Enteritidis, *Salmonella* Typhimurium, *C. jejuni*, avian pathogenic *Escherichia coli*, *E. coli* O157:H7, *Eimeria tenella*, avian influenza subtype H9N2 (Eterradossi et al., 1997; Tsubokura et al., 1997; Fulton et al., 2002; Lee et al., 2002; Gurtler and Fehlhaber, 2004; Kariyawasam et al., 2004; Kassaify and Mine, 2004; Rahimi et al., 2007; Mahdavi et al., 2010). Typically, IgY is produced by hens immunized with specific antigens or whole cells and these antibodies are passively transferred to the egg yolk in a high concentration (~100 mg per egg; Akita and Nakai, 1993a). Because of relative stability of IgY in acidic pH and to the proteolytic activity of pepsin and trypsin, chicken IgY represents an attractive alternative to antibiotic therapy in the preslaughter control of foodborne pathogens (Schade et al., 2005; Chalghoumi et al., 2009a). In addition, it is believed that passive immunization via the administration of pathogen-specific chicken egg-yolk-derived IgY could reduce the use of antibiotics in food animals, which may result in the reduction of bacterial resistance to antibiotics (Schade et al., 2005). Avian IgY can be purified as a water-soluble fraction from egg yolks obtained from immunized hens and administered orally or via drinking water (Akita and Nakai, 1993b; Stalberg and Larsson, 2001; Gee et al., 2003) or egg yolks can be directly freeze- or spray-dried to produce hyper-immunized egg yolk powder (HEYP) for administration via feed (Kassaify and Mine, 2004; Cook et al., 2007). The addition of protein-rich egg yolk powder also increases the resistance of IgY to acid and proteolytic inactivation and increases its heat stability, thereby making it feasible to use such a preparation direct for oral administration via feed (Schade et al., 2005).

Limited studies have investigated the use of egg-yolk-derived IgY against *C. jejuni* colonization in chickens (Tsubokura et al., 1997; Kassaify and Mine, 2004). In the first study, Tsubokura et al. (1997) used water-soluble purified preparations of polyclonal IgY produced from egg yolks of chickens immunized with the inactivated whole-cell lysate of *C. jejuni* for prophylactic and therapeutic treatment of chickens experimentally infected with *C. jejuni*. In the prophylactic trial, 0.5 g of purified water-soluble IgY preparation was preincubated with 10⁶ cfu of *C. jejuni* and administered orally as single dose to 14-d-old chickens. In the therapeutic experiments, *C. jejuni*-infected chickens were given 0.2 g of the same IgY preparation, as a single dose, 4 d postinfection. These authors reported some reduction in the mean number of *C. jejuni* in the prophylactic trial, whereas therapeutic treatment trial showed a 2-log reduction (from the initial 10⁷ to 10⁵ cfu per gram of feces) after oral administration with anti-*C. jejuni* IgY. This effect lasted for a brief period because the *C. jejuni* counts returned to pretreatment levels with 10⁵ bacteria per gram of feces after 24 and 48 h, and 10⁶ cfu at 72 h after administration of IgY (Tsubokura et al., 1997). In a second study, Kassaify and Mine (2004) examined the effects of feed supplemented with nonimmunized egg yolk powder ( NEYP) on intestinal colonization of *C. jejuni* in laying hens. Laying hens were orally challenged with 10⁷ cfu of *C. jejuni* and treated continuously for 4 wk postinfection using feed supple-
mented with NEYP obtained from nonimmunized hens at a concentration ranging from 1 to 10% (wt/wt). No reduction in colonization of *C. jejuni* was observed upon feeding with 1 and 2.5% (wt/wt) NEYP. A moderate reduction in *C. jejuni* mean log<sub>10</sub> count in the fecal samples (from 4.1 cfu/g to ~2.1 cfu/g) was observed after treatment with 5 and 7.5% NEYP, whereas the mean log<sub>10</sub> count in the fecal samples of the groups fed with 10% (wt/wt) NEYP supplement decreased from 4.1 to ~1.4 cfu/g by the end of the 4-wk study period, suggesting that long-term treatment with 10% NEYP may result in at least some reduction of *C. jejuni* colonization in chickens.

The mechanism of protection induced by the water-soluble purified preparations of IgY raised against *C. jejuni* whole-cell lysates used by Tsubokura et al. (1997) is not known, but appears to be due to the potentially neutralizing activity of polyclonal IgY against multiple *C. jejuni* antigens including proteins involved in colonization. On the contrary, the effects of NEYP on *C. jejuni* colonization as reported by Kassaify and Mine (2004) is presumably due to the actions of both nonspecific IgY and the nonspecific antibacterial factors that may be present in egg yolk. These studies raise a possibility that the use of egg yolk powder enriched with IgY specifically directed against *C. jejuni* colonization-associated proteins or CAP may have synergistic effects and provide better protection against *C. jejuni* colonization. Our hypothesis in this study was that oral administration of HEYP enriched with anti-*C. jejuni* CAP-specific IgY will be efficacious in reducing the *C. jejuni* loads in the intestine of chickens. Previously, we successfully produced HEYP against 5 CAP including FlaA, CadF, FlpA, CmeC, and MoMP (Al-Adwani et al., 2013). In this study we produced 2 additional HEYP against JlpA and PeblA proteins. The objective of this study was to test the efficacy of HEYP enriched with IgY against 7 *C. jejuni* CAP to eliminate or reduce *C. jejuni* colonization in chickens.

**MATERIALS AND METHODS**

**Production of Egg-Yolk-Derived Anti-*C. jejuni* CAP-Specific IgY**

Previously, we produced chicken egg-yolk-derived IgY against 5 *C. jejuni* CAP including CadF, CmeC, FlaA, FlpA, and MOMP (Al-Adwani et al., 2013). Similarly, egg-yolk-derived IgY against JlpA and PeblA CAP were produced following the similar methods described previously (Al-Adwani et al., 2013). Briefly, recombinant *C. jejuni* CAP were expressed in the *E. coli* expression host and purified by cobalt-column affinity chromatography. Each recombinant CAP (~100 μg) resuspended in 0.5 mL of PBS was mixed with 0.5 mL of Freund’s complete adjuvant (Sigma, St. Louis, MO). At 35 wk of age, *C. jejuni*-free hens were intramuscularly injected with 0.25 mL of the emulsion at 4 sites in the breast muscle (total 1 mL/bird). Booster immunizations were given at 3, 4, and 6 wk after first immunization with Freund’s incomplete adjuvant (Sigma) emulsified with ~100 μg of each recombinant protein (Al-Adwani et al., 2013). Egg yolks collected from immunized hens were lyophilized to obtain egg yolk powder enriched with JlpA- and PeblA-specific antibodies. The water-soluble fraction of HEYP and NEYP collected from all the immunized and nonimmunized hens, respectively, were tested for the presence of antibodies against recombinant *C. jejuni* CAP by indirect enzyme-linked immunosorbent assay as described previously (Al-Adwani et al., 2013).

**Preparation of Feed**

The HEYP against each *C. jejuni* CAP, NEYP, and a cocktail containing equal parts of 5 or 7 HEYP were mixed with feed (Flock Raiser Sunfresh Crumble feed, Purina Mills, St. Louis, MO) at the final concentration of 10% (wt/wt). Treated feed containing egg yolk powder was freshly prepared for each experiment.

**Chicken Experiments**

Three experiments were performed in this study. All the experimental procedures were performed in accordance with the protocols approved by the Washington State University-Institute for Animal Care and Usage Committee. Specific-pathogen-free (SPF) fertile eggs were obtained from Sunrise Farms Inc. (Catskill, NY) and hatched at Washington State University animal facility. One-day-old chickens were transferred to chicken experiment facility and divided into different groups. Individual chick groups were placed into isolation cages on wire mesh (Hazleton Systems Inc., PA). Water and feed were provided ad libitum until the end of the experiment. Before infecting the chickens, cloacal swabs were collected from all birds and tested for *C. jejuni* by selective plating and identification following standard protocols for *C. jejuni* isolation as described previously (Al-Adwani et al., 2013). All the experimental birds were found *C. jejuni* negative. In all experiments, chickens were orally inoculated with *C. jejuni* strain F38011, which is known to colonize chicken intestine (Young et al., 1999; Ziprin et al., 1999; Konkel et al., 2007). *Campylobacter jejuni* F38011 strain was grown under microaerophilic conditions at 42°C on Campy-Cefex agar plate (Hardy Diagnostics, Santa Maria, CA) for 48 h before infection. The inoculum was prepared by making *C. jejuni* suspension in Bolton’s broth with selective supplement (Oxoid Limited, Hampshire, UK), serially diluted and plated onto Campy-Cefex plates for enumeration of cfu.

**Experiment 1.** Ninety-six 1-d-old SPF chickens were subdivided into 8 groups of 11 chicks each and 1 group with 8 chicks. At d 3, 8 groups of chicks were inoculated orally with 10⁸ cfu of *C. jejuni*. At d 7, cloacal swabs were taken from each bird using sterile cotton-tipped swabs (Fisher Scientific, Waltham, MA) and suspended
in 1 mL of Bolton broth with selective supplement (Oxoid Limited) to determine the presence of \textit{C. jejuni}. From d 12 to 14 (3 d), 5 groups of chickens were treated using feed supplemented with HEYP containing IgY against CadF, CmeC, FlaA, FlpA, and MOMP proteins, respectively. In addition, egg yolk powder was suspended in sterile H2O and vortexed vigorously to form a homogenized 10% solution and then orally administered daily to each group as a single oral dose (0.5 mL/bird) per day, from d 12 to 14. The sixth group was treated with the mixture containing equal amounts of the 5 HEYP and the seventh group was treated with NEYP. The eighth group served as nontreated positive control, whereas the ninth group was inoculated with Bolton broth and served as nontreated negative control. At d 14, all birds were euthanized by CO2 asphyxiation; ceca were aseptically collected and processed for isolation and enumeration of \textit{C. jejuni}.

\textbf{Experiment 2.} Twenty-four 1-d-old SPF chickens were equally distributed into 4 groups (n = 6 chicks/group). From d 1 of age, the first group was treated with feed supplemented with cocktail containing equal amounts of 7 HEYP against CadF, CmeC, FlaA, FlpA, and MOMP, JlpA, and PeblA, whereas the second group was treated with NEYP. The remaining 2 groups served as nontreated positive control and nontreated negative controls, respectively. To ensure homogeneous mixing of HEYP and NEYP with feed, 5% (vol/wt) canola oil (IGA, Chicago, IL) was added to the feed. Except for the nontreated negative control group, chickens in all other groups were orally inoculated with \num{1E8} cfu of \textit{C. jejuni} at d 15. Bolton broth was administered to chickens in the nontreated negative control group. To confirm \textit{C. jejuni} colonization, cloacal swabs were taken from all birds using sterile cotton swabs at d 19 and processed for isolation of \textit{C. jejuni}. All birds were euthanized by asphyxiation with carbon dioxide at d 21 (i.e., d 7 postinoculation, \textit{pi}); ceca were aseptically collected and processed for enumeration of \textit{C. jejuni}.

\textbf{Experiment 3.} For this experiment, chickens were divided into prophylaxis and treatment groups. The main difference between the 2 groups was that chickens in prophylaxis groups received treated feed during the entire study period, whereas chickens in the treatment groups received treated feed only after \textit{C. jejuni} infection (i.e., postinfection treatment). Sixty 1-d-old SPF chickens were equally divided (n = 10/group) into 6 groups of which 2 served as prophylaxis groups, 2 served as treatment groups, 1 served as nontreated negative control, and 1 served as nontreated positive control. Within prophylaxis and treatment groups, the first subgroup was treated with feed supplemented with equal amounts of 7 HEYP containing IgY against CadF, CmeC, FlaA, FlpA, MOMP, JlpA, and PeblA proteins and the second subgroup was given feed supplemented with NEYP. To ensure homogeneous mixing of egg yolk powder, the feed was premixed with canola oil (IGA, Chicago, IL) at a final concentration of 5% (vol/wt). All groups except nontreated negative control were orally inoculated with \num{1E5} \textit{C. jejuni} at d 4. Bolton broth was administered to chickens in nontreated negative control. Cloacal swabs were taken from all chickens using sterile cotton swabs at d 10 for confirmation of \textit{C. jejuni} colonization. At d 14, all birds were euthanized by CO2 asphyxiation, and ceca were aseptically collected and processed for enumeration of \textit{C. jejuni} counts.

\textbf{Bacteriological Examination and Enumeration of \textit{C. jejuni}}

In each experiment, 100 μL of cloacal swab suspensions were directly plated onto Campy Cefex agar plate (Hardy Diagnostics Inc., San Diego, CA) and incubated at 42°C with 10% CO2 for 48 h.

For enumeration of \textit{C. jejuni} at the end of each experiment, individual ceca was weighed and homogenized in 3.0 mL of Bolton’s broth containing selective supplement. The samples were serially diluted (10^{-1} to 10^{-8}) in Bolton’s broth, and 10 μL of each diluent was plated onto Campy Cefex agar plates followed by incubation in microaerophilic condition at 42°C for 36 h. The cfu per gram of ceca was calculated by adjusting the volume of diluents and weight of ceca. Presumptive \textit{C. jejuni} colonies isolated from Campy Cefex plates were confirmed to be \textit{C. jejuni} by PCR amplification of the \textit{C. jejuni}-specific \textit{hipO} gene using primers and PCR procedure described previously (Persson and Olsen, 2005).

\textbf{Data Analysis}

Mean absorbance values of water-soluble fraction from HEYP and NEYP were compared using 1-way ANOVA followed by Tukey’s test (Prism5 GraphPad Inc., San Diego, CA). Bacterial counts were converted to \(\log_{10}\) cfu, and means ± SE were determined per gram of ceca. For each experiment, the statistically significant difference in the counts (cfu) between groups was calculated by ANOVA with Dunnett’s multiple comparison post hoc test (NCSS 2007, Kaysville, UT). A \(P < 0.05\) was considered as statistically significant.

\textbf{RESULTS AND DISCUSSION}

In this study the efficacy of HEYP containing 7 anti-CAP-specific IgY and NEYP were evaluated against \textit{C. jejuni} colonization in chickens. Feed supplemented with 10% HEYP were used to treat the chickens in 3 individual experiments and the HEYP were specific to 7 important CAP that were previously reported to be associated with \textit{C. jejuni} colonization in chickens.

In the first experiment, all the chickens challenged at 3 d of age were colonized with \textit{C. jejuni} by 7 d of age, as confirmed by positive culture from cloacal swabs. To determine the efficacy of short-term passive immuno-
therapy using HEYP enriched with anti-C. jejuni CAP-specific IgY, the chickens were treated, from d 12 to 14 (i.e., 9 d pi until 12 d pi), with feed supplemented with 10% (wt/wt) of HEYP against CadF, CmeC, FlaA, FlpA, and MoMP proteins individually and in the form of a cocktail containing equal parts of the above 5 HEYP. The mean colonization counts (log_{10} cfu ± SE) of C. jejuni of HEYP-CadF, HEYP-CmeC, HEYP-FlaA, HEYP-FlpA, HEYP-MOMP, HEYP-cocktails, NEYP, and positive control groups were 8.3 ± 0.2, 8.2 ± 0.3, 8.8 ± 0.2, 8.9 ± 0.2, 8.5 ± 0.2, 8.0 ± 0.2, 8.2 ± 0.2, and 8.5 ± 0.1 cfu/g, respectively (Figure 1). These results revealed that irrespective of the treatment with individual HEYP or cocktail of 5 HEYP, there were no significant differences (P < 0.05) in C. jejuni cecal colonization in treated groups compared with the untreated positive control group (Figure 1). These results are in contrast to the previously published report in which oral administration of a single dose of purified water-soluble fraction containing polyclonal anti-C. jejuni IgY resulted in at least a short-term (24 to 48 h) reduction of C. jejuni intestinal colonization by 2 logs (from the initial 10^7 to 10^5 cfu per gram of feces; Tsubokura et al., 1997). Additionally, the cecal C. jejuni counts from NEYP-treated chickens (8.2 ± 0.2) in this study were not significantly different from the nontreated positive control (8.5 ± 0.1) or any of the HEYP-treated groups. These results are also in contrast with Kassaify and Mine (2004), who reported that in-feed treatment with 10% NEYP starting from 1 wk postinfection resulted in 1.5 to 3 log reduction in fecal C. jejuni counts. These results suggest that short-term passive immunotherapy with individual or mixture of anti-CAP specific HEYP or NEYP at the dose and regimen used in this study was not sufficient to significantly reduce the cecal C. jejuni counts in chickens that are heavily colonized with C. jejuni.

Therefore, we conducted a second experiment where chickens were prophylactically treated via in-feed supplemented with a cocktail of HEYP or NEYP from d 1 onward until 3 wk of age. For this experiment we produced 2 additional HEYP against Peb1A and JlpA proteins as described previously (Al-Adwani et al., 2013). Indirect ELISA using purified Peb1A and JlpA pro-
teins as an antigen revealed that the level of anti-CAP-specific IgY in both HEYP was significantly higher \((P < 0.0001)\) compared with the NEYP (Figure 2), suggesting that similar to other HEYP reported previously, these 2 HEYP were also enriched with anti-Peb1A and anti-JlpA antibodies. Consequently we prepared a mixture containing equal amounts of 7 HEYP, which was administered orally (10% wt/wt) via feed from d 1 of age until the end of the experiment. Chickens were orally challenged with \(10^8\) cfu of \(C.\) jejuni on d 15 and cecal \(C.\) jejuni counts were measured on d 21 (i.e., 7 d pi). The mean log\(_{10}\) cfu of \(C.\) jejuni from groups treated with HEYP (8.92 ± 0.2) and NEYP (7.91 ± 1) were not significantly different from the nontreated positive control (8.8 ± 0.3; Figure 3). These results suggest that long-term prophylactic treatment with either HEYP or NEYP at the dose and regimen used in this experiment was not sufficient to significantly reduce cecal \(C.\) jejuni counts. Interestingly, Kassaify and Mine (2004) reported that in-feed prophylactic treatment with 10% NEYP for 4 wk before infection resulted in 3 to 4 log reduction in fecal \(C.\) jejuni counts. Although our results are in contrast to the report of Kassaify and Mine (2004), it is important to note that in the current study birds were treated prophylactically only for 2 wk. Overall, the results of this second trial suggest that prophylactic treatment with HEYP or NEYP at the dose and regimen used in this experiment were not sufficient to significantly reduce the cecal \(C.\) jejuni colonization in chickens.

The results of first 2 trials indicated that when chickens were challenged with \(10^8\) cfu of \(C.\) jejuni strain, the ceca from challenged chickens were colonized consistently with high level of \(C.\) jejuni (range = 7.9081 ± 1.1 to 9.48 ± 0.15 cfu/g). In case of untreated positive control, the average intestinal \(C.\) jejuni counts in our study was 4 to 5 log\(_{10}\) higher when compared with those reported by Kassaify and Mine (2004). Doses of \(C.\) jejuni as low as \(10^4\) or \(10^5\) cfu have been reported to result in consistent colonization in 1-d-old chicks (Stern et al., 1988). Therefore, to rule out if the high dose used in our experiments may have counteracted the neutralizing action of HEYP, we reduced the challenge dose to \(10^5\) cfu per chicken in the third experiment. In this experiment the chickens in both prophylactic and treatment groups were challenged with \(C.\) jejuni at d 4, but with a lower dose (\(10^5\) cfu per chicken). The cloacal swabs from all infected chickens were tested positive for \(C.\) jejuni at d 10 (i.e., 6 d pi). At d 14, the \(C.\) jejuni counts in the ceca from both prophylactic (9.38 ± 0.16) and treatment group (9.24 ± 0.2) treated with HEYP were not significantly different compared with the nontreated positive control (9.34 ± 0.2) or NEYP-treated group (9.13 ± 0.34; Figure 4). Comparison of \(C.\) jejuni counts from nontreated positive controls between experiment 1 (8.5 ± 0.1), experiment 2 (8.8 ± 0.3), and experiment 3 (9.34 ± 0.2) revealed that lowering the infection dose of \(C.\) jejuni did not result in lower \(C.\) jejuni colonization levels. Overall, the results of this experiment indicated that the long-term prophylactic (14 d) or therapeutic (11 d) use of a cocktail of 7 HEYP or NEYP at the dose and regimen used in this experi-

![Figure 2](image-url) **Figure 2.** Antibody response in water-soluble fraction of IgY extracted from hyperimmunized egg yolk powder (HEYP) against recombinant colonization-associated proteins (\(JlpA\) lipoprotein A, \(JlpA\), and \(Campylobacter\) putative adhesion protein, Peb1A) and from nonimmunized egg yolk powder (NEYP) using indirect ELISA. The ELISA showed significantly higher levels of anti-\(Campylobacter jejuni\) colonization-associated protein specific IgY in HEYP compared with IgY extracted from NEYP \((P < 0.0001)\). Vertical bars indicate the SE. *Asterisk indicates statistical significance at \(P < 0.0001)\.

![Figure 3](image-url) **Figure 3.** Effect of oral administration of feed supplemented with 10% (wt/wt) cocktail of 7 anti-\(Campylobacter jejuni\) colonization-associated protein specific antibodies in the form of hyperimmunized egg yolk powder (HEYP) compared with the nonimmunized egg yolk powder (NEYP) on \(Campylobacter jejuni\) cecal colonization in experimentally infected chickens. Horizontal bars indicate mean counts for each group. The differences in the \(C.\) jejuni counts were not statistically significant \((P < 0.05)\).
ment was not sufficient to significantly reduce cecal *C. jejuni* counts.

It is important to consider certain caveats before any direct comparison of our results with the previously published studies can be made. It should be noted that none of the previous studies tested HEYP enriched with IgY that are specifically directed against *C. jejuni* CAP, and *C. jejuni* counts were determined either from feces or whole intestine, instead of cecum, which is the primary site of *C. jejuni* colonization and the major source of cross-contamination of carcasses at postharvest processing (Beery et al., 1988). In all of the experiments conducted in this study, we tested the cecal counts of *C. jejuni*. Additionally, Kassaify and Mine (2004) used NEYP, which contained no antibodies against *C. jejuni*, whereas Tsubokura et al. (1997) used water-soluble fraction of IgY purified from egg yolk from chickens immunized with whole-cell lysates of *C. jejuni*. In contrast, our study used lyophilized egg yolk powder enriched with anti-*C. jejuni* CAP specific antibodies. While the results of this manuscript were being compiled, Hermans et al. (2014) reported that oral administration of feed supplemented with nonlyophilized egg yolks derived from chickens immunized with different fractions of whole-cell lysates of *C. jejuni* resulted in >5 log reduction in cecal *C. jejuni* counts when compared with untreated control groups that received only nonimmunized egg yolks. In addition, transmission of *C. jejuni* from infected chickens to the contact chickens was either completely prevented or significantly reduced at 3 d pi, which is very short. However, long-term protection afforded by immunized egg yolks against *C. jejuni* colonization was not determined. Interestingly, these authors identified several immunodominant proteins, which included at least 2 of the proteins used in the current study (MOMP and FlaA). Whereas the role of majority of the proteins identified by Hermans et al. (2014) is unknown and intriguing, further research is needed to identify specific proteins that not only play a role in cecal colonization of *C. jejuni*, but are also immunodominant and induce protective immune response to effectively reduce the *C. jejuni* colonization in chickens.

In this study, all of the proteins, including FlpA, CadF, FlaA, CmeC, MOMP, Pelb, and JlpA, were previously reported to play an important role in *C. jejuni* adhesion and colonization (Nachamkin et al., 1993; Ziprin et al., 1999; Lin et al., 2002; Flanagan et al., 2009; Islam et al., 2010). Most importantly, it has been reported that transfer of maternal antibodies reactive against the *C. jejuni* proteins including FlaA, CadF, CmeC, and PorA may protect against *C. jejuni* colonization in chickens until ~2 wk of age (Sahin et al., 2003; Shoaf-Sweeney et al., 2008; Cawthraw and Newell, 2010). However, reduction in colonization was not achieved using HEYP that were specific to all of the above CAP. Chalghoumi et al. (2009b) used HEYP-supplemented feed at 5 concentrations (0 to 5%) ad libitum for 4 wk, where chickens were cochallenged with *Salmonella* spp. at d 3 of treatment. They demonstrated that in long-term treatment of broiler chickens with HEYP containing anti-*Salmonella* spp., IgY did not reduce cecal colonization by *Salmonella*. The authors speculated that the IgY might have been degraded, denatured, or both during the passage through the intestine of the chickens or the lower concentration of specific IgY within the HEYP may contribute to this effect. Similar factors could potentially alter the activity of HEYP in the current study. However, previous studies have shown that stability of IgY is increased when mixed with alkaline solutions or in protein-rich (egg yolk components) medium (Schade et al., 2005). Therefore, further studies will be required to confirm the stability and activity of anti-*C. jejuni* CAP-specific IgY in the intestinal tract of treated chickens. In our study, all attempts including individual IgY treatment and combined IgY treatment were undertaken; however, none of the approaches significantly reduced cecal *C. jejuni* colonization. Although it is currently unknown what concentration of IgY would be optimal to reduce the *C. jejuni* colonization, for all HEYP tested in current study, the titer of anti-*C. jejuni* CAP-specific antibodies was consistently >1,024. Further studies will be needed to optimize the dose percentage and the time course of the IgY treatment.

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REFERENCES


