Use of a Caprine Serum Fraction-Immunomodulator to Reduce Mortality in Commercial and Large-Bodied Turkey Lines Infected with *Pasteurella multocida*¹²

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**Abstract:** The effectiveness of an injected caprine serum fraction-immunomodulator (CSF-I2) as an immunostimulant in male and female F-line and commercial turkey poult, infected with fowl cholera (*Pasteurella multocida*) was examined in separate trials. In the first two of three controlled trials, the effects of an i.m. injection of CSF-I2 given 24 h prior to a *P. multocida* challenge, administered by s.c. injection, on mortality and days to death in F-line turkeys was determined. The CSF-I2 reduced mortality but did not affect average number of days to death of F-line turkeys across trials and sexes when administered 24 h prior to a *P. multocida* challenge. In the third trial, the effects of an i.m. injection of CSF-I2 given to a commercial line of turkeys 24 h prior to an s.c. injection of *P. multocida* on mortality and days to death were determined. While positive control commercial turkey poult, experienced an 85% level of mortality across sex, the administration of CSF-I2 did not significantly reduce percentage mortality or average number of days to death. The difference in the effects of CSF-I2 on mortality in F-line and commercial turkey poult challenged with *P. multocida* suggests that CSF-I2 did not impart immunostimulation to commercial turkeys as it did in F-line turkeys that were infected with *P. multocida*. Therefore, genetic variation in turkeys may be an important consideration before using CSF-I2 as an immunomodulator to protect juvenile turkeys against fowl cholera.

**Key words:** Caprine serum, caprine serum fraction-immunomodulator, mortality, *Pasteurella multocida*, turkeys

**Introduction**

Exploration continues in immunological and pharmaceutical research for bio-molecules that may potentiate the immune response in animals. Caprine serum fraction-immunomodulator 2 (CSF-I2), a non-adjuvanted immunostimulant (immunglobulin, albumin and most cytokines excluded) derived from the serum of adult goats. The material can be lyophilized, reconstituted in water and used as an injectable prophylactic for bacterial challenges. Caprine serum fraction-immunomodulator 2 does not exhibit antimicrobial properties, but it has been shown to be effective in reducing mortality, modulating lymphocyte decreases and heterophil increases and preventing CD4⁺ depressions in specific-pathogen-free layer chickens in response to a fowl cholera (*Pasteurella multocida*) infection (Willeford et al., 2000). Furthermore, it has been evaluated for its ability to impart immunostimulation in other species (i.e., mice; Parker et al., 2002).

Turkey poult selected for increased BW (F-line) have been found to be highly susceptible to *P. multocida* (Nestor et al., 1999). The F-line, a sire sub-line of a randombred control line (RBC2), was selected over 31 generations for increased 16-wk BW (Nestor, 1977, 1984; Nestor et al., 1996). Li et al. (2000) reported that male and female 6-wk-old F-line turkey poult, exhibited higher mortality and fewer numbers of days to death following challenge with *P. multocida* than did the RBC2 line. Nestor et al. (1999) also reported that at 6 wk of age, the F-line birds were heavier at the time of challenge with *P. multocida* and had higher mortality following challenge with *P. multocida* than did a line obtained from a commercial breeder. The selection criteria for the sire line used in commercial turkey production was unknown, but was based on increased growth rate. Genetic selection for increased BW is known to reduce disease resistance in poultry (Saif et al., 1984; Qureshi and Havenstein, 1994). The goal of the current study was to determine the effectiveness of CSF-I2 in imparting immunostimulation in male and female F-line and commercial turkey poult infected with fowl cholera.

**Materials and Methods**

**CSF-I2:**

**F-line and commercial CSF-I2 trials:** The CSF-I2 was fractionated from adult (> 1 yr of age) goat serum via dialysis at a 6-8kDa cut-off. The material which is 37.5% proteinaceous, was lyophilized to a powder and stored at -70°C until reconstituted in purified water for use (Willeford et al., 2000). Each 1.0mL of injected CSF-I2
solution contained 20 mg/mL of material. Sham CSF-I2 injections contained only 1.0 ml of purified water. Sham and CSF-I2 solution injections were administered i.m. in the right breast muscle 24 h before bacterial challenge.

**Bacteria:**

**F-line and commercial preliminary dosage and CSF-I2 trials:** Inocula from field isolates of *P. multocida* capsular serogroup A, somatic serotype 3, 4 were used. Birds challenged with *P. multocida* were injected s.c. in the back of the neck with 1.0 ml of *P. multocida* inoculum. In the F-line and commercial CSF-I2 trials, *P. multocida* challenge inoculations were given 24 h after sham or CSF-I2 solution injection. Sham *P. multocida* inoculations used in the F-line and commercial CSF-I2 trials contained 1.0 ml of sterile broth.

**F-line trials:** Bacteria were grown in veal infusion broth for 24 h at 37°C and were then washed 3 times with sterile PBS (pH 7.3), divided into aliquots and stored at -70°C until being thawed for use. Inoculums contained 1.2 x 10^8 bacteria per ml in trial 1 and 1.2 x 10^7 bacteria per ml in trial 2. The number of bacteria used per bird was as previously specified for 6-wk-old F-line turkey poultts (Nestor et al., 1999; Li et al., 2000). Growth characteristics of the *P. multocida* isolate used were given by Sacco et al. (1991).

**Commercial preliminary dosage and CSF-I2 trials:** Bacteria were grown in brain-heart infusion broth for 24 h at 37°C and 1.0 ml of the culture was transferred to a fresh medium and incubated for 6 h. In the preliminary trial, the number of bacteria per ml of fresh inoculum was as specified for each of the 6 treatments and in the CSF-I2 trial, each 1.0 ml of fresh inoculum contained approximately 4.5 x 10^7 bacteria, which was the approximate concentration at which an 80% level of mortality was attained in the preliminary trial.

**Housing, management, and treatments:**

**F-line and commercial preliminary dosage and CSF-I2 trials:** All poultts were provided ad libitum access to feed and water, and all diets met or exceeded established National Research Council (1994) recommendations for turkey diets. Experimental protocols were approved by Animal Care and Use committees at Ohio State University for the F-line turkey trials and at Mississippi State University for the commercial turkey trials. Poultts exhibiting severe clinical morbidity were euthanized by cervical dislocation and were included in the record of mortalities. Birds that remained alive for 14 d in the F-line trials and commercial preliminary trial and for 10 d in the CSF-I2 trial after *P. multocida* challenge at 6 wk were similarly euthanized.

**F-line and commercial CSF-I2 trials:** At 6 wk of age, turkey poultts were subjected to one of the following treatments:

1) Not challenged with *P. multocida* (sham-injected) and not treated with CSF-I2 solution (sham-injected).
2) Not challenged with *P. multocida* (sham-injected) and treated with CSF-I2 solution,
3) Challenged with *P. multocida* and not treated with CSF-I2 solution (sham-injected), or
4) Challenged with *P. multocida* and treated with CSF-I2 solution. The sexes were intermingled in all pens. Because only one room (unit of replication) was used for treatments 1 and 2, they were not included in the statistical comparison of treatments. Only treatments 3 and 4 were compared statistically.

**F-line trials:** In each of 2 trials, F-line turkey poultts were used. Birds were brooded on floor pens in conventional housing, and at 5 wk of age all birds were wing-banded, and those that were challenged with *P. multocida* at 6 wk of age (treatments 3 and 4) were moved to an isolation facility where they were housed on floor pens. Control birds (treatments 1 and 2) remained on floor pens in the separate conventional housing and were ventilated, fed, and watered separately from the challenged birds to preclude the possibility of a *P. multocida* infection. The birds were fed the first 2 diets in a multi-diet feeding system with declining protein, as described by Naber and Touchburn (1970). For treatment 1, there were 13 males and 19 females in an individual pen in trial 1 and 13 males and 20 females in an individual pen in trial 2. For treatment 2, there were 16 males and 16 females in an individual pen in trial 1 and 11 males and 13 females in an individual pen in trial 2. For treatments 3 and 4, there were 13 replicate floor pens per treatment in trial 1, and 2 replicate floor pens per treatment in trial 2. In each trial, there were 6 birds per sex in each replicate pen for treatments 3 and 4.

**Commercial preliminary dosage and CSF-I2 trials:** Throughout each trial, commercial turkey poultts were raised on litter spread on the floors of isolation rooms within an isolation facility where they were ventilated, fed, and watered separately. All poultts were fed basal turkey diets, with compositional adjustments made every 3 wk.

**Commercial preliminary dosage trial:** The concentration of *P. multocida* bacteria in inocula necessary to achieve an approximate 80% level of mortality in 6 wk old female commercial turkey poultts was determined. From 1 d to 3 wk posthatch, 60 birds were kept in one room; from 3-6 wk posthatch, 30 birds were kept in each of 2 rooms and from 6-8 wk posthatch, 10 birds were housed in each of 6 rooms according to
treatment. At 6 wk of age, the commercial turkey poult
each in the 6 rooms were challenged with one of the
following inoculum treatments, containing different
numbers of bacteria per mL of inoculum: 1) 1.2x10^7, 2)
1.2x10^6, 3) 1.2x10^5, 4) 1.2x10^4, 5) 1.2x10^3, or 6) 1.2x10^2.

**Commercial CSF-I2 trial:** At 2d of age, poult
ts were separated by sex and placed in 2 different rooms
according to sex through 2 wk of age and at 2 wk of age,
96 poultts (48 males and 48 females) were wing-banded
and randomly assigned to 6 rooms, so that there were
16 birds in each room (8 males and 8 females). All of
the birds in 5 of the rooms (P. multocida treatment
rooms) were subsequently challenged with P. multocida
at 6 wk of age. In addition, 16 control birds (8 males and
8 females), which did not receive P. multocida challenge
injections at 6 wk of age, were maintained in a separate
isolation room (control room). In each of the 5 P.
multocida treatment rooms, 4 females and 4 males
received sham solution injections and 4 females and 4
males received CSF-I2 solution injections. At the same
time, 4 females and 4 males similarly received sham or
CSF-I2 solution injections in the non-P. multocida
challenge control room.

**Data collection:**

**F-line trials:** During the fifth wk of age, each poult was
weighed and bled via the brachial vein before being
moved to their respective facilities. A pre-challenge
ELISA serological test was determined on blood from
each bird to ensure a disease-free status. Mortality was
recorded daily in each trial and percentage mortality and
average number of days to death were determined
through 2 wk post-challenge. The number of days to
death following challenge for birds surviving at the end
of each trial was considered as being 14.

**Commercial preliminary dosage trial:** For each of the
inoculum treatments, bird mortality was monitored at
least 3 times daily between 6 and 8 wk of age for
calculation of cumulative percentage mortality.
Observations for gross anatomical lesions were
conducted on 3-8 birds in each room that were recorded
as mortalities before and after challenge. Fresh liver,
heart, and bone marrow samples were cultured from
necropsied birds that were observed for lesions for the
specific isolation of P. multocida.

**Commercial CSF-I2 trial:** Mortality and BW of individual
live and dead birds in each room were monitored at
least 3 times daily through 10 days post-challenge. Blood
samples were collected from 4 tagged birds per room
prior to challenge, and at 3, 8, 24, 48, 72, 96 and 120h
post-challenge for determination of plasma refractive
index. Percentage 10d cumulative mortality and average
days to death through d10 post-challenge were
calculated for each sex within each treatment replicate
room. The number of days to death following challenge
for birds surviving at the end of each trial was
considered as being 10. All birds in each of the 5 P.
multocida treatment rooms that were dead or remained
alive after 10d post-challenge were necropsied. Upon
necropsy, gross anatomical lesions were noted in all
birds that died before or after challenge and fresh liver,
heart, and bone marrow samples were cultured from
necropsied birds for the specific isolation of P.
multocida. Furthermore, at the end of the trial, all
remaining live challenged birds were likewise posted for
the culturing of tissues and for lesion observation and
recording. The 4 birds (2 males and 2 females) from
the individual control room were similarly processed.
Liver weight was determined in birds that remained alive after
10 d post-challenge.

**Statistical analysis for F-line and commercial
preliminary dosage and CSF-I2 trials:** In the F-line trials
(trials 1 and 2), a completely randomized experimental
design was used and in the commercial CSF-I2 trial
(trial 3), a randomized complete block experimental
design was used. In the F-line and commercial CSF-I2
trials, data were analyzed in a 2x2 factorial arrangement
to test for the individual and interactive effects of the 2
sexes and the 2 CSF-I2 levels (CSF-I2 vs. no CSF-I2) in
birds that were challenged with P. multocida.

Treatments 1 and 2 in each trial were not included in the
statistical analysis, because birds in those treatments
were housed in only 1 pen or room. The F-line data were
analyzed withing each trial. Also, employing trial as a
block, the F-line data were further analyzed across trials.
The data of both trials were pooled then analyzed
together, with trial considered as a random effect. Data
were subjected to one-way ANOVA when treatment
effects across sex, sex effects across treatment, or
treatment effects within sex were analyzed. Individual
sample data within each of the replicate units was
averaged prior to analysis. Least-squares means were
compared using Fisher’s protected LSD test in the event
of significant global effects (Steel and Torrie, 1980). All
data were analyzed using the MIXED procedure of SAS
software (SAS Institute, 2003). Global effects and
differences among least-squares means were
considered significant at P ≤ 0.05.

**Results**

**F-line trials:** The ELISA serological tests confirmed that
control and challenged birds were negative and positive,
respectively, for the presence of P. multocida. Across
both trials, there was a significant interaction between
sex and CSF-I2 treatment for percentage mortality in F-
line turkey poultts challenged with P. multocida (P<0.02).
This interaction reflects the occurrence of significant
effects due to treatment on percentage mortality of F-line
turkey poults across sex in trial 1 (P = 0.04) and trial 2 (P = 0.04) and across both trials (P = 0.005). Within each trial and in both trials combined, the CSF-I2 significantly reduced mortality across sex in birds challenged with *P. multocida* (Table 1).

However, there was no significant interaction between sex and CSF-I2 and no significant CSF-I2 treatment main effect on average number of days to death in birds challenged with *P. multocida*. Nevertheless, there was a significant (P < 0.003) main effect due to sex on average number of days to death. The average days to death across sex was not affected by CSF-I2 in birds challenged with *P. multocida* in either trial or across both trials (Table 2). Percentage mortality was significantly greater in males than in females across treatment in trial 1 (P < 0.02) and across both trials (P < 0.007), but was not significantly greater in males than in females in trial 2 (Table 3). Furthermore, average days to death was found to be significantly greater in females than in males across treatment in trial 2 (P < 0.03) and across both trials (P < 0.007), but was not significantly greater in females than in males in trial 1 (Table 3). Across both trials, CSF-I2 significantly reduced mortality in female (P < 0.003) but not in male F-line turkeys challenged with *P. multocida* (Table 4). Conversely, across both trials, the average days to death was not significantly affected by CSF-I2 in male or female F-line turkeys challenged with *P. multocida* (Table 4).

**Commercial preliminary dosage trial:** From the preliminary dosage trial, it was determined that treatment 5 achieved approximately 80% bird mortality. The numbers of bacteria in treatment 5 were approximately 1.2x10^8. Gross anatomical lesion observation and fresh liver, heart and bone marrow cultures showed evidence of *P. multocida* infection and confirmed the presence of *P. multocida* bacteria in challenged birds.

**Commercial CSF-I2 trial:** Typical patterns of morbidity and gross anatomical lesions were observed in all birds that had been challenged with *P. multocida*; however, birds that had not been challenged appeared normal in all aspects. Furthermore, fresh liver, heart and bone marrow culture tests confirmed that selected control birds that did not receive a *P. multocida* challenge were negative for *P. multocida*, whereas swab samples of all 3 tissues tested in all the birds in each of the 5 challenged rooms were positive for the presence of *P. multocida*. Also, in birds that received a *P. multocida* challenge, there were no significant interactions between sex and CSF-I2 treatment. There were also no significant main effects due to sex or CSF-I2 treatment on relative liver weight of those birds alive at Day 10 post-challenge, on daily and cumulative changes in BW between 0 and 10d post-challenge, or on plasma refractive index between 3 and 120h post-challenge. The only exception was a significant main effect due to sex on change in BW between d2 and 3 post-challenge (P < 0.03). In that time interval, there was a 19g average loss of BW in females and a 105g average increase of BW in males.

No mortalities were recorded for birds that were not challenged with *P. multocida* (treatments 1 and 2). Because only one unit (room) was used to house birds belonging to treatments 1 and 2, those treatments were not included in the statistical comparison of treatments. In trial 3, there were no significant interactions between sex and CSF-I2 treatment and no significant main effects due to sex or CSF-I2 treatment on average percentage 10d cumulative mortality or average days to death through d10 post-challenge in pouls challenged with *P. multocida*. The administration of CSF-I2 did not significantly reduce percentage mortality across sex in commercial turkey pouls challenged with *P. multocida* (treatments 3 and 4; Table 5).

Examination of the effects of treatment across sex on mean number of days to death of the turkey pouls also revealed that there was no significant effect due to the injection of CSF-I2 prior to *P. multocida* challenge (treatments 3 and 4; Table 6). Analysis of the possible effects of bird sex across treatments 3 and 4 on percentage mortality and days to death showed that there were no significant differences between sexes for either parameter (Table 7). Furthermore, when treatment effects were analyzed within each sex separately, it was shown that CSF-I2 did not significantly affect mortality or days to death in either male or female commercial turkey pouls challenged with *P. multocida* (Table 8).

**Discussion**

As evidenced by the results for both percentage mortality and number of days to death, female F-line turkey pouls are less susceptible to *P. multocida* than are males. The difference in the number of bacteria in the challenge inocula used in trials 1 (1.2x10^8) and 2 (1.2x10^9) could have led to the differential sex effect on mortality between the 2 trials. A corresponding difference between trials would be expected for days to death; however, because significant effects on both mortality and days to death were not found in trial 1, the 1 log difference in bacteria used between the trials may not have influenced the relationship between sex and mortality or between sex and days to death in challenged birds. The percent mortalities of non-CSF-I2-injected F-line birds in trials 1 and 2 following a *P. multocida* challenge (64.4 and 95.8%, respectively) further suggest that a 1 log reduction in organism numbers (trial 1-1.2x10^8 vs. trial 2-1.2x10^9) was not sufficient to reduce mortality in challenged birds. Therefore, should a 50-80% mortality rate after *P. multocida* challenge be desired for the future testing of CSF-I2 in F-line birds, a greater than 1 log reduction in organism numbers below that used in trial 1 should be considered.
Table 1: Percentage mortality (dead : total birds) of F-line turkey poults across sex in trials 1 and 2 and across both trials in the following treatment groups: 1) Not challenged with P. multocida and not treated with CSF-I2, 2) Not challenged with P. multocida and treated with CSF-I2, 3) Challenged with P. multocida and not treated with CSF-I2, and 4) Challenged with P. multocida and treated with CSF-I2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Both trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) No P. multocida and no CSF-I2</td>
<td>0 (0/2)</td>
<td>0 (0/33)</td>
<td>0 (0/85)</td>
</tr>
<tr>
<td>2) No P. multocida and CSF-I2</td>
<td>0 (0/32)</td>
<td>0 (0/24)</td>
<td>0 (0/56)</td>
</tr>
<tr>
<td>3) P. multocida and no CSF-I2</td>
<td>94.4 (34/36)*</td>
<td>95.6 (23/24)*</td>
<td>95.9 (57/60)*</td>
</tr>
<tr>
<td>4) P. multocida and CSF-I2</td>
<td>80.6 (29/36)*</td>
<td>75.0 (18/24)*</td>
<td>78.3 (47/60)*</td>
</tr>
</tbody>
</table>

*Means within column with no common superscript differ significantly (P ≤ 0.05). †Treatments 1 and 2 were not included in the statistical comparison of treatments. ‡SEM based on pooled estimate of variance for treatments 3 and 4 = 2.78; n = 3 replicate units for calculation of means within treatments 3 and 4. §SEM based on pooled estimate of variance for treatments 3 and 4 = 2.95; n = 2 replicate units for calculation of means within treatments 3 and 4. ¶SEM based on pooled estimate of variance for treatments 3 and 4 across trials = 2.04; n = 5 replicate units for calculation of means within treatments 3 and 4.

Table 2: Days to death of F-line turkey poults across sex in trials 1 and 2 and across both trials in the following treatment groups: 1) Not challenged with P. multocida and not treated with CSF-I2, 2) Not challenged with P. multocida and treated with CSF-I2, 3) Challenged with P. multocida and not treated with CSF-I2, and 4) Challenged with P. multocida and treated with CSF-I2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Both trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) No P. multocida and no CSF-I2</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>2) No P. multocida and CSF-I2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3) P. multocida and no CSF-I2</td>
<td>4.61</td>
<td>4.96</td>
<td>4.75</td>
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<tr>
<td>4) P. multocida and CSF-I2</td>
<td>5.26</td>
<td>5.02</td>
<td>5.10</td>
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*Treatments 1 and 2 were not included in the statistical comparison of treatments. †There were no significant differences between treatments in separate or combined trials. ‡SEM based on pooled estimate of variance for treatments 3 and 4 = 0.397; n = 3 replicate units for calculation of means within treatments 3 and 4. §SEM based on pooled estimate of variance for treatments 3 and 4 = 0.430; n = 2 replicate units for calculation of means within treatments 3 and 4. ¶SEM based on pooled estimate of variance for treatments 3 and 4 across trials = 0.287; n = 5 replicate units for calculation of means within treatments 3 and 4.

Table 3: Percentage mortality, dead : total birds, and days to death of male and female F-line turkey poults challenged with P. multocida across CSF-I2 treatment (treatments 3 and 4) in trials 1 and 2 and across both trials.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Both trials</th>
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<tbody>
<tr>
<td>Mortality (%)</td>
<td>94.4*</td>
<td>80.6*</td>
<td>91.7</td>
</tr>
<tr>
<td>Dead : total birds</td>
<td>34/36</td>
<td>20/36</td>
<td>22/24</td>
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<tr>
<td>Days to death</td>
<td>4.35</td>
<td>5.52</td>
<td>4.04*</td>
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*Means for each sex within each trial and across both trials for each parameter with no common superscript differ significantly (P ≤ 0.05). †SEM based on pooled estimate of variance for males and females in trial 1 = 2.78; n = 3 replicate units for calculation of means within each sex. ‡SEM based on pooled estimate of variance for males and females in trial 2 = 2.95; n = 2 replicate units for calculation of means within each sex. §SEM based on pooled estimate of variance for males and females across both trials = 1.88; n = 5 replicate units for calculation of means within each sex. ¶SEM based on pooled estimate of variance for males and females in trial 1 = 0.4094; n = 3 replicate units for calculation of means within each sex. ‡SEM based on pooled estimate of variance for males and females across both trials = 0.224; n = 2 replicate units for calculation of means within each sex. ¶SEM based on pooled estimate of variance for males and females across both trials = 0.252; n = 5 replicate units for calculation of means within each sex.

A 1.0mL injection of 200mg/mL CSF-I2 24h prior to challenge with P. multocida reduced mortality in the F-line poults by more than 20%, which indicates that CSF-I2 confers resistance to fowl choler in the F-line turkeys. However, this effect was largely due to a decrease in female mortality and this occurred despite a lower mortality in P. multocida-challenged females than males, which was most evident in trial 1. Although the use of CSF-I2 prior to a P. multocida challenge reduced mortality, it did not significantly affect the number of days to death in the F-line turkeys and its use remained ineffectual despite a significantly higher number of days to death for females than for males, which was most notable in trial 2. The average percentage of mortality of non-CSF-I2-injected poults across trial in the F-line was 85.0% and in the commercial line was 85.0%. These mortalities were in response to ≤ 1.2x10^5 and 4.5x10^5 bacteria per 1.0mL of challenge inoculum, respectively. This would suggest that the commercial birds may be more susceptible to a P. multocida challenge at 6 wk of age than are F-line birds. This suggestion is in
Table 4: Percentage mortality, dead: total birds, and days to death of male and female F-line turkey poults across trials 1 and 2 in the following treatment groups: 1) Not challenged with *P. multocida* and not treated with CSF-12, 2) Not challenged with *P. multocida* and treated with CSF-12, 3) Challenged with *P. multocida* and not treated with CSF-12, and 4) Challenged with *P. multocida* and treated with CSF-12.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Males</th>
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<th>Females</th>
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<tbody>
<tr>
<td></td>
<td>% Mortality</td>
<td>Days to death</td>
<td>% Mortality</td>
<td>Days to death</td>
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<tr>
<td></td>
<td>(Dead : total birds)</td>
<td></td>
<td>(Dead : total birds)</td>
<td></td>
</tr>
<tr>
<td>1) No <em>P. multocida</em> and no CSF-12</td>
<td>0 (0/26)</td>
<td>-</td>
<td>0 (0/36)</td>
<td>-</td>
</tr>
<tr>
<td>2) No <em>P. multocida</em> and CSF-12</td>
<td>0 (0/27)</td>
<td>-</td>
<td>0 (0/29)</td>
<td>-</td>
</tr>
<tr>
<td>3) <em>P. multocida</em> and no CSF-12</td>
<td>98.7 (26/30)</td>
<td>3.77</td>
<td>93.3 (28/30)</td>
<td>5.73</td>
</tr>
<tr>
<td>4) <em>P. multocida</em> and CSF-12</td>
<td>90.0 (27/30)</td>
<td>4.87</td>
<td>66.7 (20/30)</td>
<td>6.65</td>
</tr>
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</table>

Means with column with no common superscript differ significantly (P ≤ 0.05). Treatments 1 and 2 were not included in the statistical comparison of treatments. SEM based on pooled estimate of variance for calculations of means within treatments 3 and 4; n = 5 replicate units for calculation of means within treatments 3 and 4. SEM based on pooled estimate of variance for treatments 3 and 4 = 2.89; n = 5 replicate units for calculation of means within treatments 3 and 4. SEM based on pooled estimate of variance for treatments 3 and 4 = 0.383; n = 5 replicate units for calculation of means within treatments 3 and 4.

Table 5: Percentage mortality (dead: total birds) of commercial turkey poults across sex in the following treatment groups: 1) Not challenged with *P. multocida* and not treated with CSF-12, 2) Not challenged with *P. multocida* and treated with CSF-12, 3) Challenged with *P. multocida* and not treated with CSF-12, and 4) Challenged with *P. multocida* and treated with CSF-12 in trial 3.1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Mortality</th>
<th>Days to death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Dead: total birds)</td>
<td></td>
</tr>
<tr>
<td>1) No <em>P. multocida</em> and no CSF-12</td>
<td>0 (0/8)</td>
<td>-</td>
</tr>
<tr>
<td>2) No <em>P. multocida</em> and CSF-12</td>
<td>0 (0/8)</td>
<td>-</td>
</tr>
<tr>
<td>3) <em>P. multocida</em> and no CSF-12</td>
<td>85.0 (34/40)</td>
<td>7.10</td>
</tr>
<tr>
<td>4) <em>P. multocida</em> and CSF-12</td>
<td>82.5 (33/40)</td>
<td>-</td>
</tr>
</tbody>
</table>

1Treatments 1 and 2 were not included in the statistical comparison of treatments. One unit (room) was used for determination of mortality within treatments 1 and 2. There were no significant differences between treatments 3 and 4. SEM based on pooled estimate of variance for treatments 3 and 4 = 7.10; n = 5 replicate units for calculation of means within treatments 3 and 4.

Table 6: Days to death of commercial turkey poults across sex in the following treatment groups: 1) Not challenged with *P. multocida* and not treated with CSF-12, 2) Not challenged with *P. multocida* and treated with CSF-12, 3) Challenged with *P. multocida* and not treated with CSF-12, and 4) Challenged with *P. multocida* and treated with CSF-12 in trial 3.1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days to death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) No <em>P. multocida</em> and no CSF-12</td>
<td>-</td>
</tr>
<tr>
<td>2) No <em>P. multocida</em> and CSF-12</td>
<td>-</td>
</tr>
<tr>
<td>3) <em>P. multocida</em> and no CSF-12</td>
<td>3.98</td>
</tr>
<tr>
<td>4) <em>P. multocida</em> and CSF-12</td>
<td>4.43</td>
</tr>
</tbody>
</table>

1Treatments 1 and 2 were not included in the statistical comparison of treatments. One unit (room) was used for determination of days to death within treatments 1 and 2. There were no significant differences between treatments 3 and 4. SEM based on pooled estimate of variance for treatments 3 and 4 = 0.582; n = 5 replicate units for calculation of means within treatments 3 and 4.

Table 7: Percentage mortality, dead: total birds, and days to death of male and female commercial turkey poults challenged with *P. multocida* across CSF-12 treatment (treatments 3 and 4) in trial 3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality (%)</td>
<td>75.0</td>
<td>92.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead: total birds</td>
<td>30/40</td>
<td>37/40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days to death</td>
<td>5.18</td>
<td>3.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1There were no significant differences between sexes. SEM based on pooled estimate of variance for both sexes = 8.20; n = 5 replicate units for calculation of means within both sexes. SEM based on pooled estimate of variance for both sexes = 0.576; n = 5 replicate units for calculation of means within both sexes.

Disagreement with that of Nestor et al. (1999), who concluded that mortality following challenge with *P. multocida* was higher in the F-line than a contemporary commercial line. Nestor et al. (1999) also stated that there may have been variation in resistance among commercial sire lines from the major primary turkey breeders at the time of that study. The differences in the disease resistance between the F and commercial lines observed by Nestor et al. (1999) and those of the current study indicate that the commercial birds used in the 2 studies might also have different levels of disease resistance. The physiological bases underlying these differences have not been determined.

Nevertheless, the results of the current trials suggest that 1.0 mL i.m. injections of CSF-12 solutions at 20mg/mL concentrations 24 h prior to *P. multocida* challenge at 8 wk of age are effective in reducing mortality in F-line turkey poults, but may be ineffective in reducing mortality in commercial turkey poults. The different resistances of the birds in these 2 lines to the bacterial challenge may have also led to differences in their responses to CSF-12. Willeford et al. (2000) concluded that one or more small molecular weight compounds isolated from CSF-12 were able to reduce mortality in specific-pathogen-free-layer chicken infected with *P. multocida*. Although it was stated that the
Table 8: Percentage mortality, dead : total birds, and days to death of male and female commercial turkey poults in the following treatment groups: 1) Not challenged with *P. multocida* and not treated with CSF-I2, 2) Not challenged with *P. multocida* and treated with CSF-I2, 3) Challenged with *P. multocida* and not treated with CSF-I2, and 4) Challenged with *P. multocida* and treated with CSF-I2 in trial 3-4.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Mortality (Dead : total birds)</td>
<td>Days to death</td>
</tr>
<tr>
<td>1) No <em>P. multocida</em> and no CSF-I2</td>
<td>0 (0/4)</td>
<td>-</td>
</tr>
<tr>
<td>2) No <em>P. multocida</em> and CSF-I2</td>
<td>0 (0/4)</td>
<td>-</td>
</tr>
<tr>
<td>3) <em>P. multocida</em> and no CSF-I2</td>
<td>88.0 (18/20)</td>
<td>4.65</td>
</tr>
<tr>
<td>4) <em>P. multocida</em> and CSF-I2</td>
<td>70.0 (14/20)</td>
<td>5.70</td>
</tr>
</tbody>
</table>

*SEM based on pooled estimate of variance for treatments 3 and 4 = 10.90, n = 5 replicate units for calculation of means within treatments 3 and 4. SEM based on pooled estimate of variance for treatments 3 and 4 = 0.900, n = 5 replicate units for calculation of means within treatments 3 and 4. SEM based on pooled estimate of variance for treatments 3 and 4 = 7.91, n = 5 replicate units for calculation of means within treatments 3 and 4. SEM based on pooled estimate of variance for treatments 3 and 4 = 0.633, n = 5 replicate units for calculation of means within treatments 3 and 4.

Mechanism by which CSF-I2 causes immunostimulation was unclear, Willeford et al. (2000) suggested that one possible route by which CSF-I2 sustains an immunocompetent status is by maintaining the presence of helper T cells in serum. Because serum helper T cell populations were not monitored in any of the current trials, this factor should be investigated in subsequent experiments to help provide a basis of explanation for the disparity in the effectiveness of CSF-I2 to impart immunostimulation in the F and commercial line turkeys infected with fowl cholera.

**References**


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