ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 7 (8): 818-824, 2008
ISSN 1682-8356

Use of a Caprine Serum Fraction-Immunomodulator to Reduce Mortality in Commercial and Large-Bodied Turkey Lines Infected with *Pasteurella multocida*[1,2]

E.D. Peebles[1], K.O. Willeford[2], R.W. Keirs[3], K.E. Nestor[4], Y.M. Saif[4], C. Wang[3], C.J. Matyi[2], J.W. Anderson[4], M.T. Kidd[1] and R. Pulikanti[1]
[1]Department of Poultry Science, [2]Department of Biochemistry and Molecular Biology, [3]College of Veterinary Medicine, Mississippi State University, Mississippi State, Mississippi, 39762, USA
[4]Department of Animal Sciences, Ohio State University, Wooster, Ohio, 44691, USA

Abstract: The effectiveness of an injected caprine serum fraction-immunomodulator (CSF-I2) as an immunostimulant in male and female F-line and commercial turkey poults infected with fowl cholera (*Pasteurella multocida*) was examined in separate trials. In the first 2 of 3 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 24h 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 24h prior to an s.c. injection of *P. multocida* on mortality and days to death were determined. While positive control commercial turkey poults 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 poults 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[3] (CSF-I2), is a non-adjuvanted immunostimulant (immunoglobulin, 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 CD_4+ 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 poults 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 poults 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 poults 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 20mg/mL of material. Sham CSF-I2 injections contained only 1.0mL of purified water. Sham and CSF-I2 solution injections were administered i.m. in the right breast muscle 24h 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 1.0mL of *P. multocida* inoculum. In the F-line and commercial CSF-I2 trials, *P. multocida* challenge injections were given 24h after sham or CSF-I2 solution injection. Sham *P. multocida* injections used in the F-line and commercial CSF-I2 trials contained 1.0mL of sterile broth.

F-line trials: Bacteria were grown in veal infusion broth for 24h 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. Inoculum contained $1.2x10^8$ bacteria per mL in trial 1 and $1.2x10^7$ bacteria per mL in trial 2. The number of bacteria used per bird was as previously specified for 6-wk-old F-line turkey poults (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 24h at 37°C and 1.0mL of the culture was transferred to a fresh medium and incubated for 6h. 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 1.0mL of fresh inoculum contained approximately $4.5x10^3$ 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 poults 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. Poults exhibiting severe clinical morbidity were euthanized by cervical dislocation and were included in the record of mortalities. Birds that remained alive for 14d in the F-line trials and commercial preliminary trial and for 10d 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 poults 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 poults 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 3 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 poults were raised on litter spread on the floors of isolation rooms within an isolation facility where they were ventilated, fed, and watered separately. All poults 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 poults was determined. From 1d 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 poults in each of 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, poults 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 poults (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 d 10 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 within 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 $P \leq 0.05$.

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 poults challenged with *P. multocida* ($P \leq 0.02$). This interaction reflects the occurrence of significant effects due to treatment on percentage mortality of F-line

turkey poults across sex in trial 1 ($P \leq 0.02$) and trial 2 ($P = 0.04$) and across both trials ($P \leq 0.005$). 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 ($P \leq 0.003$) 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 ($P \leq 0.02$) and across both trials ($P \leq 0.007$), 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 ($P \leq 0.03$) and across both trials ($P \leq 0.007$), 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 ($P \leq 0.003$) 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 $1.2x10^3$. 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 ($P \leq 0.03$). 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 d 10 post-challenge in poults challenged with *P. multocida*. The administration of CSF-I2 did not significantly reduce percentage mortality across sex in commercial turkey poults 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 poults 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 poults 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 poults 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 ($1.2x10^7$) 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 (94.4 and 95.8%, respectively) further suggest that a 1 log reduction in organism numbers (trial 1-$1.2x10^8$ vs. trial 2-$1.2x10^7$) 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[1]

	Trial 1[2]	Trial 2[3]	Both trials[4]
Treatment	% mortality (dead:total birds)		
1) No *P. multocida* and no CSF-I2	0 (0/32)	0 (0/33)	0 (0/65)
2) No *P. multocida* and CSF-I2	0 (0/32)	0 (0/24)	0 (0/56)
3) *P. multocida* and no CSF-I2	94.4 (34/36)[a]	95.8 (23/24)[a]	95.0 (57/60)[a]
4) *P. multocida* and CSF-I2	80.6 (29/36)[b]	75.0 (18/24)[b]	78.3 (47/60)[b]

[a,b]Means within column with no common superscript differ significantly ($P \leq 0.05$). [1]Treatments 1 and 2 were not included in the statistical comparison of treatments. [2]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. [3]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. [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[1,2]

	Trial 1[3]	Trial 2[4]	Both trials[5]
Treatment	Days		
1) No *P. multocida* and no CSF-I2	-	-	-
2) No *P. multocida* and CSF-I2	-	-	-
3) *P. multocida* and no CSF-I2	4.61	4.96	4.75
4) *P. multocida* and CSF-I2	5.26	5.02	5.16

[1]Treatments 1 and 2 were not included in the statistical comparison of treatments. [2]There were no significant differences between treatments in separate or combined trials. [3]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. [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. [5]SEM based on pooled estimate of variance for treatments 3 and 4 across trials = 0.267; 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

	Trial 1		Trial 2		Both trials	
Parameter	Males	Females	Males	Females	Males	Females
Mortality (%)[1,2,3]	94.4[a]	80.6[b]	91.7	79.2	93.3[a]	80.0[b]
Dead : total birds	34/36	29/36	22/24	19/24	56/60	48/60
Days to death[4,5,6]	4.35	5.52	4.04[b]	5.94[a]	4.22[b]	5.69[a]

[a,b]Means for each sex within each trial and across both trials for each parameter with no common superscript differ significantly ($P \leq 0.05$). [1]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. [2]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. [3]SEM based on pooled estimate of variance for males and females across both trials = 1.86; n = 5 replicate units for calculation of means within each sex. [4]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. [5]SEM based on pooled estimate of variance for males and females in trial 2 = 0.224; n = 2 replicate units for calculation of means within each sex. [6]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 20mg/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 cholera 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 95.0% and in the commercial line was 85.0 %. These mortalities were in response to $\geq 1.2 \times 10^7$ and to 4.5×10^3 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-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[1]

	Males		Females	
Treatment	% Mortality[2] (Dead : total birds)	Days to death[3]	% Mortality[4] (Dead : total birds)	Days to death[5]
1) No *P. multocida* and no CSF-I2	0 (0/26)	-	0 (0/39)	-
2) No *P. multocida* and CSF-I2	0 (0/27)	-	0 (0/29)	-
3) *P. multocida* and no CSF-I2	96.7 (29/30)	3.77	93.3 (28/30)[a]	5.73
4) *P. multocida* and CSF-I2	90.0 (27/30)	4.67	66.7 (20/30)[b]	5.65

[a,b]Means within column with no common superscript differ significantly ($P \leq 0.05$). [1]Treatments 1 and 2 were not included in the statistical comparison of treatments. [2]SEM based on pooled estimate of variance for treatments 3 and 4 = 3.73; n = 5 replicate units for calculation of means within treatments 3 and 4. [3]SEM based on pooled estimate of variance for treatments 3 and 4 = 0.356; n = 5 replicate units for calculation of means within treatments 3 and 4. [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. [5]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-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[1,2]

Treatment	%Mortality (Dead : total birds)[3]
1) No *P. multocida* and no CSF-I2	0 (0/8)
2) No *P. multocida* and CSF-I2	0 (0/8)
3) *P. multocida* and no CSF-I2	85.0 (34/40)
4) *P. multocida* and CSF-I2	82.5 (33/40)

[1]Treatments 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. [2]There were no significant differences between treatments 3 and 4. [3]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-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[1, 2]

Treatment	Days to death[3]
1) No *P. multocida* and no CSF-I2	-
2) No *P. multocida* and CSF-I2	-
3) *P. multocida* and no CSF-I2	3.98
4) *P. multocida* and CSF-I2	4.43

[1]Treatments 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. [2]There were no significant differences between treatments 3 and 4. [3]SEM based on pooled estimate of variance for treatments 3 and 4 = 0.562; 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-I2 treatment (treatments 3 and 4) in trial 3[1]

Parameter	Males	Females
Mortality (%)[2]	75.0	92.5
Dead : total birds	30/40	37/40
Days to death[3]	5.18	3.23

[1]There were no significant differences between sexes. [2]SEM based on pooled estimate of variance for both sexes = 8.20; n = 5 replicate units for calculation of means within both sexes. [3]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 1.0mL i.m. injections of CSF-I2 solutions at 20mg/mL concentrations 24 h prior to *P. multocida* challenge at 6 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-I2. Willeford *et al.* (2000) concluded that one or more small molecular weight compounds isolated from CSF-I2 were able to reduce mortality in specific-pathogen-free layer chickens 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[1,2]

	Males		Females	
Treatment	% Mortality[3] (Dead : total birds)	Days to death[4]	% Mortality[5] (Dead : total birds)	Days to death[6]
1) No *P. multocida* and no CSF-I2	0 (0/4)	-	0 (0/4)	-
2) No *P. multocida* and CSF-I2	0 (0/4)	-	0 (0/4)	-
3) *P. multocida* and no CSF-I2	80.0 (16/20)	4.65	90.0 (18/20)	3.30
4) *P. multocida* and CSF-I2	70.0 (14/20)	5.70	95.0 (19/20)	3.15

[1]Treatments 1 and 2 were not included in the statistical comparison of treatments. One unit (room) was used for determination of mortality and days to death within treatments 1 and 2. [2]There were no significant differences between treatments 3 and 4 within each sex. [3]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. [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. [5]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. [6]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

Li, Z., K.E. Nestor, Y.M. Saif, J.W. Anderson and R.A. Patterson, 2000. Serum immunoglobulin G and M concentrations did not appear to be associated with resistance to *Pasteurella multocida* in a large-bodied turkey line and a randombred control population. Poult. Sci., 79: 163-166.

Naber, E.C. and S.P. Touchburn, 1970. Ohio poultry rations. Ohio Cooperative Extension Service Bulletin 343. The Ohio State University, Columbus, OH.

National Research Council, 1994. Nutrient Requirements of Poultry. 9th Rev. ed. National Academy Press, Washington, DC.

Nestor, K.E., 1977. Genetics of growth and reproduction in the turkey. 5. Selection for increased body weight alone and in combination with increased egg production. Poult. Sci., 56: 337-347.

Nestor, K.E., 1984. Genetics of growth and reproduction in the turkey. 9. Long-term selection for increased 16-week body weight. Poult. Sci., 63: 2114-2122.

Nestor, K.E., D.O. Noble, J. Zhu and Y. Moritsu, 1996. Direct and correlated responses to long-term selection for increased body weight and egg production in turkeys. Poult. Sci., 75: 1180-1191.

Nestor, K.E., Y.M. Saif, J.W. Anderson, R.A. Patterson, and Z. Li, 1999. Variation in resistance to *Pasteurella multocida* among turkey lines. Poult. Sci., 78: 1377-1379.

Parker, T.A., K.O. Willeford, S. Parker and K. Buddington, 2002. Reducing mortality in *Salmonella enterica* Serovar Typhimurium-infected mice with a tripeptidic serum fraction. Antimicrob. Agents Chemother, 46: 1971-1973.

Qureshi, M.A. and G.B. Havenstein, 1994. A comparison of the immune performance of a 1991 commercial broiler with a 1957 randombred strain when fed "typical" 1957 and 1991 broiler diets. Poult. Sci., 73: 1805-1812.

Sacco, R.E., Y.M. Saif, K.E. Nestor, N.B. Anthony, D.A. Emmerson and R.N. Dearth, 1991. Genetic variation in resistance of turkeys to experimental challenge with *Pasteurella multocida*. Avian Dis., 35: 950-954.

Saif, Y.M., K.E. Nestor, R.N. Dearth and P.A. Renner, 1984. Case report-Possible genetic variation in resistance of turkeys to erysipelas and fowl cholera. Avian Dis., 28: 770-773.

SAS Institute, 2003. SAS Proprietary Software Release 9.1. SAS Inst. Inc., Carey, NC.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd Ed. McGraw-Hill, New York, NY.

Willeford, K.O., T.A. Parker, E.D. Peebles, C. Wang and E.W. Jones, 2000. Reduction of mortality in specific-pathogen-free layer chickens by a caprine serum fraction after infection with *Pasteurella multocida*. Poult. Sci., 79: 1424-1429.

[1]This is journal No. J-11413 from the Mississippi Agricultural and Forestry Experiment station supported by MIS-322210.

[2]Use of trade names in this publication does not imply endorsement by Mississippi Agricultural and Forestry Experiment Station of these products, nor similar ones not mentioned.

[3]US Patent 5, 219, 578.