Research Article

Impact of Serratiopeptidase Treatment on Performance and Health Parameters in Broiler Chickens

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ABSTRACT

The aim of this study was to evaluate the effect of using graded levels of enzyme Serratiopeptidase, treatment on growth performance, immune response and some blood parameters of broiler chickens. One hundred twenty 1-day-old broiler chicks were randomly assigned to 4 treatment groups; each group included 3 replicates of 10 birds. Birds were treated as the following group (1) birds were non infected and non treated used as control group. Group (2) birds were only infected with E. coli and Mycoplasma gallisepticum (MG) at 14th day of age. While birds of group (3) were infected with E. coli and *Mycoplasma gallisepticum* (MG) at 14th day of age and treated with Serrapeptase in drinking water at a dose of 2 g/liter for the first 3 days of each week. Group (4) birds were infected with E. coli and *Mycoplasma gallisepticum* (MG) at 14th day of age and were treated with Serrapeptase in drinking water at a dose of 1 g / liter for the first 3 days of each week. The results showed significant improvement in final body weight, body gain and feed conversion of birds which was treated with Serrapeptase at 2g /liter drinking water. Similar trend was noted for the effect of Serratiopeptidase on total serum proteins of infected chickens, Serratiopeptidase significantly corrected the total protein from (6.86±0.11) in the infected non treated group to (7.88±0.17 and 7.81±0.15) in groups 3 and 4 respectively. Serratiopeptidase significantly decreased the total cholesterol, serum LDH and the inflammatory markers tested (CRP and ESR). Serratiopeptidase treatment improved the immunological response to NDV vaccination, and decreased the re-isolation and shedding of MG and E. coli

Keywords: Broiler performance, Inflammatory markers, *Mycoplasma gallisepticum*, Serrapeptase.

INTRODUCTION

Serratiopeptidase (Serratia E-15 protease, also known as serralysin, serratiapeptase, serrapeptase serratia peptidase, Serratiopeptidase, or serrapeptidase) is a proteolytic enzyme (protease) produced in the laboratory by *Enterobacterium Serratia* sp. E-15 from plant material. This microorganism was originally isolated in the late 1960:s from the intestine of silkworm Bombyx mori. Serratiopeptidase allows the emerging moth to dissolve its cocoon. Serratiopeptase is produced by purification from culture of *Serratia E*-15 bacteria.

Serrapeptase has a specific, anti-inflammatory effect, superior to that of other proteolytic enzymes. This immunologically active enzyme is completely bound to the alpha 2 macroglobulin in biological fluids. Histological studies revealed powerful anti-inflammatory effects of this naturally occurring enzyme.

Also this enzyme has the ability to digest non-living tissue that is a by-product of the healing process without harming sound tissue. Serrapeptase is used to dissolve non-living tissues as: scar tissue, fibrosis, blood clots, cysts and arterial plaque. It is also used as an anti-inflammatory agent against sinusitis, by thinning the mucous secretion. It is believed that serrapeptase can play a key role in dissolving the outer protective layers of cancer cells (fibrocystic breasts) to enable the immune system and other cancer fighters to better attack the cancer.

Serratiopeptidase is thought to work in three ways: (a) it may reduce inflammation by thinning the fluids formed from an injury, and by facilitating the fluid's drainage. This in turn, also speeds tissue repair. (b) it may help alleviate pain by preventing the release of pain-inducing "amines". (c) it may enhance cardiovascular health by breaking down fibrin, of blood clot without harming living tissue. This dissolves atherosclerotic plaque (which causes atherosclerosis) without causing harm to the inside of the arteries.[1,2] Serratiopeptidase may be particularly effective for those who have lung problems, as it clears out all of the inflammation, mucus and dead scar tissues, enabling the body's own natural healing system to replace the bad tissue with healthy tissue resulting in better lung function. In avian medicine; Mycoplasma gallisepticum (MG) is an important agent of complicated chronic respiratory disease CCRD, and reduced feed conversion efficiency, downgrading of broilers carcasses and increased medication costs.[3]

Complicated Chronic Respiratory Disease is characterized by formation of thick fibrinous inflammation in the air sacs, lungs, liver, heart and peritoneum leading to decreased respiration and aeration efficiency of the body and decreased productive performance. This work is the first in Egypt to be done on this enzyme for poultry regarding its possible effect to relieve fibrinous air

sacculitis in Complicated Chronic Respiratory Disease CCRD, and subsequently on productive performance of broilers.

MATERIALS AND METHODS

Birds, Diet and Experimental Design

One hundred twenty 1-day-old broiler chicks (avian 48) obtained from a local broiler chicken hatchery were randomly assigned to 4 treatment groups, each group included 3 replicates of 10 birds. The experiment was conducted in accordance with animal welfare. Birds were kept in the wire-floored battery cages. Birds were treated as follows: Group (1) birds were non infected, non treated control group. Group (2) birds were only infected with E. coli and Mycoplasma gallisepticum (MG) at 14th day of age. Group (3) birds were infected with E. coli and Mycoplasma gallisepticum (MG) at 14th day of age and were treated with Serrapeptase in drinking water at a dose of 2 g/liter for the first 3 days of each week. Birds were treated with ciprofloxacin at a dose of 1 g / liter for 5 days post-infection. Group (4) birds were infected with E. coli and Mycoplasma gallisepticum (MG) at 14th day of age and were treated with Serrapeptase in drinking water at a dose of 1 g / liter for the first 3 days of each week. Birds were treated with ciprofloxacin at a dose of 1 g / liter for 5 days post-infection.

The initial temperature of 32^{o}C was reduced sequentially according to the age of the birds until reaching 26^{o}C at 21 days of age. The chicks were kept on a 23-h light program, with free access to feed and water throughout the experiment. The birds were fed a starter diet until 21 day of age followed by a finishing diet from 21st to 35th day.

Birds were vaccinated as follows: Clone Ma5 (Eye drop) at 7 days of age: inactivated NDV vaccine at 10 days of age S/C injection;, Gumboro intermediate plus (Bursine plus vaccine) in drinking water at 12 days, and LaSota vaccine in drinking water at 21 days of age.

Experimental infection and Biochemical and serological identification for *Mycoplasma gallisepticum*

Chickens were inoculated with 0.2 ml of Frey's broth containing MG at a concentration of 108 CFU/ml of fresh culture into the right abdominal air sac at the age of 14 days.[4, 5]

Also chickens were injected simultaneously into the air sac of the left side by 0.2 ml of MacFarland tube no.1 containing 10^6 E. coli organisms /1 ml saline suspension. The chickens of the non infected control group were inoculated with 0.2 ml sterile saline. The chickens were observed daily for respiratory signs up to 3 wk PI.

At the end of 3th wk PI, five chickens from each group were sacrificed and their air sacs were examined for gross pathological lesions. The lesion scores were given for both right and left air sacs (1-4 scores individually) as well as average lesion scores of five birds from each group were cumulatively scored.[6] MG was re-isolated from air sacs and trachea on Frey's medium[7] and again were identified biochemically and serologically.

All suspected *MG* isolates were subjected to biochemical identification using digitonin sensitivity test[8], glucose fermentation and arginine hydrolysis tests.[9,10] Biochemically identified isolates were serologically tested by agar gel precipitation test (AGPT) and serum growth inhibition test (GI)[8] using rabbit hyperimmune serum prepared against commercial MG bacterin according to.[11]

Measurements and Observations

Body weight development and feed intake of chicks in different groups were weekly recorded. The weight gain (expressed in grams) was calculated as the difference between two successive body weights. Moreover, feed conversion ratio and relative growth rate (RGR) were also calculated. After the end of the experiment, at 35 days of age, birds were night fastened; the blood was collected from wing vein, without anticoagulant at room temperature for one hour, and then centrifuged at 3000 rpm/10 min. The serum was obtained in clean sterilized rubber stoppered glass vials and stored at -20°C until used for biochemical analysis. Serum total proteins and serum albumin were determined calorimetrically according to[12,13] respectively. Serum globulin was determined by subtracting the albumin value from the total proteins in the same sample, according to.[14] Total serum lipids, serum cholesterol and serum triglycerides concentrations were determined according to[15-17] respectively. LDH activity, CRP, ALT and AST activities and serum ALP in serum were done according to[18-21] respectively.

Respiratory signs and mortality rate were recorded daily. Air sac lesion scoring was recorded visually, weekly, for 3 weeks PI. *MG* re-isolation from air sacs and tracheas was carried out on Frey's broth and agar medium[7] while *E. coli* re-isolation from air sacs was carried out on MacConkey's agar media (OXOID, Basingstoke, UK) and incubated aerobically at 37°C for 24 h. The identification of *E. coli* was according to.[22]

The β procedure of (HI) test in microtiter was carried out as described by[23] for detection of antibody titers against Newcastle disease virus NDV at 35 days of age.

Statistical analysis

Data were subjected to one way analysis of variance using the General Linear Models (GLM) procedure of SAS User's guide.[24] Duncan's Multiple Range Test[25] was used to separate means when separation was relevant. Any significant differences for all measured parameters at the probability level of ($P \leq 0.05$) among the experimental groups.

RESULTS AND DISCUSSION

This work was carried out at Faculty of Veterinary Medicine, Damanhour University to investigate the effects of the enzyme Serrapeptase, treatment on growth

performance, immune response and some blood parameters of broiler chickens.

The experimental design appears as if it was lacking a 5th group that should have been treated with the enzyme serrapeptase, but the reason that we did not include this group that the product manufacturer stated that this enzyme is not used alone but it should be always used in combination with antibiotics in case of treatment of bacterial infection as it is does not have an antibacterial properties. Also, we should emphasize that the choice of ciprofloxacin as a model antibiotic in this study was decided upon the sensitivity test for both *E. coli* and *Mycoplasma gallisepticum*.

Date in table 1 showed that there was insignificant difference at the starting period of the trial while, in the final part, the final weight and relative growth rate (R.G.R) were numerically increased in body weight of group 3, which was treated with Serrapeptase 2g /liter drinking water. In this group, a significant difference in feed intake when compared with the other groups. Also this group (3) showed a better feed conversion ratio FCR (92%) relative to control, because of high body gain and low feed intake when compared with other groups. Chickens of group 3 showed an improvement in final body weight (102%) when compared with the control. Also chickens of group 3 showed highest body gain in all treated groups and had (103%) body gain relative to control. This may due to anti-inflammatory[26] and to proteolytic ability characteristics of Serrapeptase induced by the experimental infection with *E. coli* and *Mycoplasma gallisepticum.*

Table 1: Effect of Serrapeptase supplementation on broiler performance

Parameters	Groups			
	1 Control non infected, non treated	2 infected, non treated	3 Infected; treated (ciprofloxacin + Serratiopeptidase 2g / L)	4 Infected; treated (ciprofloxacin + Serratiopeptidase 1g / L)
Initial body weight	41.22	41.44	41.26	41.22
Final body weight	1380.00 ± 49.71a	1290.00 ± 54.46b	1421.00 ± 46.87a	1357.00 ± 51.92a
Weight gain	1338.78 ± 49.54a	1248.56 ± 54.27a	1379.74 ± 46.71a	1315.78 ± 51.74a
Relative growth rate	188.30 ± 0.42ab	187.11 ± 0.46b	188.51 ± 0.40a	187.85 ± 0.44ab
Feed intake	2179.00 ± 0.00c	2264.00 ± 0.00a	2070.00 ± 0.00d	2248.00 ± 0.00b
Feed conversion ratio	1.63 ± 0.07bc	1.81 ± 0.08a	1.50 ± 0.07c	1.71 ± 0.08ab
Mortality rate	11.11%	25.9%	0	11.11%

Means within the same raw carry different superscripts are significantly different (P≤ 0.05)

Table 2: Effect of Serrapeptase on serum proteins of broiler

Parameters	Groups			
	1 Control non infected, non treated	2 infected, non treated	3 Infected; treated (ciprofloxacin + Serratiopeptidase 2g / L)	4 Infected; treated (ciprofloxacin + Serratiopeptidase 1g / L)
Total protein (mg/dl)	8.02 ± 0.18^{a}	6.86 ± 0.11^{c}	7.88 ± 0.17^{ab}	7.81 ± 0.15^{ab}
Albumin (mg/dl)	4.48 ± 0.18^{a}	2.67 ± 0.09^{b}	4.52 ± 0.16^{a}	4.50 ± 0.18^{a}
Globulin (mg/dl)	3.54 ± 0.08^{b}	4.19 ± 0.06^{a}	3.36 ± 0.13^{bc}	3.31 ± 0.05^{bc}

Means within the same raw carry different superscripts are significantly different (P≤ 0.05)

Table 3: Effect of Serrapeptase treatment on serum lipids of broiler

Parameters	Groups			
	1 Control non infected, non treated	2 Infected, non treated	3 Infected; treated (ciprofloxacin + Serratiopeptidase 2g / L)	4 Infected; treated (ciprofloxacin + Serratiopeptidase 1g / L)
Total lipids (mg/dl)	330.23 ± 3.52^{c}	394.42 ± 4.32^{a}	363.16 ± 2.45^{b}	360.28 ± 2.36^{b}
Total cholesterol (mg/dl)	141.23 ± 2.57^{b}	166.34 ± 3.65^{a}	139.41 ± 3.58^{b}	$137.25\pm.3.29^{b}$
Triglycerides (mg/dl)	115.12 ± 4.62^{a}	118.34 ± 3.67^{a}	113.91 ± 5.48^{a}	116.12 ± 3.25^{a}

Means within the same raw carry different superscripts are significantly different (P≤ 0.05)

Table 4: Effect of Serrapeptase treatment on serum enzyme markers for inflammation

Parameters	Groups			
	1 Control non infected, non treated	2 Infected, non treated	3 Infected; treated (ciprofloxacin + Serratiopeptidase 2g / L)	4 Infected; treated (ciprofloxacin + Serratiopeptidase 1g / L)
LDH (U/L) (lactate dehydrogenase)	103.63 ± 5.67[b]	125.24 ± 6.24[a]	86.55 ± 4.51[c]	70.86 ± 5.59[d]
CRP (mg/L) (C Reactive protein)	8.87±1.18[d]	72.86±4.65[a]	43.59±3.87[b]	26.93±3.54[c]
ESR (mm/hr) (erythrocyte sedimentation rate)	15.12±2.33[b]	44.34±4.72[a]	18.87±3.39[b]	13.69±2.67[b]

Means within the same raw carry different superscripts are significantly different (P≤ 0.05)

Table 5: Effect of serrapeptase treatment on liver function enzymes

Parameters	Groups			
	1 Control non infected, non treated	2 Infected, non treated	3 Infected; treated (ciprofloxacin + Serratiopeptidase 2g / L)	4 Infected; treated (ciprofloxacin + Serratiopeptidase 1g / L)
ALT (U/L)	38.46±4.55b	51.53±2.34a	37.72±2.43b	36.23±2.51b
AST (U/L)	67.14±6.53b	83.31±7.21a	69.42±4.35b	65.64±3.57b
ALP (U/L)	103.31±7.64a	108.18±8.84a	105.66±5.52a	102.82±6.73a

Means within the same raw carry different superscripts are significantly different (P≤ 0.05)

Table 6: Effect of Serrapeptase treatment on (log2) HI titers, air sac lesion scores at 35 days of age and re-isolation of *E.coli* and *MG*

Parameters measured	Groups			
	1 Control non infected, non treated	2 Infected, non treated	3 Infected; treated (ciprofloxacin + Serratiopeptidase 2g / L)	4 Infected; treated (ciprofloxacin + Serratiopeptidase 1g / L)
HI titers of ND	$2^{6.6}$	2^{6}	$2^{7.3}$	$2^{5.3}$
Air sac lesion scoring	0	3	1	2
MG re-isolation	0%	100%	33.3%	66.6%
E. coli re-isolation	0%	100%	66.6%	66.6%

Figure 1: Normal chickens in group 1.

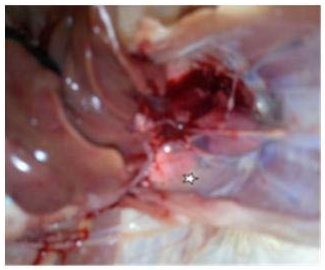

Figure 2: score 0 air sacculitis in control non infected, non treated group 2 (star).

Figure 3: Conjunctivitis in group 2 (arrow).

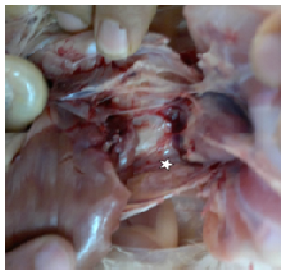

Figure 4: score 3 air sacculitis in infected non treated group 3 (star).

Serrapeptase supplementation has a positive effect on general health status of infected birds so it was improved bird performance through this experiment.

Concerning the effect of Serratiopeptidase on total serum proteins of infected chickens, (Table 2), the results revealed that Serratiopeptidase significantly corrected the total protein from (6.86±0.11) in the infected non treated group to (7.88±0.17 and 7.81±0.15) in groups 3 and 4 respectively. Regarding albumin as compared with the infected groups, the enzyme treatment also increased the values in serum from (2.67±0.09) to (4.52±0.16 and 4.50±0.18) in groups 3 and 4 respectively. On the contrary total serum globulin showed a significant increase in infected non treated group (4.19±0.06) compared to the non infected (3.54±0.08) and the enzyme treatment produced decreased it to the level of the control non infected group (3.36±0.13 and 3.31±0.05) in groups 3 and 4 respectively. As it is shown, the enzyme treatment

increased the total serum proteins and albumin to the control level; also it decreased the values of serum globulin in the 2 treated groups to the level of the control, non infected group.

Table (3) showed that Serratiopeptidase significantly decreased the total cholesterol from 166.34±3.65 in group 2 to the level very near to the non infected groups to 139.41±3.58 and 137.25±.3.29 in groups 3 & 4 respectively. Also the same pattern of action can be seen with triglycerides and total lipids.

There were shortages in literature reporting the effect of Serratiopeptidase on protein and lipid profiles.

Table (4) demonstrated that Serratiopeptidase decreased significantly the inflammatory markers tested (CRP and ESR). The C reactive protein was diminished from 72.86±4.65 in the non treated groups 2 to 43.59±3.87 and 26.93±3.54 in groups 3&4 respectively. The erythrocyte sedimentation rate showed similar changes whereas in the infected group had a mean value of 44.34±4.72 which decreased down in treated groups 3&4 to 18.87±3.39 and 13.69±2.67 respectively.

In addition to significant decrease in serum LDH (as an indicator of kidney, heart, skeletal muscle, brain, liver and lung damages) from 125.24±6.24 to 86.55±4.51 and 70.86±5.59 in groups 3 & 4 respectively.

Depending upon this mechanism of decreasing the destruction, damage and inflammation, this enzyme could increase the bioavailability of antibiotic at the sites of infection like the lungs, air sacs, heart and liver.

Regarding the effect of serratiopeptidase on liver enzymes, table (5) showed that serratiopeptidase and antibiotic significantly decreased the ALT in from 51.53±2.34 U/L in the infected non treated group to 37.72±2.43 and 36.23±2.51 U/L in the infected and treated groups 3 & 4 respectively. Also the same type of results was shown with AST, where the mean of values of the infected non treated group was 83.31±7.21 U/L which declined by the enzyme treatment to 69.42±4.35 and 65.64±3.57 U/L in groups 3& 4 respectively. ALP was also lowered with the enzyme and antibiotic treatment but insignificantly.

Serratiopeptidase, a proteolytic enzyme derived from non-pathogenic enterobacteria Serratia sp E-15 found in silkworms,[27] has anti-inflammatory and anti-edemic activity in a number of tissues. Anti-inflammatory mechanism involve degradation of inflammatory mediators, suppression of edema, activation of fibrinolysis, reduction of immune complexes and proteolytic modification of cell-surface adhesion molecules which guide inflammatory cells to their targets.[26] Serratiopeptidase showed significant anti-inflammatory effect in soft tissue injury to upper limb, lower limb or both reflected in decrease in swelling more as compared to results observed by Aceclofenac.[28]

Lactic acid dehydrogenase (LDH) is an intra cellular enzyme that is widely distributed in the tissues of the body, particularly in the kidney, heart, skeletal muscle, brain, liver and lung. Its increase usually indicates cellular death and subsequently leakage of the enzyme from the cell. Extra protein is often released from the site of inflammation; these proteins can be readily detected in the bloodstream and are therefore referred to as inflammatory markers. Perhaps the most commonly used marker of inflammation is C-reactive protein (CRP). CRP is synthesized in the liver and despite being a minor plasma protein; levels are dramatically increased within 6 hours after the onset of inflammation. The final increase can sometimes be as much as 60-fold. Furthermore CRP is much more specific than some of the other commonly used markers of inflammation such as the erythrocyte sedimentation rate (ESR). Falling CRP levels are a useful indication of response to antibacterial or anti-inflammatory therapy. Serratiopeptidase administration succeeded to improve the biochemical parameters related to liver functions, lipid profiles and inflammatory markers reflected in significant increased total protein and albumin and reduced globulin, significant decreased lipid profiles and significant decreased inflammatory markers in infected chickens, Tables (2-5).

On the contrary[29] reported that Serratiopeptidase did not have anti-inflammatory effect in rat paw edema model compared with Diclofenac sodium.

The researches on this enzyme in the field of avian medicine are not available; hence our discussion is focused on the human medicine. Similar observations have been made on Serratiopeptidase by [30-31] on patients of fibrocystic breast disease and concluded that Serratiopeptidase to be superior to placebo for improvement of breast pain, swelling and induration. Another trial by[32] also who concluded the efficacy of Serratiopeptidase as an anti-inflammatory enzyme. [33] conducted a double blind study to determine the effect of Serratiopeptidase on postoperative swelling and pain in patients who were treated for rupture of knee ligament. The patients were given Serratiopeptidase and showed 50% reduction in swelling and became pain-free more rapidly compared with controls. In animal studies also efficacies of Serratiopeptidase as an anti-inflammatory have been described.[34] Another animal study showed that Serratiopeptidase is orally effective and possesses an anti-inflammatory activity, which is nearly equivalent to Diclofenac sodium in both acute and chronic phases of inflammation.[35]

Serratiopeptidase has been shown to enhance the activity of several antibiotics including Ampicillin, Ciclacillin, Cephalexin and cefotiam.[36] This enzyme can actually team up with antibiotics and deliver increased concentrations of antibiotics to the site of the infection.

Regarding clinical examination and post-mortem findings, it was noticed that respiratory signs (as coughing, sneezing and head swelling) started 3 days PI in groups

2&3 and started 5 days PI in group 4 and were milder in group 4 than the other 2 groups along the experiment (Figures 1&3). The average HI log 2 titers for Newcastle disease virus (NDV) were $2^{6.6}$ in chickens of group1, and 2^{6} in chickens of group 2, while it was $2^{7.3}$ in chickens of group 3, and $2^{5.3}$ in chickens of group 4.

The cumulative air sac lesion scores were 0, 1, 2, 3 in chickens of groups 1, 3, 4 and 2 respectively (Table 5) (Figures 2 & 4).

As seen from the results, it is clear that the most severe air sac lesion was in group 2 that was infected but non treated only, and the least severe lesions were in group 3 that received serrapeptase at 2g / L DW with ciprofloxacin. The re-isolation of *MG* from tracheal swabs of infected chickens was negative, from chickens of group 1, and 100% in chickens of group 2, while it was 33.3% from chickens of group 3, and 66.6% from chickens of group 4. Also the group 3 revealed the least re-isolation rate for *MG* which indicates usefulness of synergism between ciprofloxacin and serrapeptase at a dose of 2g / L in decreasing the establishment in the respiratory tract of chickens and shedding of *MG* into the surrounding atmosphere. Also the re-isolation of *E. coli* from chickens of group 1 was negative, and 100% in chickens of group 2, while the concentration of the enzyme in drinking water did not affect the rate of *E. coli* re-isolation as it was 66.6% in chickens of both groups 3&4 (Table 6).

CONCLUSION

From the above mentioned results, it can be concluded that treatment of chickens with both Serratiopeptidase + ciprofloxacin for 3 days / week for 5 weeks in the broiler life against bacterial infections, significantly enhanced the performance parameters like growth rate, and feed conversion efficiency. Also the treatment improved the immunological response to NDV vaccination, and decreased the re-isolation and shedding of *MG and E. coli.* Also the liver function enzymes, serum biochemical parameters, anti-inflammatory parameters and survival rate of chickens were all improved.

This study recommends the use of this natural enzyme in the field of avian medicine in association with the antibiotics to alleviate the hazards induced by infections especially those causing CCRD as *MG* and *E. coli.*

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