Research Note

Influence of different rearing systems on natural immune parameters in broiler turkeys

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ABSTRACT The aim of this study was to determine serological values of lysozyme, hemolytic complement levels (alternative pathway), and bactericidal activity of serum in turkeys kept in different rearing systems (industrial, backyard, and experimental). Results showed that the values for serum bactericidal activity and hemolytic complement levels increased with age, and their values were higher in experimental and in industrial turkeys than in turkeys reared in backyard. Lysozyme concentration showed a similar pattern; its value was higher in the industrial and experimental groups than in the backyard group. Data obtained suggest that rearing system can have an influence on the natural immune parameters considered; experimental and industrial groups showed a similar trend, differentiated from that observed in the backyard group. In the backyard group, the values observed may suggest that hybrid turkeys, selected for high production, have difficulty with being reared outside where predators (foxes and weasels) and weather conditions could be responsible for a stress situation.

Key words: broiler turkey, natural immunity, welfare

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INTRODUCTION

In recent years increasing attention has been focused on animal welfare in response to intensification and specializations in husbandry, done mainly for economic purposes. A high stocking density and the use of highly concentrated feed, responsible for unsuitable management, have been frequently considered to influence health and welfare in poultry production (e.g., poor litter quality, high ammonia; Thomas et al., 2004; Villagrá et al., 2009). Monitoring of natural immunity parameters can give useful information on the health and, as a consequence, welfare of animals (Olff, 1999; Padgett and Glaser, 2003; Broom, 2006). Chronic stress can influence the natural immune system, predisposing the animals to conditioned pathologies because it represents the first and rapid immune response against extraneous organisms (Kimbrell and Beutler, 2001). The variation of several parameters of the nonadaptive immunity, such as heterophil:lymphocyte blood ratio, lysozyme (Fevolden et al., 1994; Ng et al., 1999), hemolytic complement levels (Tort et al., 1996; Star et al., 2006), and serum bactericidal activities (Hajji et al., 1990) have been investigated in response to uncomfortable environmental factors in different animals. The bactericidal activity showed a significant variation in newly introduced breed gilts in relation to environment and food changes (Battistacci et al., 2007). Various factors play a role in influencing levels of these parameters: different breeds of turkeys showed significant phenotypic variation in lysozyme and hemolytic complement levels as well several growth promoters (Bayyari et al., 1997; Sotirov et al., 1998; Sotirov et al., 2001). The effect of the rearing system on lysozyme and hemolytic complement levels was also investigated in broiler chickens raised in batteries and on hard floor (Stoyanchev et al., 1997) and in different hybrid turkeys raised on a slat floor and on litter (Yotova et al., 2004). To evaluate differences in innate immunity response in relation to rearing conditions, this study aimed to investigate the lysozyme, hemolytic complement levels, and serum bactericidal activities in broiler turkeys raised in industrial, backyard, and controlled experimental conditions to point out the possible differences in serum levels.

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MATERIALS AND METHODS

Experimental Design

The trial was performed over a 100-d period during the autumn and winter using 1-d-old British United Turkey broiler turkeys (British United Turkey Ltd., Chester, UK). Forty female meat turkeys (referred to as group A) were investigated in an industrial farm, consisting of 13,500 birds, with a natural light and ventilation system. The selected birds were kept in a separate wired area throughout the trial. The stock density during the first 2 wk was 8 to 10 birds/m^2, after which it was approximately 6 birds/m^2 until slaughter at 100 d. Twenty 1-d-old turkeys (referred to as group B) were collected from the same commercial farm and raised in premises where light, temperature, ventilation, and density were optimized according to Anonymous (2000). The area was 4 m^2 and was managed so that in the first week the stock density was approximately 8 animals/m^2 and 4 animals/m^2 until d 100. Ten 1-day-old broiler female turkeys (referred to as group C), collected from the same industrial farm, were raised inside for the first 2 wk and were subsequently moved to a backyard pen (6 m long × 4 m wide) subdivided into 2 connected spaces, one of which opened. During the day the turkeys could enter a large external space surrounded by wire.

All groups were given rhinotracheitis virus and Newcastle disease virus vaccinations. Vaccination for hemorrhagic enteritis and a recall vaccination for Newcastle disease virus were also administered after 1 mo by the intramuscular route. The feed formulation (Valigi Group Italy, Perugia, Italy) was the same for all groups and changed in relation to different growth periods. The first period diet provided 26% CP and 2,800 kcal/kg of ME, whereas the second period diet provided 23% CP with 2,900 kcal/kg of ME. In the third period the CP was reduced to 18% and the ME was increased to 3,100 kcal/kg.

Blood Sampling

All groups were sampled at 1 (**T0**), 14 (**T1**), 23 (**T2**), 50 (**T3**), and 100 (**T4**) d of age using vacuum tubes without anticoagulant (Vacuette, Greiner Bio-One, Frickenhausen, Germany). Blood samples were incubated at room temperature for 2 h and centrifuged at 3,520 × *g* for 16 min, and the serum samples were stored under sterile conditions in aliquots at −80°C until use.

Serum Lysozyme

The serum lysozyme was assessed by the lysoplate assay (Osserman and Lawlor, 1966). Briefly, serum samples were reacted with a suspension of *Micrococcus lysodeikticus* inside an agar gel in 10-cm Petri dishes and then distributed in duplicate in 3-mm holes, 2 cm apart, at a regular distance of 1.5 cm from the dish edge. The reaction was carried out in a humidified incubator for 18 h at 37°C instead of in the environmental temperature, as described in the original protocol. The diameter of the lysed areas around serum samples and lysozyme standards of known concentration in phosphate buffer (0.066 *M*, pH 6.3) was assessed by calipers or rules. Under these conditions, lysozyme concentration (μg/mL) was proportional to the diameter of lysed areas and was determined from a standard curve created with reference preparations of egg white lysozyme (Sigma-Aldrich, St. Louis, MO).

Total Hemolytic Complement Levels

Total hemolytic complement levels (alternative pathway) were assessed as described previously (Seyfarth, 1976) using rabbit erythrocytes. The volumes of the reagents were modified to perform the test in microtiter plates at a final volume of 125 μL/well (100 μL of serum dilutions + 25 μL of 3% rabbit erythrocytes). The 0 and 100% hemolysis controls were set up in each plate at the same volume in veronal buffer (pH 7.3) and distilled water, respectively. Titers were expressed as 50% hemolytic units per 100 microliters (the test volume of sera). Reference standard sera were used as control toward different batches of rabbit erythrocytes.

Serum Bactericidal Activity

The test for determining serum bactericidal activity (**SBA**), used for a long time as a plate count assay (Dorn et al., 1980), was adapted in our laboratory to a turbidimetric assay in microtiter format (Amadori et al., 1997). Briefly, nonpathogenic *Escherichia coli* was grown until log phase in 20 mL of brain heart infusion (**BHI**) broth (Biolife Italiana, Milan, Italy) and frozen at −80°C in sterile skim milk. For each test 1 aliquot was thawed, resuspended in 15 mL of BHI medium, and incubated at 37°C until optical density of 590 nm was doubled. Then, bacteria were diluted 1:100 in sterile saline solution. Test reagents were distributed into wells of sterile, U-bottomed microtiter plates according to the following scheme: 50 μL of test serum (in duplicate) added to 50 μL of veronal buffer, 100 μL of BHI broth, and 10 μL of 1:100-diluted bacterial suspension. Controls of sterility were set up without bacteria (negative control). Controls of bacterial growth (positive control) were set up without serum. The missing components were replaced by Veronal buffer at the same volumes. Plates were incubated in a humidified box at 37°C for 18 h. They were then read spectrophotometrically in an ELISA reader at 690 nm, with blank set on the sterility control. The percentage of SBA was derived from the following formula (OD = optical density):

$$\%\ \text{SBA} = 100 - \left\{\frac{\text{OD test sample}}{\text{OD growth control}} \times 100\right\}.$$

Statistical Analysis

Statistical analysis was performed using one-way ANOVA at all time points. Differences between means were significant at $P < 0.05$ and at $P < 0.001$.

RESULTS

Three parameters of the innate immunity were analyzed in turkeys raised in industrial, backyard, and controlled experimental conditions: lysozyme, total hemolytic complement levels, and serum bactericidal activity (Tables 1, 2, and 3). Lysozyme concentration showed a similar pattern in groups A, B, and C at T0 (Table 1). Lysozyme concentration was significantly higher in groups A and B than in group C at T1, T2, and T3, and it subsequently declined at T4. Results showed that the levels of hemolytic complement increased in the 3 turkey groups with age; in particular, values in group C were significantly lower than in groups A and B at T0 but were higher at T1 and T2. Values in groups A and B were significantly higher than values in group C at T3 and T4 (Table 2). In this trial the bactericidal activity increased progressively in all groups. The highest value was detected at T3 in groups A and B whereas in group C it increased at T4 (Table 3).

DISCUSSION

The relationship among welfare, immunity, and health has been considered by many authors (Olff, 1999; Padgett and Glaser, 2003; Broom, 2006). In this study we investigated possible variations of 3 serological immune parameters (bactericidal activity, lysozyme, and hemolytic complement levels) in relation to different turkey rearing systems. The bactericidal property of blood may be attributed to the presence of complement factors and small quantity of natural antibodies (Michael et al., 1962) and other occurring antibacterial substances such as β-lysine and lectin (Donaldson et al., 1964; Kawasaki et al., 1989). Results showed that bactericidal activity increased with age until T3 in groups A and B and until T4 in group C, reflecting a progressive development of the immune system as demonstrated in previous investigations (Skeeles et al., 1980; Moscati et al., 2008). The decrease of the bactericidal level in groups A and B at T4 could be justified by space limitations as the turkeys grew in size. The importance of stocking density as a stress factor influencing animal welfare is controversial. Dawkins et al. (2004) concluded that the stocking density in chickens is less important than other factors, such as nutrition and genetics, acting in the poultry environment. On the contrary, Villagrá et al. (2009) demonstrated that an excessive density causing a chronic stress situation was responsible for a pronounced corticosterone response in chickens. The serum bactericidal activity, lower than that observed for the other 2 groups, was noticed in backyard turkeys, probably reflecting a stressful adaptation to external environmental factors such as temperature ranges. Cold temperature can act as a stress factor in animals genetically selected to grow in an in-

Table 1. Statistical analysis of lysozyme (mean ± SD) in 3 turkey groups[1]

	Lysozyme (μg/mL)		
Sample[2]	Group A	Group B	Group C
T0	3.45 ± 1.2	3.45 ± 1.2	3.76 ± 3.7
T1	8.47 ± 2.9	9.74 ± 5.2	1.85*** ± 2.2
T2	9.07 ± 2.8	6.89 ± 1.7	1.79*** ± 0.7
T3	7.79 ± 3	6.88 ± 2.2	1.49*** ± 0.4
T4	3.81 ± 0.8	3.86 ± 1.3	3.19 ± 3.5

[1]The time-course of lysozyme was investigated in 3 turkey groups. Group A: 40 female meat turkeys raised on an industrial farm. Group B: twenty 1-d-old turkeys collected from the industrial farm and raised under optimized conditions according to Anonymous (2000). Group C: ten 1-d-old turkeys collected from the industrial farm, raised inside for 2 wk, and subsequently moved to a backyard pen.

[2]T0 = d 1; T1 = d 14; T2 = d 23; T3 = d 50; T4 = d 100.

***$P < 0.001$.

Table 2. Statistical analysis of hemolytic complement level (mean ± SD) in 3 turkey groups[1]

	Hemolytic complement levels (CH50[3] μg/100 μL)		
Sample[2]	Group A	Group B	Group C
T0	14.02 ± 6.6	14.02 ± 6.6	2.33* ± 0.7
T1	15.06 ± 3.5	13.83 ± 3.7	18.30 ± 7.4
T2	19.84 ± 2.7	19.86 ± 1.2	29.86*** ± 7.1
T3	80.68 ± 19	93.05 ± 12.6	32.86*** ± 12.2
T4	100.00 ± 3.77	100.00 ± 5.35	45.18*** ± 12.5

[1]The time-course of hemolytic complement levels was investigated in 3 turkey groups. Group A: 40 female meat turkeys raised on an industrial farm. Group B: twenty 1-d-old turkeys collected from the industrial farm and raised under optimized conditions according to Anonymous (2000). Group C: ten 1-d-old turkeys collected from the industrial farm, raised inside for 2 wk, and subsequently moved to a backyard pen.

[2]T0 = d 1; T1 = d 14; T2 = d 23; T3 = d 50; T4 = d 100.

[3]CH50 = total complement activity.

*$P < 0.05$; ***$P < 0.001$.

Table 3. Statistical analysis of bactericidal activity (mean values ± SD) in 3 turkey groups[1]

	Serum bactericidal activity (%)		
Sample[2]	Group A	Group B	Group C
T0	2.63 ± 4	2.63 ± 4	10.18 ± 7.84
T1	20.33 ± 9.2	19.20 ± 10.6	11.92 ± 6.2
T2	37.39 ± 16.3	30.60 ± 18	21.73 ± 10.1
T3	61.67 ± 28.6	61.38 ± 8.6	27.61* ± 18.9
T4	40.03 ± 5.1	51.07 ± 7.2	37.48 ± 11.9

[1]The time-course of hemolytic complement levels was investigated in 3 turkey groups. Group A: 40 female meat turkeys raised on an industrial farm. Group B: twenty 1-d-old turkeys collected from the industrial farm and raised under optimized conditions according to Anonymous (2000). Group C: ten 1-d-old turkeys collected from the industrial farm, raised inside for 2 wk, and subsequently moved to a backyard pen.

[2]T0 = d 1; T1 = d 14; T2 = d 23; T3 = d 50; T4 = d 100.

*$P < 0.05$.

dustrial system, such as pigs (Moscati et al., 2004). Furthermore, the presence of predators (mammalian or birds) as stress agents should have been considered. The hemolytic complement levels show the amount of free complement in the serum that is not linked to antibodies and potentially reactive. Its basal blood level can be considered positively related to natural immune system activity. High levels of this activity are indicative that the birds have not consumed the complement available in the serum for specific immune reactions against various pathogens through the conventional pathway (Ricklin et al., 2010). Our results denoted that the levels of hemolytic complement increased with age in all groups in relation to progressive immune system development. Hemolytic complement levels in group C were unexpectedly lower than those in groups A and B at T0 because, using industrials turkeys and not inbred, such biological events are possible. Even though hemolytic complement values in group C increased consistently over time and at T1 and T2 they were higher than values in groups A and B, it is evident that the values were less overall than in groups A and B. This could be justified by the lack of particular antigenic influences on hemolytic complement levels, probably attributed to the strict application of hygienic and sanitary regulations in experimental and industrial conditions, as already observed by Yotova et al. (2004). In the backyard conditions, by that time other antigenic pressure of environment could have been able to robustly trigger the hemolytic complement levels of turkeys. Further studies are needed to establish the effective role played by environmental antigenic pressure on hemolytic complement levels. Finally, it should be underlined that the value detected for serum bactericidal activity and hemolytic complement in group C can reflect a difficulty to adapt to outside conditions for turkeys that are genetically selected for high production and accustomed to being raised indoors. The lysozyme is able to damage bacterial cell walls by attacking the peptidoglycan. Because this enzyme is largely present in granules of several kind of cells, its serum concentration can give useful information about granulocyte activities and about the functionality of the monocyte-macrophage system. Therefore, it can be reasonably considered as a possible marker for the quantification of pathogens in the environment (Cohn, 1968; Gordon et al., 1974). In this study the lysozyme concentrations showed a trend independent of age in all groups, as already detected in swine by Moscati et al. (2004). Compared with group C, groups A and B had significantly higher levels of lysozyme activity at T1, T2, and T3 (Table 1). In our study the values detected for lysozyme concentration were higher than the values detected by Sotirov et al. (1998) and Yotova et al. (2004), probably because of the differences in the analysis protocols and the genetic lines tested. Our data showed that dissimilarity of the rearing systems seemed to have an influence on the innate immune system parameters monitored in our work. Results obtained for groups A and B showed a similar trend, differentiated from that observed in group C. Previous studies highlighted the importance of the rearing systems in meat turkeys in the different levels of hemolytic complement and lysozyme, when the birds were bred on slat floor and on a litter (Yotova et al., 2004). It is largely known that the immune system is strictly related to animal welfare because it reflects the capability to react against external noxae (Broom, 2006). It is difficult to establish which kind of farming could influence the natural immunity parameters considered, but we could hypothesize that turkeys genetically selected for high production experienced difficulty in adapting to backyard rearing. In backyard conditions, predators (foxes and weasels) and other external factors (weather conditions) could be responsible for a stress situation, whereas excessive stocking density and high competition can act as the most critical factors in industrial farms. Further studies are needed to determine the role of the natural immune parameters as reliable markers of welfare and health status to create a regular profile that is useful for monitoring both in the field.

REFERENCES

Amadori, M., L. Archetti, M. Frassinelli, M. Bagni, E. Olzi, M. Caronna, and M. Lanterni. 1997. An immunological approach to the evaluation of welfare in Holstein Frisian cattle. J. Vet. Med. B 44:321–327.

Anonymous. 2000. Manual British United Turkeys. Warren Hall, Chester, UK.

Battistacci, L., M. Sensi, M. Timi, S. Marchi, and L. Moscati. 2007. Adaptation ability of natural immune system in *Cinta senese* swine and commercial hybrid pigs in outdoor breeding farms. Page 44 in Proc. 6th International Symposium on the Mediterranean Pig.

Bayyari, G. R., W. E. Huff, N. C. Rath, J. M. Balog, L. A. Newberry, J. D. Villines, J. K. Skeeles, N. B. Anthony, and K. E. Nestor. 1997. Effect of the genetic selection of turkeys for increased body weight and egg production on immune and physiological responses. Poult. Sci. 76:289–296.

Broom, D. M. 2006. Behaviour and welfare in relation to pathology. Appl. Anim. Behav. Sci. 97:71–83.

Cohn, Z. A. 1968. The differentiation of macrophages. Page 101 in Proc. Symposium of the International Society of Cell Biology. K. B. Warren, ed. University of Michigan, Ann Arbor.

Dawkins, M. S., C. A. Donnelly, and T. A. Jones. 2004. Chicken welfare is influenced more by housing conditions than by stocking density. Nature 427:342–344.

Donaldson, D. M., R. S. Jensen, B. M. Jensen, and A. Matheson. 1964. Serological relationships among β-lysin, plakin, and leukin. J. Bacteriol. 88:1049–1055.

Dorn, W., G. Mehlhorn, and C. Klemm. 1980. Blood bactericidal activity in calves. Arch. Exp. Veterinarmed. 34:635–650.

Fevolden, S. E., K. H. Røed, and B. Gjerde. 1994. Genetic components of post-stress cortisol and lysozime activity in Atlantic salmon: Correlation to disease resistance. Fish Shellfish Immunol. 4:507–519.

Gordon, S., A. Todd, and Z. A. Cohn. 1974. In vitro synthesis and secretion of lysozyme by mononuclear phagocytes. J. Exp. Med. 139:1228–1248.

Hajji, N., H. Sugita, S. Ishii, and Y. Deguchi. 1990. Serum bactericidal activity of carp (*Cyprinus carpio*) under supposed stressful rearing conditions. Pages 50–54 in Bulletin of the College of Agriculture and Veterinary Medicine. Vol. 47. Nihon University, Nichidai-Nojuho Tokyo, Japan.

Kawasaki, N., T. Kawasaki, and I. Yamashina. 1989. A serum lectin (mannan-binding protein) has complement-dependent bactericidal activity. J. Biochem. 106:483–489.

Kimbrell, D. A., and B. Beutler. 2001. The evolution and genetics of innate immunity. Nat. Rev. Genet. 2:256–267.

Michael, J. G., J. L. Whitby, and M. Landy. 1962. Studies on natural antibodies to gram-negative bacteria. J. Exp. Med. 115:131–146.

Moscati, L., L. Battistacci, P. Casagrande Proietti, M. Bazzuchi, C. Canali, and M. P. Franciosini. 2008. Investigation on some parameters of natural immunity in meat turkeys. Pages 7–8 in Proc. 7th International Symposium on Turkey Diseases.

Moscati, L., M. Sensi, M. Timi, C. Maresca, and L. Battistacci. 2004. Benessere animale: Stress da adattamento in un allevamento di suini a ciclo aperto in Umbria. Pages 157–158 in 6th Congresso Nazionale S.I.Di.L.V. Abano Terme (PD).

Ng, V., D. Koh, G. Chan, H. Y. Ong, S. E. Chia, and C. N. Ong. 1999. Are salivary IgA and lysozyme biomarkers of stress among nurses? J. Occup. Environ. Med. 41:920–927.

Olff, M. 1999. Stress, depression and immunity: The role of defense and coping styles. Psychiatry Res. 85:7–15.

Osserman, E. F., and D. P. Lawlor. 1966. Serum and urinary lysozyme (muramidase) in monocytic and monomyelocytic leukemia. J. Exp. Med. 124:921–952.

Padgett, D. A., and R. Glaser. 2003. How stress influences the immune response. Trends Immunol. 24:444–448.

Ricklin, D., G. Hajishengallis, K. Yang, and J. D. Lambris. 2010. Complement: A key system for immune surveillance and homeostasis. Nat. Immunol. 11:785–797.

Seyfarth, M. 1976. Komplementtitration. Pages 145–148 in Immunologische Arbeitsmethoden. H. Friemel, ed. VEB Gustav Fischer Verlag, Jena, Germany.

Skeeles, J. K., R. G. Stewart, J. Brown, R. K. Page, and D. Russel. 1980. Hemolytic complement activity in broiler chickens and turkeys. Poult. Sci. 59:1221–1225.

Sotirov, L. K., S. T. Denev, I. Tsachev, M. Lalev, M. Oblakova, and Z. Porfirova. 2001. Effect of different growth promoters on lysozyme and complement activity. II. Studing in turkeys. Revue Méd. Vét. 152:67–70.

Sotirov, L. K., M. T. Lalev, M. G. Oblakova, Z. Porfirova, S. Tanchev, and G. Nikolov. 1998. Lysozyme and complement activity in different turkey breeds. Rév. Méd. Vét. 149:309–312.

Star, L. S., M. G. B. Nieuwland, B. Kemp, and H. K. Parmentier. 2006. Effect of stress on haemolytic complement activity in layer lines. Page 73 in Proc. 9th Avian Immunology Research Group Meeting.

Stoyanchev, T. K., L. K. Sotirov, I. T. Yotova, and K. Uzunova. 1997. Natural resistance peculiarities in broiler chickens of different blood-group genotypes raised in batteries and on hard floor. Bull. J. Agric. Sci. 3:647–649.

Thomas, D. G., V. Ravindran, D. V. Thomas, B. J. Camden, Y. H. Cottam, P. C. H. Morel, and C. J. Cook. 2004. Influence of stocking density on the performance, carcass characteristics and selected welfare indicators of broiler chickens. N. Z. Vet. J. 52:76–81.

Tort, L., J. O. Sunyer, E. Gómez, and A. Molinero. 1996. Crowding stress induces changes in serum haemolytic and agglutinating activity in the gilthead sea bream *Sparus aurata.* Vet. Immunol. Immunopathol. 51:179–188.

Villagrá, A., J. L. Ruiz de la Torre, G. Chacón, M. Lainez, A. Torres, and X. Manteca. 2009. Stocking density and stress induction affect production and stress parameters in broiler chickens. Anim. Welf. 18:189–197.

Yotova, I. T., L. K. Sotirov, T. K. Stoyanchev, N. A. Bozakova, D. J. Yarkov, K. T. Stoyanchev, M. G. Oblakova, and M. T. Lalev. 2004. Study on the level of natural humoral immunity in turkey broilers bred on two floor types. Bulg. J. Vet. Med. 7:51–56.