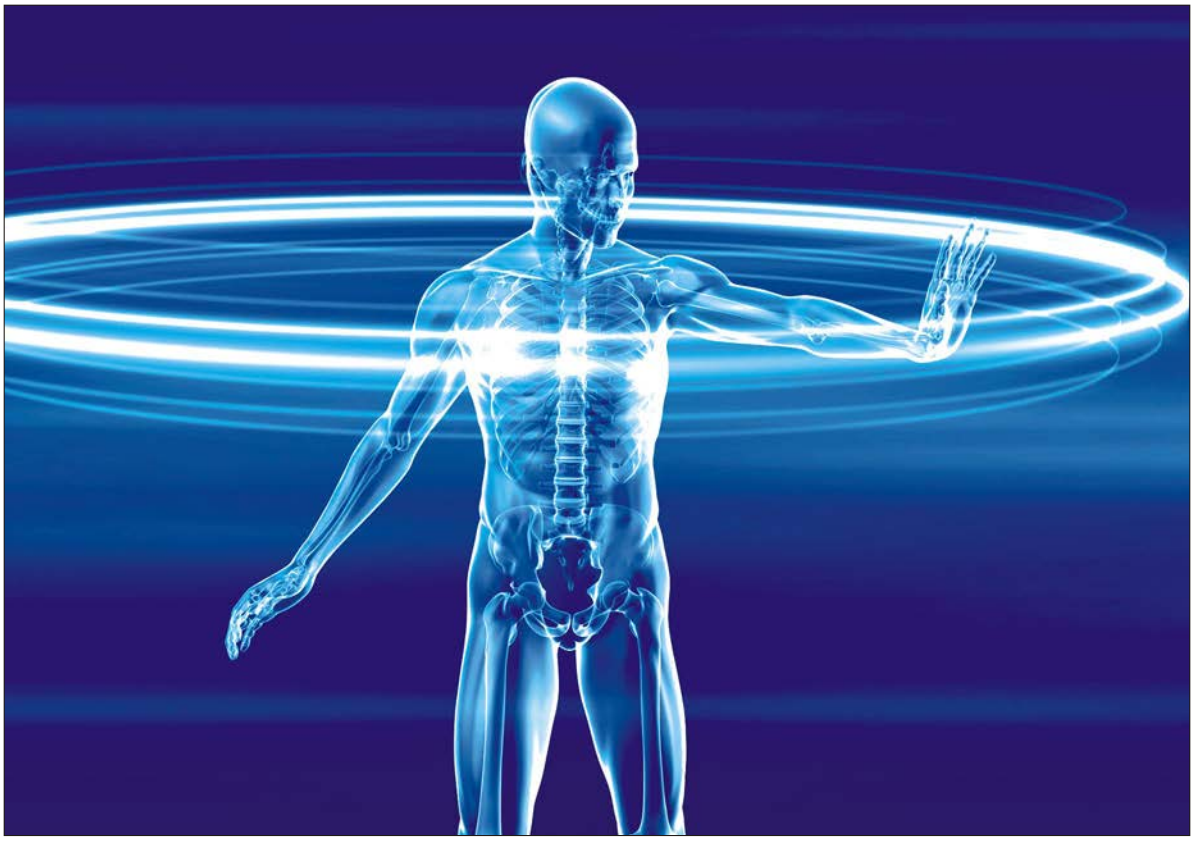

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Immunomodulatory Effect of Effective Microorganisms (EM®) in Chickens

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ABSTRACT

The study was conducted to determine the immunomodulatory effects of Effective Microorganisms (EM®) on Horro and Fayoumi chickens at Agricultural Research Center and National Veterinary Institute (Debre Zeit). A total of 450 chickens (225 from each breed) were used in this study. Birds were grouped according to treatment: EM-treated (with feed, with water, with feed and water) and non-treated (only SRBC treated and Non-EM, Non-SRBC) controls. EM was given daily from the 3rd week of age for 5 weeks. Birds were injected with SRBC on the 4th and 6th week to see the total, IgG and IgM antibody responses. Antibody measurements were made using hemagglutination technique. The findings show that: (1) EM application has significantly increased antibody responses to SRBC, (2) there was no difference in antibody responses between the two breeds, or between the three modes of EM application. From these, it can be concluded that EM has a positive immunomodulatory effect when provided to chicks with feed or water. This study did not consider the cellular arm of the immune response and EM response to infections with specific pathogens was not investigated, collectively demanding further research in these and other issues if EM has to be used as good feed additive.

Key words: Effective microorganisms, horro, fayoumi, immune response

INTRODUCTION

Available experimental data show that our indigenous birds have limited genetic capacity for both egg and meat production (Negussie, 1999). However, local chickens have several invaluable characteristics appropriate to traditional low input/low output farming systems, which are not found in any exotic breed (Tadelle, 2003). Horro is one of local chicken known for its reasonably good performance among ecotypes studied in Ethiopia (Tadelle, 2003). The problem of poor adaptability to confinement and susceptibility of the Horro ecotype to some infectious diseases compared to the Fayoumi breed has prompted the DZARC to undertake a comparative study on their immune responses using Effective microorganisms, a laboratory cultured mixture of microorganisms consisting mainly of lactic acid bacteria, photosynthetic bacteria and yeast. Studies in other areas have already shown that the use of probiotics and Effective microorganisms could improve the immune responses of chicken to various infections and enhance better sanitation in poultry houses (Kabir *et al*., 2004; Mohiti *et al*., 2007; Chichlowski *et al*., 2007). The objective of this study was to evaluate the immune competence (Humoral immune response) of Horro chicks compared with Fayoumi breed with or without EM supplementation.

MATERIALS AND METHODS

Study animals, Housing and feeding of experimental chicks: Day old chickens obtained from Fayoumi and Horro chickens were randomly assigned to experimental pens and reared in floor pens filled with hay as litter material with a density of 6 birds m^{-2}. A standard starter feed and water *ad libitum* till 8 weeks of age (end of experimental study). Standard bio-security protocol was employed throughout the experimental period; however, the chicks were not vaccinated for any of the prevalent diseases.

Experimental design and treatment groups: Experimental groups of Fayoumi and Horro chickens with different modes of EM treatment were arranged in a Completely Randomized Design (CRD) with three replications of 15 birds each (Table 1).

Supplementation of Effective Microorganisms (EM®): The different EM preparations were made according to EM research organization (EMROSA, 2003). Birds were provided with EM with feed, with water or with feed and water daily starting from the age of 3 weeks till the end of the experiment (week 8). For this, 1% EM-Bokashi in feed was made for EM with feed, 0.1% EM-activated solution in water for EM in water and half of the above concentrations for EM supplemented in both feed and water. Control groups (F-NT-C, F-SRBC, H-NT-C and H-SRBC) did not receive EM.

Table 1: Experimental groups of Fayoumi and Horro chickens with different modes of EM treatment

			Weeks					
Groups	No. animal group	No. animal sampled	3	4	5	6	7	8
F-EM-F	45	10	Bleeding	Bleeding, immunized with SRBC	Bleeding	Serum, immunized with SRBC	Bleeding	Bleeding
F-EM-W	45	10	Bleeding	Bleeding, immunized with SRBC	Bleeding	Bleeding, immunized with SRBC	Bleeding	Bleeding
F-EM-FW	45	10	Bleeding	Bleeding, immunized with SRBC	Bleeding	Bleeding, immunized with SRBC	Bleeding	Bleeding
F-SRBC	45	10	Bleeding	Bleeding, immunized with SRBC	Bleeding	Bleeding, immunized with SRBC	Bleeding	Bleeding
F-NT-C	45	10	Bleeding	Bleeding	Bleeding	Bleeding	Bleeding	Bleeding
H-EM-F	45	10	Bleeding	Bleeding, immunized with SRBC	Bleeding	Bleeding, immunized with SRBC	Bleeding	Bleeding
H-EM-W	45	10	Bleeding	Bleeding, immunized with SRBC	Bleeding	Bleeding, immunized with SRBC	Bleeding	Bleeding
H-EM-FW	45	10	Bleeding	Bleeding, immunized with SRBC	Bleeding	Bleeding, immunized with SRBC	Bleeding	Bleeding
H-SRBC	45	10	Bleeding	Bleeding, immunized with SRBC	Bleeding	Bleeding, immunized with SRBC	Serum	Serum
H-NT-C	45	10	Bleeding	Bleeding	Bleeding	Bleeding	Serum	Serum

F-EM-F: Fayoumi given EM in feed (*3), F-EM-W: Fayoumi given EM in water (*3), F-EM-FW: Fayoumi given EM in Both feed and water (*3), F-SRBC: Fayoumi SRBC treated control (*3) F-NT-C: Fayoumi non-treated control (*3), H-EM-F: Horro given EM in feed (*3), H-EM-W: Horro given EM in water (*3), H-EM-FW: Horro given EM in Both feed and water (*3), H-NT-C: Horro non-treated control (*3), H-SRBC: Horro SRBC treated control (*3)

Measurement of immune response

Preparation of SRBC and immunization of chickens: Sheep red blood cells were isolated and a 0.5% suspension of the SRBC was made with PBS at the National Veterinary Institute (Debre Zeit, Ethiopia) using a standard technique. All groups of birds except controls were then immunized intramuscularly (in the breast area) with an initial dose of 0.5 mL of 0.5% SRBC in PBS one week after EM supplementation was started (week 4). Negative control groups received the same amount of PBS in place of SRBC. The booster dose of SRBC was given on week 6, i.e., 14 day after the initial injection of the SRBC antigen.

Measurement of antibody responses against SRBC: Serum samples were collected from brachial vein weekly starting from the age of 3 weeks. The first sample (week 3) was meant to represent antibody response against SRBC before the start of any treatment (EM or SRBC). The second sample represents antibody response against SRBC after one week of EM supplementation but without SRBC injection. Measurements were made for total and IgG and IgM antibodies according to the method described previously (Yamamoto and Glick, 1982; Qureshi and Havenstein, 1994; Lepage *et al*., 1996).

Statistical analysis and model: Serum Ig titers (Ig-total, IgG, IgM) were monitored in a 2*5 ANOVA (two strains*4 EM supplementations and additional two SRBC subjected but non EM injected controls) . All data were analyzed by ANOVA with the repeated model mixed procedure of SAS software (2000 version 8) and compared by least square means at $p<0.05$.

RESULTS

Antibody responses to EM supplementation (Total, IgG and IgM)

Total immunoglobulin titer: Antibody titers for samples taken before any treatment or before SRBC treatment were much lower compared to those after treatment with EM. EM-treated groups had significantly higher total immunoglobulin titers compared to the non-treated control groups for each breed ($p<0.05$). The peaks were observed 3 weeks after the start of EM and 2 weeks after the first SRBC injection. However, it gradually declined till week 8 even though it remained significantly higher than control groups (Fig. 1). There was no significant difference for total antibody titer between EM treatment groups in each breed and between breeds for each mode of EM application (feed, water, both). One exception to this is, the significantly higher antibody titer in Fayoumi breed compared to Horro when EM is given with water ($p<0.05$). On the other hand, total antibody titer in groups treated with SRBC where significantly higher than the non treated control groups in both breeds during the study period ($p<0.05$). As the total antibody titer was very low before SRBC treatment, it was difficult to obtain readings for the fractions (IgG and IgM). Therefore, data for weeks 3 and 4 are not included here. Starting from the 5th week of age (2 weeks of EM and 1 week of SRBC), the pattern of IgG titer is similar to that of the total antibody measurement (Fig. 2). All EM supplemented groups had higher IgG titer than control and SRBC groups for both breeds ($p<0.05$) except on week 5 where there was no significant difference between EM-treated and non-treated groups. There was no significant difference for IgG antibody titer between EM treatment groups in each breed and between breeds for each mode of EM application. However, on week 8 there was significantly higher IgG titer in Fayoumi breed than in Horro when EM is given with water ($p<0.05$). IgM response peaked one week following the first SRBC injection and then declined to control levels within the following two weeks (Fig. 3). EM application,

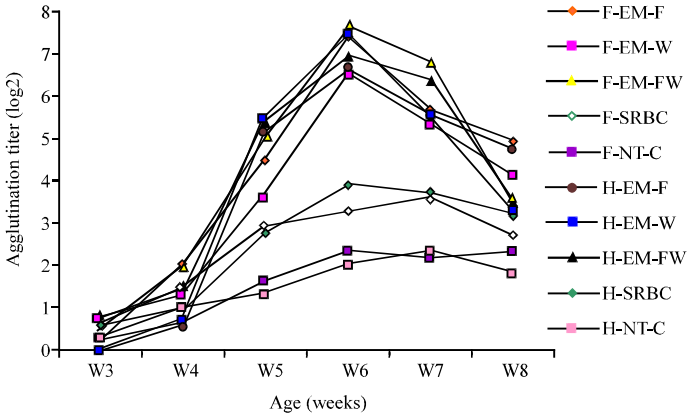

Fig. 1: Total antibody titre of Fayoumi (F) and Horro (H) chicken groups supplemented with EM in feed (EM-F), in water (EM-W), in feed and water (EM-FW) and non-treated control (C) and SRBC groups

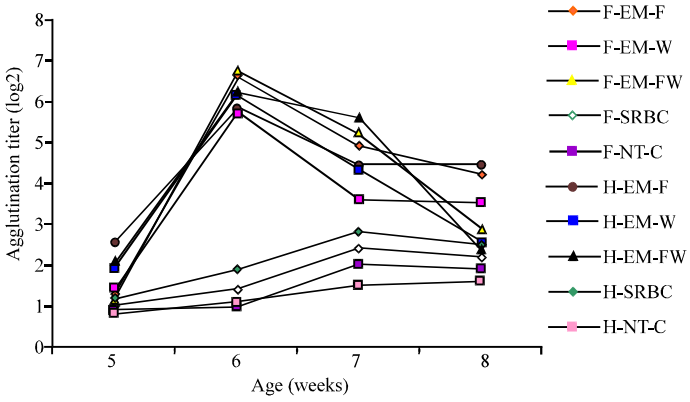

Fig. 2: Dynamics of IgG antibody in Fayoumi (F) and Horro (H) chicken groups supplemented with EM in feed (EM-F), in water (EM-W), in feed and water (EM-FW), non-treated control (C) and SRBC groups

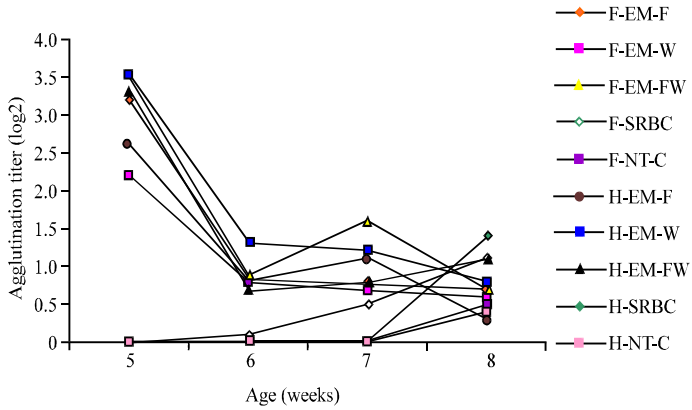

Fig. 3: Dynamics of IgM antibody in Fayoumi (F) and Horro (H) chicken groups supplemented with EM in feed (EM-F), in water (EM-W), in feed and water (EM-FW), non-treated control (C) and SRBC groups

regardless of the mode of application, has resulted in significantly higher IgM titer on weeks 5 and 6 compared with controls in both breeds of chicken ($p<0.05$). For those groups treated with EM, there was no significant difference in IgM titer between the different modes of EM supplementation and between the two breeds.

DISCUSSION

Effect of EM on immune competence of horro and fayoumi chickens: The significance of EM/probiotic supplementation in boosting immune response to various antigens such as SRBC and specific pathogens has been demonstrated in various studies. Anjum (1998) has reported that geometric mean titer of antibody against New Castle Disease in EM treated chickens was twofold higher compared with untreated control layers. Similarly, Kabir *et al.* (2004), Perdigon *et al.* (1995), Panda *et al.* (2000), Cross (2002), Yunis *et al.* (2000), Koenen *et al.* (2004) and Huang *et al.* (2004) reported that probiotics stimulate the immunity of chickens when compared with non-probiotic groups. In this study, EM supplementation irrespective of the mode of application had a positive effect on total antibody as well as IgG and IgM levels in both Fayoumi and Horro chicken. This is clearly visualized when mean agglutination titers are compared to those of non-EM controls. A previous study demonstrated that day-old chicks immunized with probiotics had increased serum and intestinal antibodies reactive to tetanus toxoid and *Clostridium perfengens* alpha toxin (Haghighi *et al.*, 2006). This result is also in line with the findings of Ahmad (2006) who reported EM increases production of antibodies usually of IgG and IGM classes and interferon γ. Similarly, (Haghighi *et al.*, 2005) also reported that Probiotic treated birds showed significantly higher serum antibody to SRBC than birds that were not treated with probioties. Likewise, serum IgG and IgM reactive to tetanus toxoid and alpha toxin were increased in probiotic treated, unimmunized chickens compared to levels in untreated controls (Haghighi *et al.*, 2006). The sharp increase in the level of IgM in all EM-treated groups one week after SRBC injection observed in this study is in accordance with other studies. IgM appears after 4-5 days following exposure to a disease organism/antigen and then disappear by 10-12 days (Butcher and Miles, 2003).The mechanisms by which EM/probiotics improve the immune responses of birds are not clearly understood. The addition of probiotics to diets may benefit the host animal by stimulating appetite, improving intestinal microbial balance and stimulating non-specific/specific immune system (Nahashon *et al.*, 1992; Afrc, 1989; Toms and Powrie, 2001). The present study compared the Fayoumi breed (originated from Egypt) known for its adaptability to various environmental conditions and its relative resistance to some known diseases (Pinard-Van Der Laan *et al.*, 1998; Lakshmanan *et al.*, 1996) with the poorly known local chicken, the Horro. The findings indicated that except at week 8 where the Fayoumi chicken responded better than the Horro when EM is given with water or with feed and water, there was no significant difference between the two breeds for total and specific antibody responses against SRBC irrespective of the type of EM supplementation. It seems that both the local Horro and the Exotic Fayoumi have similarly responded to EM supplementation. The exceptions at week 8 could be due to the amount of EM-treated water consumed. Similarly, Deif *et al.* (2007) have reported that Hubbard broiler chicks had significantly higher total ant-SRBC antibody titer at 7 and 14 days post primary and secondary SRBC injection compared to Cobb breed. Such discrepancy in results may arise due to differences in experimental animals, experimental protocols and/or study duration (5 weeks in our case). It was observed that mode of application had generally no remarkable influence on the immunomodulatory effect of EM in both Fayoumi and Horro chicken. This may imply that mixing the EM activated solution in water or feed (as medium of administration) did not significantly affect the immunomodulatory activity of EM.

CONCLUSION AND RECOMMENDATION

EM treatment caused a significant increase in total, IgG and IgM antibody levels (against SRBC as a general mitogen) compared to non-treated controls in both breeds. These responses were not different between breeds and between modes of EM application. Therefore, EM can be used as a feed additive regardless of the mode of application (feed/water) to boost the immune responses of these birds. However, it remains to be seen if the addition of EM to feed or water could also improve the immune competence of these birds to specific pathogens. However, this study was conducted with limited number of chicks and with relatively young birds (less than two months of age) due to limitations in availability of reagents. Therefore, to arrive at better understanding and conclusion on the role of EM and its future utilization in poultry farming. It is essential to enhance resistance of chicks to the most important diseases such as viral and bacterial origin. therefore, experimental works should involve the role of EM to boosting immune responses to specific pathogens.

REFERENCES

Afrc, R.F., 1989. Probiotics in man and animals. J. Applied Microbiol., 66: 365-378.

Ahmad, A., 2006. Effect of probiotics on broilers performance. Int. J. Poult. Sci., 5: 593-597.

Anjum, A.D., 1998. Layer production with EM-technology. Proceedings of the 7th International Conference on Technology of Effective Microorganisms, October 28-31, 1998, Bali, Indonesia.

Butcher, G.D. and R.D. Miles, 2003. The avian immune system. University of Florida. The Institute of Food and Agricultural Sciences Extension. http://edis.ifas.ufl.edu/pdffiles/VM/VM01600.pdf.

Chichlowski, M., J. Croom, B.W. McBride, G.B. Havenstein and M.D. Koci, 2007. Metabolic and physiological impact of probiotics or direct-fed-microbials on poultry: A brief review of current knowledge. Int. J. Poult. Sci., 6: 694-704.

Cross, M.L., 2002. Microbes versus microbes: Immune signals generated by probiotic lactobacilli and their role in protection against microbial pathogens. FEMS. Immunol. Med. Microbiol., 34: 245-253.

Deif, E.A., A. Galal, M.M. Fathi and A. Zein-El-Dein, 2007. Immunocompetence of two broiler strains fed marginal and high protein diets. Int. J. Poult. Sci., 6: 901-911.

EMROSA, 2003. Effective microorganisms research organization of South Africa. Emrosa Inc., South Africa.

Haghighi, H.R., J. Gong, C.L. Gyles, M.A. Hayes and H. Zhou *et al*., 2005. Modulation of antibody-mediated immune response by probiotics in chickens. Clin. Diagn. Lab. Immunol., 12: 1387-1392.

Haghighi, H.R., J. Gong, C.K. Gyles, M.A. Hayes and H. Zhou *et al*., 2006. Probiotics stimulate production of natural antibodies in chickens. Clin. Vaccine Immunol., 13: 975-980.

Huang, M.K., Y.J. Choi, R. Houde, J.W. Lee, B. Lee and X. Zhao, 2004. Effect of lactobacilli and an acidophilic fungus on the production performance and immune responses in broiler chickens. Poult. Sci., 83: 788-795.

Kabir, S.M.L., M.M. Rahman, M.B. Rahman, M.M. Rahman and S.U. Ahmed, 2004. The dynamics of probiotics on growth performance and immune response in broilers. Int. J. Poult. Sci., 3: 361-364.

Koenen, M.E., J. Kramer, R. van der Hulst, L. Heres, S.H.M. Jeurissen and W.J. Boersma, 2004. Immunomodulation by probiotic lactobacilli in layer and meat type chickens. Br. Poult. Sci., 45: 355-366.

Lakshmanan, N., M.G. Kaiser and S.J. Lamont, 1996. Marek's Disease Resistance in MHC-Congenic Lines from Leghorn and Fayoumi Breeds. In: Current Research on Marek's Disease, Silva, R.F., H.H. Cheng, P.M. Coussens, L.F. Lee and L.F. Velicer (Eds.). American Association of Avian Pathologists, Kennett Square, PA., USA., pp: 57-62.

Lepage, K.T., S.E. Bloom and R.L. Jr. Taylor, 1996. Antibody response to sheep red blood cells in a major histocompatibility (B) complex aneuploid line of chickens. Poult. Sci., 75: 346-350.

Mohiti, A.M., S.A. Hosseini, H. Ltfollahian and F. Shariatmadari, 2007. Effect of probiotics yeast vitamin C supplements on performance and immune response of laying hen during high environmental temperature. Int. J. Poult. Sci., 6: 895-900.

Nahashon, S.N., H.S. Nakaue and L.W. Mirosh, 1992. Effect of direct-fed microbials on nutrient retention and production parameters of laying pullets. Poult. Sci., 71: 111-111.

Negussie, D., 1999. Evaluation of the performance of local, Rod Island Red and Fayuomi breeds chicken under different management regimes in the highlands of Ethiopia. M.Sc. Thesis, Swedish University of Agriculture Sciences, Uppsala.

Panda, A.K., M.R. Reddy, S.V.R. Rao, M.V.L.N. Raju and N.K. Praharaj, 2000. Growth, carcass characteristics, immunocompetence and response to *Escherichia coli* of broilers fed diets with various levels of probiotic. Arch. Geflugelkd., 64: 152-156.

Perdigon, G., S. Alvarez, M. Rachid, G. Aguero and N. Gobbato, 1995. Immune system stimulation by probiotics. J. Dairy Sci., 78: 1597-1606.

Pinard-Van Der Laan, M.H., J.L. Monvoisin, P. Pery, N. Hamet and M. Thomas, 1998. Comparison of outbreed lines of chickens for resistance to experimental infection with Coccidiosis (*Eimeria tenella*). Poult. Sci., 77: 185-191.

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 boiler diets. Poult. Sci., 73: 1805-1812.

Tadelle, D., 2003. Phenotypic and genetic characterization of local chicken ecotypes in Ethiopia. Ph.D. Thesis, Humboldt-University in Berlin, Germany.

Toms, C. and F. Powrie, 2001. Control of intestinal inflammation by regulatory T cells. Microb. Infect., 3: 929-935.

Yamamoto, Y. and B. Glick, 1982. A comparison of the immune response between two lines chickens selected for differences in the weight of the bursa of Fabricius. Poult. Sci., 61: 2129-2132.

Yunis, R., A. Ben-David, E.D. Heller and A. Cahaner, 2000. Immunocompetence and viability under commercial conditions of broiler group differing in growth rate and in antibody response to *Escherichia coli* vaccine. Poult. Sci., 79: 810-816.