

# INTERDIGITAL SKIN TEST FOR EVALUATION OF DELAYED HYPERSENSITIVITY AND MONITORING CELL-MEDIATED IMMUNE RESPONSES IN CHICKENS\*\*

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**Abstract:** A skin test to assess cell mediated delayed hypersensitivity (DH) used to evaluate immune response of chickens. Results of many studies indicated, that skin testing is especially useful as a simple in vivo screening to evaluate normal and suppressed T-cell mediated DH. Chickens were sensitized with using mitogens, B and T-cell dependent antigen by intradermal injection. The most feathered skin of chickens is too thin for adequate intradermal injections, so the wattle is the standard site for skin testing, however, in younger than 2 or 3 weeks old chickens, the wattle is undeveloped and intradermal injection and measurement of response are difficult. A simple interdigital skin used by many of the authors.

Skin swelling response and DH reaction were measured in mm before injections and after. The skin test and DH in vivo results oedema-initiating characteristics of sensitizing agents, which increase in skin thickness detectable after 4- 6 hours of application. Many of investigation results suggests that healthy chickens are able to have strong immune response and support the concept that some changes in the cell-mediated immune response and other pathogens may potentially affect immune response.

**Key words:** skin test, delayed hypersensitivity, immune response

## Introduction

In scientific and clinical work in vivo, in chickens, interdigital test, test

of delayed hyper sensitivity (DH) can be used to determine causes of different agents. DH is auxiliary test in diagnostics of avian tuberculosis, *Tizard R.I. (1996.)*. It is carried out around the comb *Janković et al. (1963)*, in the region of wattle *Cottler et al. (1985)*, wing crease *Paramentier et al. (1993.)* and in the region of interdigital toe skin *Corrier E.D. and De Loach R.J. (1990)*. Use of mitogene substances of T cell or B cell dependent antigen without previous sensibilization of macro organisms, is also called delayed basophile hypersensitivity and often both terms are used in numerous research papers. Response of sensibility on inoculation spot of mitogene substance phytohemaglutinine – lektine extracted from red beans *Phaseolus Vulgaris* is local increase and thickening of skin due to accumulation of T lymphocytes and cells of inflammatory process. Occurrence of edema is caused by increase of permeability of blood vessels due to release of vasoactive amines, such as serotonin, histamine, released from mastocystes, as well as due to sensibilization with antibodies or T cell dependent antigens *Ptak et al. (1991)*. Interdigital application of PHA and sole between 3. and 4. toe was performed on young chickens at the ages of 10,14, 17, 24 and 31 days, where wattle wasn't still developed. Response was read 24 and 48 hours subsequent to intradermal application and investigated skin indicated infiltration of basophile leukocytes as stated in research by *Stadecker M.J. and Leskowitz S. (1974)*, *Corrier E.D. and DeLoach R.J. (1990)* and *Sinha B.K. et al. (1988)*. Investigation in vivo of reactivity to sensibilization of PHA in light and heavy strains by *Rusov et al. (1994)* showed that in peripheral blood of chickens total number/count and differential relation of leukocytes didn't change, and that citomorphological content of skin infiltrates 12 hours after PHA inoculation indicated increased number/count of heterophils, lower leukocyte and macrophage count and mast cells. Results of the investigation showed that Isabrown chickens were slightly more immune reactive than Hybro and Ross chickens. In monitoring of immune reactivity of poultry in intensive rearing quick answers can be obtained relating to evaluation of the status of activity of T lymphocytes. In investigation of *Miljković et al. (1993,1994)* DH was investigated on skin of young chickens using PHA, dosage was determined, place of interdigital inoculation as well as time for response reading.

## Results and Discussion

In conditions of intensive broiler production, when economical losses are possible due to stress, immune suppressive etiological agents, immune

suppressive substances in forage mixtures for chickens, with regular inoculation/vaccine application, nutrition disbalances, use of antibiotics, constant struggle is imposed upon us to preserve and maintain integrity of immune system of chickens as well as health of chickens, welfare and economically efficient production.

Research today is going in direction of finding the balance towards preservation/maintenance and development of immune system in relation to development and reproduction of animals, in accordance with optimization of productivity and welfare such as reduction of diseases due to justification of production. Role of nutritive deficiency within physiological needs relating to development and reproduction has been marginalized. Physiological needs of chickens have been investigated and, biotin was discovered as the first and iron as the second limiting factor in development of pathogen causes *Klasing K. And Humphrey B.D (1998)*. Same author stated that nutritive needs/requirements in selenium, vitamins E and C as well as bioactive antioxidants which protect the integrity of lipid cell component including cell membranes in acute stage of infection, necessary for replication of activated leukocytes, cytotoxic T lymphocytes, macrophages, neutrophils and murder cells which release catabolic enzymes, nitrogen oxide, peroxides and free radicals. Nutritive factors such as fish fatty acids which stimulate auxiliary lymphocytes and production of antibodies also influence cell communication, whereas fatty acids from maize stimulate macrophages to secrete  $IL-1$  and stimulate secretion of prostaglandin E. Research today is directed towards reduced use of antibiotics which are substituted with probiotics. Use of vaccines in struggle against virus and certain bacterial diseases has broad application. This requires preservation of immune system using new alternative substances. Alternative solutions are investigated so in prevention of avian flue caused by highly pathogen virus H5N1 in humans, use of antiviral effect of catechin from green tea is now openly mentioned *Baik Lin Seong (2006)*.

Using the technique of immune histochemical characteristic with mono clone antibodies to chicken leukocytes in the tissue of swollen wing crease and method of rapid detection of immune reactivity in chickens, *DH test, Parmentier H.K. et al. (1998)* obtained extra vascular localization of leukocytes 24 hours subsequent to sensitization using several different mitogene substances. In use of PHA local activation and proliferation of T lymphocytes was obtained, CD4 + cells and CD 8+ cells, TCR -1 cells, heterophils but not B cells. In genetic selection of hybrid lines of chickens according to their humeral immune response to sheep red blood cells, and

categorization into higher and lower reactive selection, one of the information parameters of the activity is interleukin 1 (Il-1) and tumour necrotic factor  $\alpha$  (TNF  $\alpha$ ). Induced skin DH in vivo, performed by PHA inoculation, after 1-2 hours, and then after 4-6 hours, can show activity of Il-1 and TNF  $\alpha$  *Parmentier H.K. et al.* (1998). Ability of production of these cytokines between chickens which in selection were selected because of their ability for higher production of antibodies, found application in genetic selection on chicken resistance.

Local response occurring in antigen specific DH, 1-2 hours after sensibilization and medium immune response after 4-6 hours is already inclusion of immune globulin in activation and proliferation of antigen specificity of DH, *Parmentier H.K. et al.* (1998).

Our previous research related to establishing of presence of short term immune suppressive activity of Mareck vaccine which was demonstrated by DH applied PHA through reduced thickening of intradigital skin in chickens *Jojić-Maličević L.* (1998).

The same effect of the Mareck vaccine was presented through stereological parameters of structural parts of Timusu, Burzi Fabrisijus as well as through number and density of lymph follicles of the spleen *Miljković B.* (2001).

## Conclusion

Except the use of DH test in detection of avian tuberculosis, this test is also used in determination of the status of immune competency. Test is applied in detection of natural activity of non-specific immune response and it is used in selection activities, selection of birds as less or more reactive hybrid lines, as well as in research of avian biology, ecology and toxicology.

# INTERDIGITALNI KOŽNI TEST U PROCENI KASNE PREOSETLJIVOSTI – I MONITORING CELULARNOG IMUNOG ODGOVORA PILIĆA

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## Rezime

Kožni test, test kasne preosetljivosti ( KP ) koristio se u proceni celularnog imuniteta kod pilića. Mnogobrojne studije ukazuju da je kožni test posebno koristan, jednostavan skrining test in vivo, za procenu normalnog i suprimiranog T ćelijskog imuniteta. Pilići se senzibilišu korišćenjem mitogena , B i T zavisnog antigena, intradermalnom aplikacijom. Najveći deo kože kod pilića je vema tanak za adekvatnu intradermalnu aplikaciju, pa su podbradnjaci standardno mesto za testiranje, međutim kod mladih pilića od 2-3 nedelje podbradnjaci su nerazvijeni pa je otežana aplikacija i merenje. Mnogi autori su koristili jednostavan interdigitalni test. Zadebljanje kože i kasna preosetljivost se mere u mm pre i nakon aplikacije. Kožni test in vivo kod merenju daje edem-inicijalnu karakteristiku sezitivnog agensa, koji se u vidu zadebljanja kože detektuje nakon 4-6 sati od aplikacije. Mnogobrojni rezultati sugerišu da su zdravi pilići sposobni da daju jak imuni odgovor, što odgovara mogućem konceptu da izmena u celularnom odgovoru kao i patogeni uzročnici remete imuni odgovor.

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