

# ajava

Asian Journal of Animal and Veterinary Advances



Academic  
Journals Inc.

[www.academicjournals.com](http://www.academicjournals.com)

**Biomarkers Identified by Proteomic Study of Spleen Lymphocyte from Broilers Infected with *Salmonella gallinarum* after Feeding Korean Mistletoe (*Viscum album coloratum*)**

<sup>1</sup>Hyun-Kyung So, <sup>2</sup>P.K. Mandal, <sup>1</sup>O. Baatartsogt, <sup>1</sup>Hee-Kyong Lim, <sup>2</sup>Chi-Ho Lee, <sup>3</sup>Jun-Heon Lee and <sup>1</sup>Kangduk Choi

<sup>1</sup>School of Biotechnology, Hankyong National University, Anseong-City, 456 749, Korea

<sup>2</sup>Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul, Korea

<sup>3</sup>Division of Animal Science and Resources, College of Agriculture and Life Sciences, Chungnam National University, Daejeon 305-764, Korea

---

**Abstract:** To find the alternative for antibiotic this study was carried out to investigate the differentially expressed proteome between *Salmonella gallinarum* infected and uninfected control in the spleen lymphocytes of ROS broiler chicks fed with Korean mistletoe using proteomic approach. Total four protein spots were detected with differential expression from the chicken spleen lymphocyte in 2DE gels after silver staining. These proteins were characterized by MALDI-TOF MS and MS/MS. Two known proteins were up-regulated viz., Fatty Acid Binding Protein (FABP) and MRP-126 and 2 proteins were down regulated viz., ribosomal protein12, pyruvate kinase. In this experimental fowl typhoid infection in broilers fed with Korean mistletoe through proteomics approach significant differential expression of four proteins were found which appears to be candidate molecules for fowl typhoid.

**Key words:** Broiler, spleen lymphocyte, fowl typhoid, candidate molecules, Korean mistletoe

---

## INTRODUCTION

In poultry, it is very important to improve immunity so as to prevent infectious diseases. Antibiotics have been widely used in poultry feeds primarily to control disease and more recently to promote growth and improve feed conversion. Use of antibiotics becoming highly restricted in many countries including Korea, therefore, alternatives to antibiotics are of great interest to the poultry industry. Use of immunostimulant is one solution to improve the immunity of poultry and to decrease their susceptibility to infectious diseases (Liu, 1999).

Mistletoe (*Viscum album*) is a half-parasitic plant that grows on deciduous trees all over the world. Korean mistletoe a subspecies of *Viscum album* has shown promise in suppressing enteric pathogens, modulating the immune response, improving the integrity of the intestinal mucosa in human and animals (Choi *et al.*, 2008; Lyu and Park, 2006). Extracts from mistletoe have been used therapeutically against various diseases, including cancer, arthrosis and cardiovascular illness (Lee *et al.*, 1999). Mistletoe extracts are widely used as adjuvant cancer therapy (Azuma and Kim, 1999; Gabius *et al.*, 1994; Hajto, 1986). Korean mistletoe has been reported to have immunomodulatory and tumor suppressive effect in human (Yoon *et al.*, 1995, 1999, 2001).

Feeding of Korean mistletoe at 1% level significantly reduced *Salmonella* counts in ceca and significantly increased lymphocyte counts (Kim *et al.*, 2007). Immunomodulatory effect of Korean mistletoe through feed was reported in Japanese eel (Choi *et al.*, 2008) and as extract in murine splenocyte (Lyu and Park, 2006). Several studies were reported about the active principles and their biological function in Korean mistletoe (Khwaja *et al.*, 1980; Yoon *et al.*, 1995; Lee *et al.*, 1999). Although mistletoe extracts are in use for some diseases and there are studies about the active principles. However, there is no report about the mode of action and its effect at molecular level.

Fowl typhoid is a septicemic disease of domestic birds caused by *Salmonella gallinarum* (SG). *Salmonella enterica* serotype *gallinarum* is a non-motile, host adapted avian pathogens belonging to *Salmonella* serogroup D (Shivaprasad, 1997). The out break of fowl typhoid is characterized by increased mortality, anorexia, greenish-yellow diarrhea and a drop in egg production. In necropsy, greenish brown swollen liver with multiple white foci, enlarged spleen and misshaped ova are common (Shivaprasad, 1997). Though, it has largely been eradicated from countries with intensive poultry industry for many years, fowl typhoid caused by SG is still of considerable economic significance to the poultry industry in many countries of Africa, the Middle East, Central and South America and Asia (Shivaprasad, 1997). The quick detection of this pathogen is therefore extremely important. Use of classical methods based on biochemical tests/assays are tedious and time consuming (Christensen *et al.*, 1992; Shah *et al.*, 2001). Moreover, recent reports of intermediate strains with variable biochemical pattern casts doubts on the validity of these biochemical assays (Jia *et al.*, 1993; Shah *et al.*, 2005). In recent years, there is extensive effort to supplement the use of antibiotic by using herbs to stimulate resistance against *Salmonella* with varied success but the molecular mode of action of those herbs are not understood.

Proteomics, the large scale analysis of gene function, is central to functional genomics. Parallel quantitative display of proteins is considered the most promising strategy for biomarker discovery. Recently, proteome analysis, which refers to large-scale study of protein expression and function has gained great interest (Pandey and Man, 2000). This is used for the determination of biochemical processes involved in pathogenesis of diseases (Sinz *et al.*, 2002). The comparative characterization of protein patterns in tissues has the potential to serve as the basis for new diagnostic tools and in designing of disease specific therapies (Sinz *et al.*, 2002). Two-dimensional gel electrophoresis (O'Farrell, 1975) followed by in-gel proteolytic digestion and mass spectrometric analysis (Mann *et al.*, 2001) has become a powerful method for the identification of proteins present in specific tissues or organs (Wang and Chait, 1994). Proteomics is gaining popularity in the research on animal and poultry diseases. Because of its biomedical utility, the poultry will imminently join those vertebrates that have representative genomes sequenced (Burt and Pourquie, 2003).

The cellular and molecular mechanisms of fowl typhoid is not yet well understood. Gene expression in response to *Salmonella in vivo* and *in vitro* in chicken mainly focused on cytokinase and in various tissues have been reported by Kaiser *et al.* (2000), Withanage *et al.* (2004) and Cheeseman *et al.* (2006). However, no detailed study on proteome analysis of fowl typhoid and the immunomodulatory effect of feeding Korean mistletoe against *Salmonella* has not been reported yet. The spleen plays a vital role in pathogenesis of *Salmonella* infection of chickens (Hendersson *et al.*, 1999). Therefore, this study was carried out to investigate the differentially expressed proteome between *S. gallinarum* infected and uninfected control in the spleen lymphocytes of chicken fed with Korean mistletoe using proteomics approach for identifying suitable biomarker.

## MATERIALS AND METHODS

### Experimental Plan

This study was conducted during 2006-2007. One hundred and twenty, one-day-old *S. gallinarum* free Ross broiler chicks were procured from Yanggi Hatchery, Pyeongtaek, S. Korea.

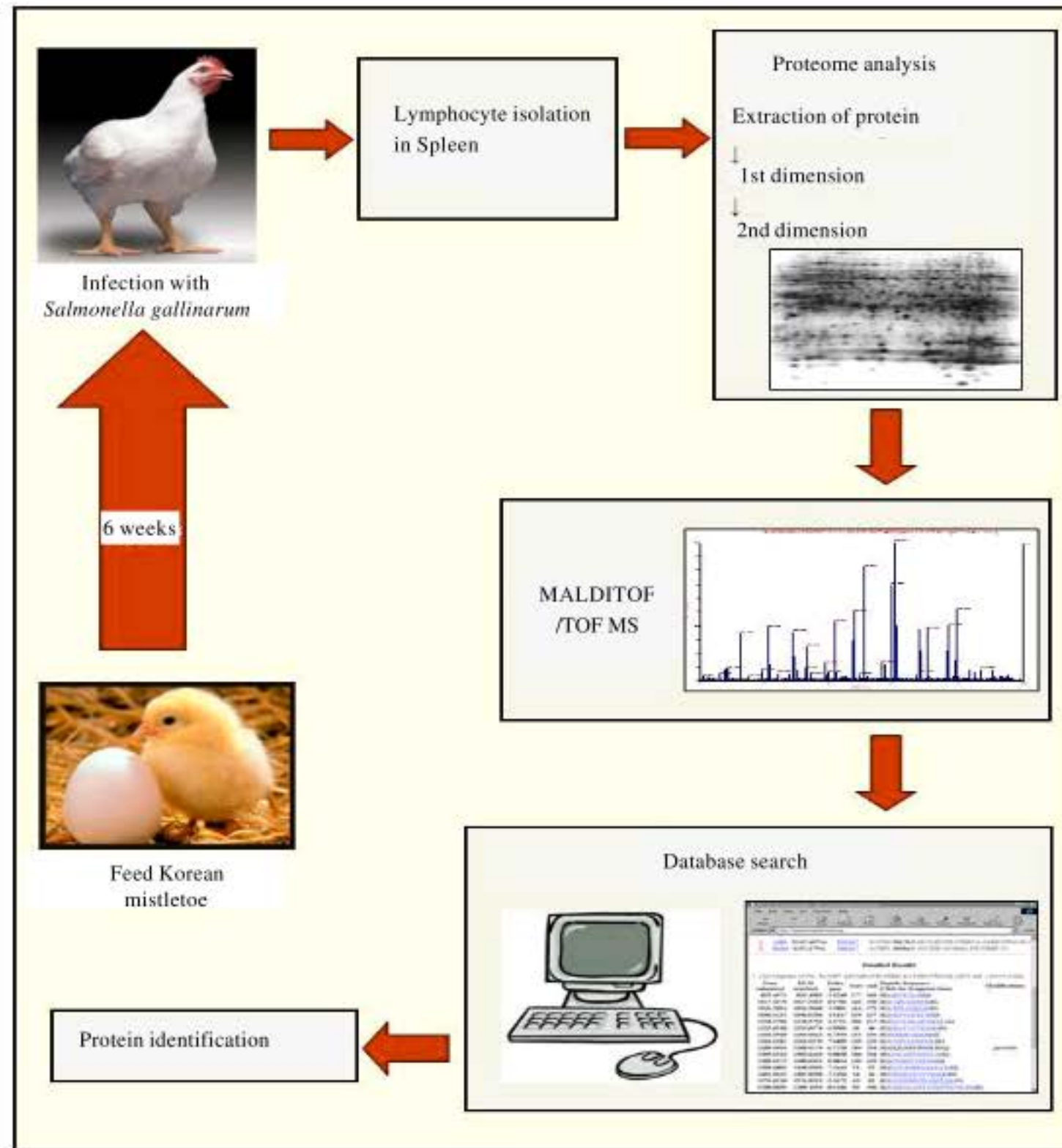


Fig. 1: Experimental design and plan for proteomic study of spleen lymphocyte from broiler chicken spleen lymphocyte of *S. gallinarum* infected and noninfected chicken fed with 1% of Korean mistletoe through feed for 6 weeks

After 6 weeks of feeding with 1% Korean mistletoe the chicks were randomly divided into two groups. One group was infected by intramuscular injection with 1 mL of *S. gallinarum* ( $2.9 \times 10^7 \text{ mL}^{-1}$ ) and the other group was kept away as uninfected control (Fig. 1).

#### Protein Sample Preparation

Randomly, 5 chickens were selected from each group after 1 week of infection and spleens were collected after appropriate anaesthesia. Chicken spleens were pooled together and lymphocytes were separated (Histopaque H-1070, Sigma) and homogenized in a mortar containing liquid nitrogen and mixed with sample buffer containing 0.3% SDS, 50 mM Tris-HCl (pH 8.0), 1 mM PMSF (phenylmethylsulfonyl fluoride, Roche, Germany) and 200 mM DTT (dithiothreitol). The mixture was centrifuged in 15,000 g at 4°C for 10 min. Supernatant of mixture was boiled at 100°C for 10 min.

Then supernatant was transferred to ice and incubated with solution containing 40 U DNase I (Roche, Germany), 14 U RNase A (Roche, Germany), 50 mM Tris-HCl (pH 8.0), 0.1 mM MgCl<sub>2</sub> for 30 min. The supernatant was precipitated with 50% trichloro acetic acid (TCA, Sigma). The protein pellet was washed with ice-cold acetone to remove contaminants. The washed pellet was dried using speed vac and used for 2-DE analysis.

## **2D Electrophoresis**

Proteins isolated from chicken spleen lymphocyte were solubilized in rehydration buffer [8 M urea, 2% CHAPS (3-3-cholamidopropyl-dimethylammonio-1-propanesulfonate, Amersham Pharmacia, Sweden), 60 mM DTT, 0.5% IPG buffer (Amersham Biosciences, Sweden) with a trace amount of bromophenol blue]. The protein concentration was measured with the help of a protein assay kit (Bio-Rad, USA) using BSA as a standard. Absorbance was measured at 595 nm wavelength using Spectramax plus (Molecular devices). The protein solution containing 300 µg of protein was applied on 18 cm immobilized pH gradient (IPG) strip gel (pH 3-10) for Iso-Electric Focusing (IEF) using IPGphor system (Amersham Biosciences, Sweden). In the first dimension, the IEF of proteins were performed in five steps (rehydration for 12 h, 200 V for 1 h, 500 V for 1 h, 1000 V for 1 h and 8000 V for 9 h).

After IEF separation, the gel strips were equilibrated in a tube containing 50 mM Tris-HCl, pH 8.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS and 1% (w/v) DTT for 15 min with gentle shaking, followed by 15 min in the same solvent containing 2.5% (w/v) iodoacetamide in place of the DTT. The second dimension electrophoresis was run on a 13% SDS polyacrylamide gel using a Ettan DALT electrophoresis (Amersham Bioscience, USA). After completion of electrophoresis the gel was stained using a silver staining kit (Amersham Bioscience, USA). The visualization of protein spots was done by silver staining.

## **Image Analysis**

Silver-stained gels were scanned using a Power look III image scanner (UMAX data system, Taiwan). Image treatment, spot detection and protein quantification were carried out using Image Master 2D Elite software (Amersham Biosciences). The molecular weights of proteins on the gel were determined by comparing with the standard markers (Sigma, USA) run in parallel to the sample. The pI of the proteins were determined by comparing the migration of protein spots on 18 cm IPG strips (pH 3-10). Spot volumes and intensity were determined from more than three gels by repeating the experiments.

## **In-Gel Digestion**

Spots of interest were excised manually with clean tips, cut off into fine slices with a razor blade and then transferred to micro-centrifuge tubes. Gel slices were washed with distilled water and kept frozen at -20°C till further use. Silver stained gel slices were destained in microtubes with 15 mM potassium ferricyanide and 50 mM sodium thiosulfate. When the brownish color disappeared, the gel slices were rinsed with distilled water, then kept in 200 mM ammonium bicarbonate for 20 min, after that the slices were crushed using micro-pestles (Eppendorf, Hamburg). For rehydration, the gel pieces were incubated in 100 mM ammonium bicarbonate and 10 mM DTT for 1 h at 56°C. Alkylation was performed in 100 mM ammonium bicarbonate and 55 mM iodoacetamide for 40 min in the dark at room temperature. After dehydration in acetonitrile, the gel pieces were dried under vacuum (Thermo Savant SpeedVac Plus, Savant, Holbrook, NY). Samples were digested with sequencing grade trypsin (Promega, Madison, WI, enzyme:substrate ratio >1:20) at 37°C, overnight, in 50 mM ammonium

bicarbonate. Trypsinized gel pieces were extracted through a repeated process of hydration-dehydration and sonication. Supernatants were transferred into new tubes and dried completely under vacuum for 6 h.

#### **MALDI-TOF-MS and MALDI-TOF/TOF**

The resulting tryptic peptides were dissolved in 0.5% trifluoroacetic acid (TFA) solution and then desalted using the ZipTipC18 (Millipore, Bedford, MA) tip. Peptides were eluted directly onto MALDI target by  $\alpha$ -cyano-4-hydroxy-cinnamic acid (CHCA) matrix solution (10 mg mL<sup>-1</sup> CHCA in 0.5% TFA/ 50% acetonitrile (1:1, v/v)). All mass spectra were acquired at a reflection mode by a 4700 Proteomics Analyzer (Applied Biosystems, Framingham, MA). External calibration was performed using a standard peptide mixture of des-Arg bradykinin, angiotensin-I, glu-fibrino-peptide-B, adrenocorticotrophic hormone (ACTH) clip 1-17, ACTH clip 18-39 and ACTH clip 7-38. Internal calibration was also performed using two autolysis peaks of trypsin [(M+H)<sup>+</sup> = 842.5099 and 2211.1046]. When the protein spots were not identified by Peptide Mass Fingerprinting (PMF), fragmentation patterns of tryptic peptide molecular ions [(M+H)<sup>+</sup>] were analyzed by MS/MS (tandem mass spectrometry) methods for obtaining their partial sequences using MALDI-TOF/TOF technique. All samples were irradiated with UV light (355 nm) of an Nd:YAG laser with a repetition rate of 200 Hz. Approximately 1,000 and 3,000 laser shots were averaged to normal mass spectra and MS/MS spectra, respectively. The samples were analyzed at 25 kV of source acceleration voltage with two-stage reflection in the MS mode. In the MS/MS experiment, collision energy, which was defined by the potential difference between the source acceleration voltage (8 kV) and the floating collision cell (7 kV), was set to 1 kV.

#### **Search of Database and Identification of Proteins**

The proteins were identified by searching NCBI non-redundant database using MASCOT PMF (Matrix Science, London) and MS-Fit (Protein Prospector; UCSF, San Francisco, CA) softwares. All mass spectra were searched in the database of *Mus musculus*. The search parameters were considered to allow the modifications of N-terminal Gln to pyroGlu, oxidation of methionine, acetylation of protein N-terminus, carbamidomethylation of cysteine and acrylamide-modified cysteine. The criteria for positive identification of proteins were set as follows: (1) minimum of matching peptide masses, (2) 50 ppm mass accuracy and (3) molecular weight and pI obtained from image analysis. For MS/MS search, fragmentation of selected peptide molecular ion peak was used to identify the protein in the same manner by searching NCBI nonredundant database using MASCOT MS/MS ion search program.

## **RESULTS**

This experiment was conducted to identify biomarker for fowl typhoid with the feeding of Korean mistletoe by applying proteomics approach. In the present study experimental fowl typhoid was successfully induced by intramuscular inoculation of *Salmonella gallinarum* in 6 weeks old chickens and the same has been confirmed by pathogen isolation, gross necropsy finding and clinical symptoms. Proteins isolated from lymphocytes of spleen of control and *Salmonella gallinarum* infected chickens were used for 2 Dimensional Electrophoresis (DE) and compared the proteomic expression. Most of the proteins identified between pH 3-10 are in the molecular weight of 14-60 kDa. Four differentially expressed protein spots were identified in infected chicken using 2DE Image Master (Fig. 2A, B). Peptide sequences were identified after MALDI-TOP and MS-MS analysis. Two known proteins were up-regulated *viz.*, Fatty Acid Binding Protein (FABP) and myeloid related protein (MRP-126) or calgranulin B (CAGB) and 2 proteins were down regulated *viz.*, ribosomal protein12,

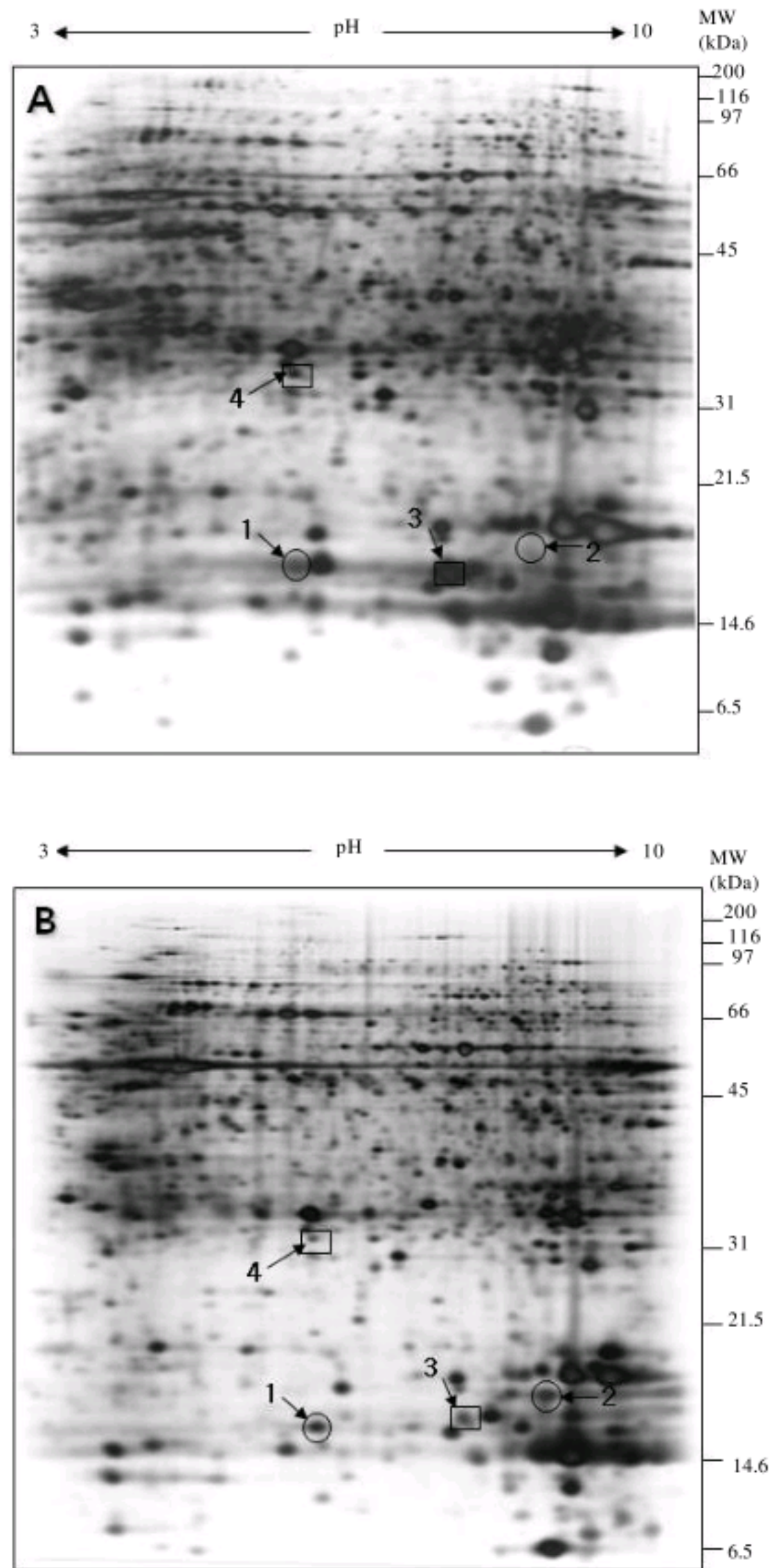


Fig. 2: The numbered spots were selected from 2D electrophoresis gel images of chicken spleen lymphocyte to compare between *S. gallinarum* (A) infected and (B) noninfected chicken fed with 1% of Korean mistletoe through feed for 6 weeks

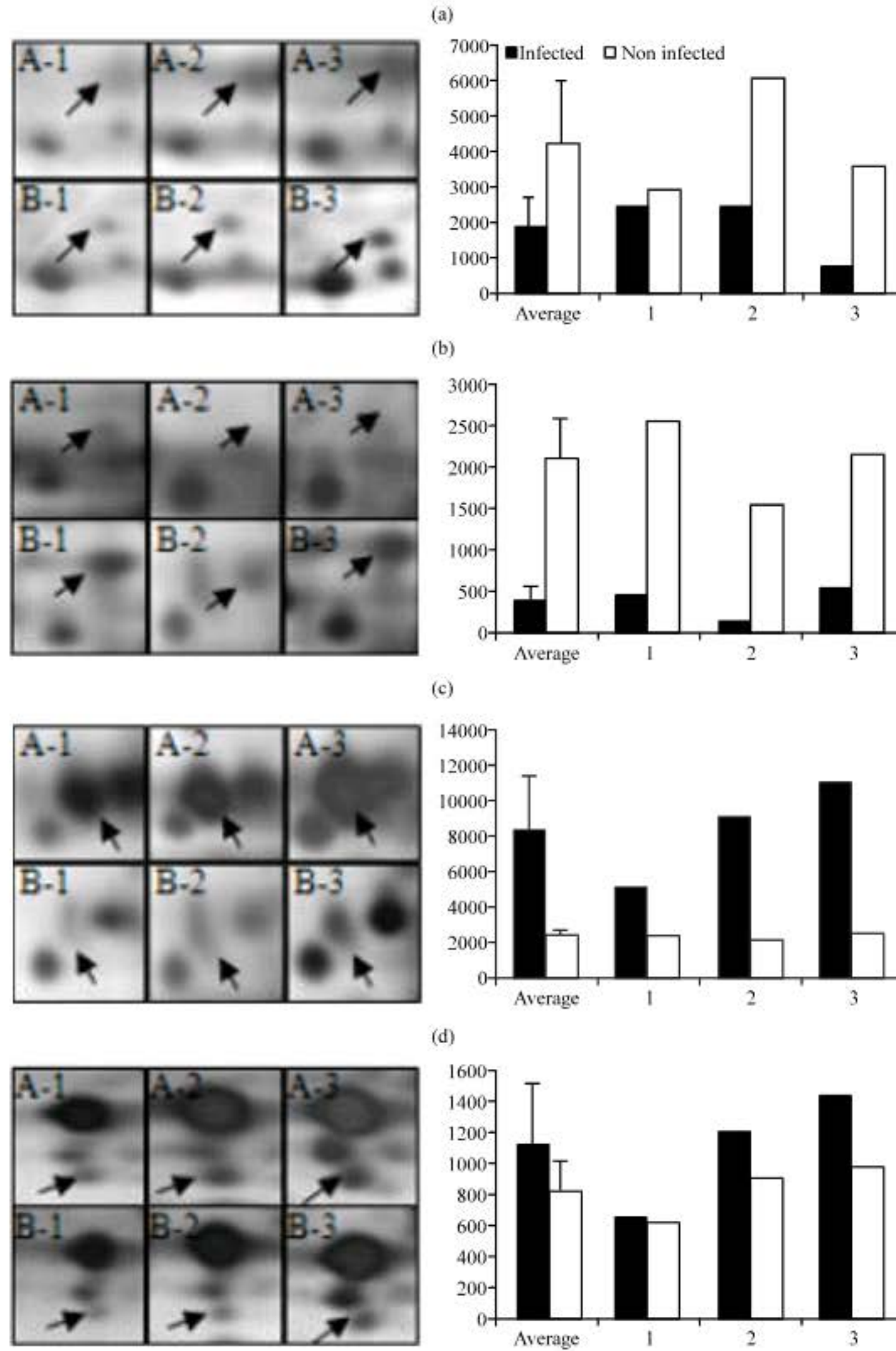


Fig. 3: Comparison of four differentially expressed protein spots selected from 2D electrophoresis gel images of chicken spleen lymphocyte to compare between *S. gallinarum* infected and noninfected chicken fed with 1% of Korean mistletoe through feed for 6 weeks. (a) Spot 1, (b) spot 2, (c) spot 3 and (d) spot 4

pyruvate kinase (Table 1, Fig. 3a-d). The findings in this study have lead to the identification of novel genes which may be useful as biomarker.



Table 1: Differentially expressed protein spots identified by 2D electrophoresis of chicken spleen lymphocyte of *S. gallinarum* infected and noninfected chicken fed with 1% of Korean mistletoe through feed for 6 weeks

Protein identity	Accession No.	MW (kDa)	pI	Function
<b>Up-regulated proteins</b>				
Fatty acid-binding protein	AAT67999	14.81	5.92	Regulate lipid metabolism and transport
Similar to protein MRP-126	XP-424012	14.06	6.44	Drug efflux pump
<b>Down-regulated proteins</b>				
Ribosomal protein S12	XP_419736	14.54	6.81	tRNA selection process
Pyruvate kinase	NP_990800	57.98	7.29	Glycolysis pathway regulation

<sup>a</sup>Accession No. of Swiss-port or Genebank database

## DISCUSSION

Study of the interaction between host and pathogen at molecular level has become a major research area in functional proteomics. The virulence factors for *Salmonella* are motility, pilli/fimbriae. The ability to attach, invade and penetrate enterocytes is crucial to virulence. The *Salmonella* organism colonize small intestine, colon, muscle, spleen and gall bladder (Shivaprasad, 1997; Brown *et al.*, 2007). Liver and intestine are known to secrete cationic antimicrobial peptides but it is not yet known about the similar activity in spleen. *Salmonella* produces disease via., exotoxin, cytotoxin (verotoxin) and endotoxin. Diarrhoea occurs due to active secretion of electrolytes, malabsorption due to reduced surface of mucosa, enterocyte competence and inflammatory exudation. Septicemia (endotoxaemia) causes fever, leucopenia, haemoconcentration, lactic acidosis, coagulopathies, hypotension and death (Shivaprasad, 1997; Brown *et al.*, 2007).

In this experiment four known proteins were found with differential expression, two proteins were up-regulated *viz.*, Fatty Acid Binding Protein (FABP) and MRP-126 and two proteins were down regulated *viz.*, ribosomal protein12, (RP12) and pyruvate kinase. In this study a search was made for biomarkers in fowl typhoid, to our knowledge no report is available about the proteomic search on fowl typhoid for comparison of the present findings.

### Fatty Acid Binding Protein (FABP)

These proteins are members of the superfamily of Lipid Binding Protein (LBP). It is abundant in cell cytoplasm and plays important role in cell proliferation. It is also involved in many biological action related to regulation of lipid metabolism leading to energy homeostasis. The primary role of all the FABP family members is regulation of Fatty Acid (FA) uptake and intracellular transport and thereby modulate cell growth and proliferation (Chmurzynska, 2006). Response to signals triggers activation of specific transcription factors and FAs also act as signaling molecules (McArther *et al.*, 1999). Over expression of FABP in mammal can increase FA transport. The FAs are disturbed from plasma membrane into the cytoplasm and the FABPc proteins may accelerate FA uptake in several ways. FABP proteins stimulate not only FA desorption, but also cytoplasmic diffusion (Clarke *et al.*, 2004). In the present study the upregulated expression of FABP appears to be an effort to maintain energy balance by mobilizing the fatty acids by increasing the transport to the cytoplasm for metabolism.

### Calprotectin/MRP126/Calgranulin B (CAGB)

Calprotectin, the complex of S100A8 and S100A9 is a major calcium- and zinc-binding protein in the cytosol of neutrophils, monocytes and keratinocytes (Sampson *et al.*, 2002). Odink *et al.* (1987) identified and cloned the calgranulin B gene (CAGB) also known as myeloid related protein (MRP14). Vogl *et al.* (2007) demonstrated that mice lacking MRP8-MRP14 complexes are protected from endotoxin-induced lethal shock and *Escherichia coli* induced abdominal sepsis. Both proteins are released during activation of phagocytes and Mrp8-Mrp14 complexes amplify the endotoxin-triggered

inflammatory responses of phagocytes. Vogl *et al.* (2007) concluded that MRP8-MRP14 complexes are novel inflammatory components that amplify phagocyte activation during sepsis upstream of TNF- $\alpha$ -dependent effects. Corbin *et al.* (2008) concluded that calprotectin is a critical factor in the innate immune response to infection and that metal chelation is a mechanism for inhibiting microbial growth inside tissue. The upregulated expression of this protein in spleen lymphocytes of *Salmonella* infected chicken must be due to the *Salmonella* infection and feeding of Korean mistletoe which may be a mechanism to boost the immune response against the infection.

#### **Ribosomal Protein S-12 (RPS12)**

The mammalian ribosome is composed of approximately 80 different proteins. Herault *et al.* (1991) isolated cDNAs encoding ribosomal protein S12 (RPS12) from human lymphocyte cDNA library. They deduced that 132-amino acid human RPS12 protein is 97% identical to rat Rps12. Pogue-Geile *et al.* (1991) found increased levels of RPS12 mRNA in 4 of 4 human colorectal tumors by Northern blot analysis using a rat RPS12 probe. RPS12 is expressed as an approximately 480 bp transcript. Kenmochi *et al.* (1998) mapped the RPS12 gene to 6q using somatic cell hybrid and radiation hybrid mapping analysis. The downregulated expression of RPS12 might be a response due to *Salmonella* infection and feeding of Korean mistletoe was not very effective in this regard.

#### **Pyruvate Kinase**

Pyruvate kinase is an enzyme involved in glycolysis. It catalyzes the conversion of phosphoenolpyruvate to pyruvate in last step of TCA cycle which is irreversible. The PKLR gene encodes for both liver and RBC isozymes (Zanella *et al.*, 2001). Genetic defects of this enzyme cause the pyruvate kinase deficiency leading to slow process of glycolysis and energy deficiency. This effect is devastating for cells without mitochondria like RBC, because it has to solely depend on anaerobic glycolysis. RBC in a state of pyruvate kinase deficiency rapidly undergo hemolysis due to energy deficiency and therefore, cause hemolytic anaemia (Zanella *et al.*, 2005). In the present study a down regulated expression of pyruvate kinase protein suggest a deficiency of the enzyme in chicken due to *Salmonella* infection and the Korean mistletoe appears to be not effective against this. A rapid haemolysis may be leading to difficulty in haemoglobin metabolism in liver which is already weak due to infection which may be excreted as biliverdin instead of getting converted to bilirubin through faeces, giving greenish colour, a typical symptom of fowl typhoid.

### **CONCLUSION**

In conclusion, in experimental fowl typhoid infection in broilers fed with Korean mistletoe through proteomics approach we have found significant differential expression of four known proteins; two proteins fatty acid binding protein and MRP-126 were up-regulated and two proteins; ribosomal protein12, pyruvate kinase were down regulated which appears to be candidate molecules for fowl typhoid.

### **ACKNOWLEDGMENT**

This research was partly supported by Gyonggi Regional Research Centre (GRRC) of Gyonggi Province at Hankyong National University, Anseong.

### **REFERENCES**

Azuma, I. and J.B. Kim, 1999. Lectins isolated from Korean mistletoe (*Viscum album coloratum*) induce apoptosis in tumor cells. *Cancer Lett.*, 136: 33-40.

- Brown, C.C., D.C. Baker and I.K. Barker, 2007. Infectious and Parasitic Diseases of the Alimentary Tract-Salmonellosis. In: Pathology of Domestic Animals, Grant, M.M. (Ed.). Saunders, Ltd., London, pp: 193-195.
- Burt, D. and O. Pourquie, 2003. Genetics. Chicken genome science nuggets to come soon. Science, 300: 1669-1669.
- Cheeseman, J.H., M.G. Kaiser, C. Ciraci, P. Kaiser and S.J. Lamont, 2006. Breed effect on early cytokine mRNA expression in spleen and cecum of chickens with and without *Salmonella enteritidis* infection. Dev. Comp. Immunol., 31: 52-60.
- Chmurzyńska, A., 2006. The multigene family of Fatty Acid-Binding Proteins (FABPs): Function, structure and polymorphism. J. Applied Genet., 47: 39-48.
- Choi, S.H., K.H. Park, T.J. Yoon, J.B. Kim, Y.S. Jang and C.H. Choe, 2008. Dietary Korean mistletoe enhances cellular non-specific immune responses and survival of Japanese eel (*Anguilla japonica*). Fish Shellfish Immunol., 24: 67-73.
- Christensen, J.P., J.E. Olsen, H.C. Hansen and M. Bisgaard. 1992. Characterization of *Salmonella enterica* serovar gallinarum biovars gallinarum and pullorum by plasmid profiling and biochemical analysis. Avian Pathol., 21: 461-470.
- Clarke, D.C., D. Miskovic, X.X. Han, J. Calles-Escandon, J.F.C. Glatz and J.F.P. Luiken, 2004. Over expression associated fatty acid binding protein (FABPpm) *in vivo* increases fatty acid sarcolemmal transport and metabolism. Physiol. Genomics, 17: 31-37.
- Corbin, B.D., E.H. Seeley, A. Raab, J. Feldmann, M.R. Miller, V.J. Torres and K.L. Anderson *et al.*, 2008. Metal chelation and inhibition of bacterial growth in tissue abscesses. Science, 319: 962-965.
- Gabius, H.J., S. Gabius, S.S. Joshi, B. Koch, M. Schroeder and W.M. Manzke, 1994. From ill-defined extracts to the immunomodulatory lectin: Will there be a reason for oncological application of mistletoe. Planta Medica, 60: 2-7.
- Hajto, T., 1986. Immunomodulatory effects of Iscador: A *Viscum album* preparation. Oncology, 43: 51-51.
- Henderson, S.C., D.L. Bounous and M.D. Lee, 1999. Early events in the pathogenesis of avian salmonellosis. Infect. Immunol., 67: 3580-3586.
- Herault, Y., D. Michel, G. Chatelain and G. Brun, 1991. cDNA and predicted amino acid sequences of the human ribosomal protein genes rpS12 and rpL17. Nucleic Acids Res., 19: 4001-4001.
- Jia, Y., G. Ferracci, A. Warley, M. Ward, K.Y. Leung and S. Samsuddin, 1993. Evolutionary origin and radiation of the avian-adapted non-motile salmonellae. J. Med. Microbiol., 38: 129-139.
- Kaiser, P., L. Rothwell, E.E. Galyov, P.A. Barrow, J. Burnside and P. Wigley, 2000. Differential cytokine expression of avian cells in response to invasion by *Salmonella typhimurium*, *Salmonella enteritidis* and *Salmonella gallinarum*. Microbiology, 146: 3217-3226.
- Kenmochi, N., T. Kawaguchi, S. Rozen, E. Davis, N. Goodman, T.J. Hudson, T. Tanaka and D.C. Page, 1998. A map of 75 human ribosomal protein genes. Genome Res., 8: 509-523.
- Khwaja, T.A., J.C. Varven, S. Pentecost and H. Pande, 1980. Isolation of biologically active alkaloids from Korean mistletoe *Viscum album*, *Coloratum*. Experientia, 36: 599-600.
- Kim, J.H., D.W. Kim, K.H. Kang, B.G. Jang and D.J. Yu *et al.*, 2007. Effects of dietary Korean Mistletoe on performance of and blood characteristics in broilers. Korean J. Poult. Sci., 34: 129-136.
- Lee, H.S., Y.S. Kim, S.B. Kim, B.E. Choia, B.H. Woob and K.C. Lee, 1999. Isolation and characterization of biologically active lectin from Korean mistletoe, *Viscum album* var. *Coloratum*. Cell. Mol. Life Sci., 55: 679-682.
- Liu, X.Y., 1999. Stress and Immunity. In: Poultry Immunology, Yin, T.B. (Ed.). China Agriculture Press, Beijing, China.

- Lyu, S.Y. and W.B. Park, 2006. Mistletoe Lectin (*Viscum album coloratum*) modulates proliferation and cytokine expressions in Murine Splenocytes. *J. Biochem. Mol. Biol.*, 39: 662-670.
- Mann, M., R.C. Hendrickson and A. Pandey, 2001. Analysis of proteins and proteomes by mass spectrometry. *Annual Rev. Biochem.*, 70: 437-473.
- McArther, M.J., B.P. Atshaves, A. Frolov, W.D. Foxworth, A.B. Kier and F. Schroeder, 1999. Cellular uptake and intracellular trafficking of long chain fatty acids. *J. Lipid Res.*, 40: 1371-1383.
- O'Farrell, P.H., 1975. High-resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.*, 250: 4007-4021.
- Odink, K., N. Cerletti, J. Bruggen, R.G. Clerc, L. Tarcsay, G. Zwadlo, G. Gerhards, R. Schlegel and C. Sorg, 1987. Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis. *Nature*, 330: 80-82.
- Pandey, A. and M. Mann, 2000. Proteomics to study genes and genomes. *Nature*, 405: 837-846.
- Pogue-Geile, K., J.R. Geiser, M. Shu, C. Miller, I.G. Wool, A.I. Meisler and J.M. Pipas, 1991. Ribosomal protein genes are overexpressed in colorectal cancer: isolation of a cDNA clone encoding the human S3 ribosomal protein. *Mol. Cell. Biol.*, 11: 3842-3849.
- Sampson, B., M.K. Fagerhol, B.E. Sunderkotter, P. Golden and N. Richmond *et al.*, 2002. Hyperzincaemia and hypercalprotectinaemia: A new disorder of zinc metabolism. *Lancet*, 360: 1742-1745.
- Shah, D.H., A. Roy and J.H. Purohit, 2001. Characterization of *Salmonella gallinarum* avian strains isolated from Gujarat State. *Indian J. Comp. Microbiol. Immunol. Infect.*, 22: 131-133.
- Shah, D.H., J.H. Park, M.R. Cho, M.C. Kim and J.S. Chae, 2005. Allele-specific PCR method based on rfbS sequence for distinguishing *Salmonella gallinarum* from *Salmonella pullorum*: Serotype-specific rfbS sequence polymorphism. *J. Microbiol. Methods*, 60: 169-177.
- Shivaprasad, H.L., 1997. Pullorum Disease and Fowl Typhoid. In: *Diseases of Poultry*, Calnek, B.W. H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif (Eds.). Iowa State University Press, Ames, pp: 87-99.
- Sinz, A., M. Bantscheff, S. Mikkat and B. Ringel, 2002. Mass spectrometric proteome analysis of synovial fluids and plasmas from patients suffering rheumatoid arthritis and comparison to reactive arthritis or osteoarthritis. *Electrophoresis*, 23: 3445-3456.
- Vogl, T., K. Tenbrock, S. Ludwig, N. Leukert and C. Ehrhardt *et al.*, 2007. Mrp8 and Mrp14 are endogenous activators of Toll-like receptor 4, promoting lethal, endotoxin-induced shock. *Nature Med.*, 13: 1042-1049.
- Wang, R. and B.T. Chait, 1994. High-accuracy mass measurement as a tool for studying proteins. *Curr. Opin. Biotechnol.*, 5: 77-84.
- Wigley, P. and P. Kaiser, 2003. Avian cytokines in health and disease. *Brazilian J. Poult. Sci.*, 5: 1-14.
- Withanage, G.S., P. Kaiser, P. Wigley, C. Powers and P. Mastroeni *et al.*, 2004. Rapid expression of chemokines and proinflammatory cytokines in newly hatched chickens infected with *Salmonella enterica* serovar *typhimurium*. *Infect. Immunol.*, 72: 2152-2159.
- Yoon, T.J., Y.C. Yoo, O.B. Choi, M.S. Do, T.B. Kang, S.W. Lee, I. Azuma and J.B. Kim, 1995. Inhibitory effect of Korean mistletoe (*Viscum album coloratum*) extract on tumour angiogenesis and metastasis of haematogenous and nonhaematogenous tumour cells in mice. *Cancer Lett.*, 97: 83-91.
- Yoon, T.J., Y.C. Yoo, T.B. Kang, K. Shimazaki, S.K. Song, K.H. Lee, S.H. Kim, C.H. Park Azuma and J.B. Kim, 1999. Lectins isolated from Korean mistletoe (*Viscum album coloratum*) induce apoptosis in tumor cells. *Cancer Lett.*, 136: 33-40.

- Yoon, T.J., Y.C. Yoo, T.B. Kang, E. Her, S.H. Kim, K. Kim, I. Azuma and J.B. Kim, 2001. Cellular and humoral adjuvant activity of lectins isolated from Korean mistletoe (*Viscum album coloratum*). *Int. Immunopharm.*, 1: 881-889.
- Zanella, A., P. Bianchi, E. Fermo, A. Iurlo, M. Zappa, C. Vercellati, C. Boschetti, L. Baronciani and F. Cotton, 2001. Molecular characterization of the PK-LR gene in sixteen pyruvate kinase-deficient patients. *Br. J. Haematol.*, 113: 43-48.
- Zanella, A., E. Fermo, P. Bianchi and G. Valentini, 2005. Red cell pyruvate kinase deficiency: Molecular and clinical aspects. *Br. J. Haematol.*, 130: 11-25.