# Nutritional Control of Inflammatory Responses in Broiler Chicken

# Nutritional Control of Inflammatory Responses in Broiler Chicken

**Kazuaki TAKAHASHI**

**Graduate School of Agricultural Science, Tohoku University,
Sendai, Japan**

**Key words : broiler chicken, CLA, cysteine, inflammatory response, xylitol**

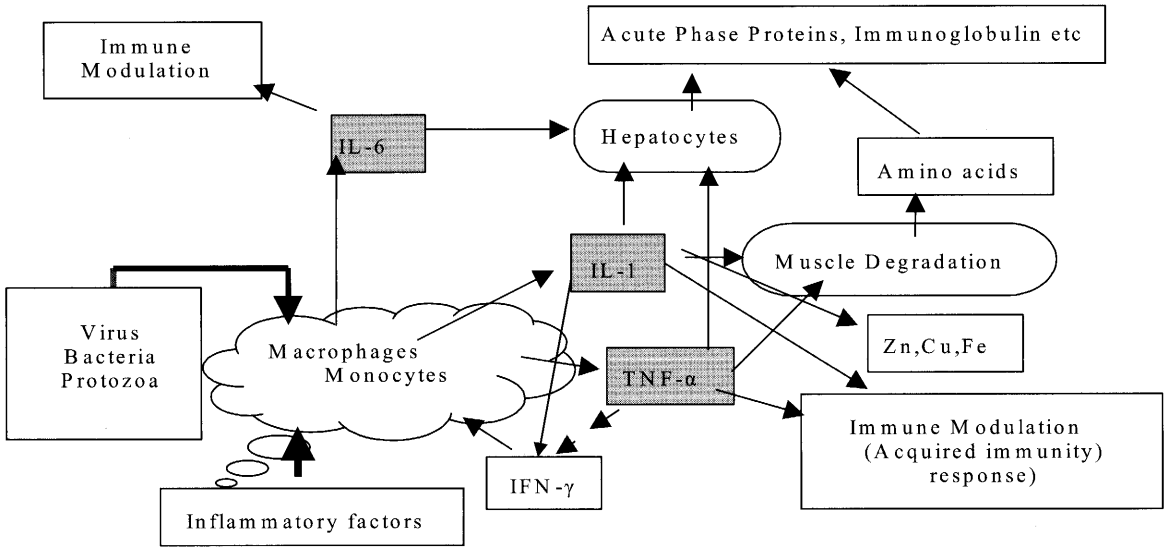
## *Introduction*

Immunological stress(es) or stimulation generally results in decrease nutrient intake, increase in catabolic process of nutrients and change nutrient partitioning. Chickens under conventional raising conditions are exposed to many kinds of stressors, such as pathogenic or non-pathogenic microorganisms, transportation to the growing site, overcrowding, vaccination, chilling and/or overheating, etc. To maintain the self-defense functions, immunecompetent cells must proliferate, express receptors for the recognition of foreign molecules, produce cytokines to regulate the responses, produce antibodies and other effectors molecules. Production of effector molecules, such as reactive intermediates of oxygen and nitrogen may increase energy and nutrient demand. Thus nutrient requirements for achieving appropriate immunological status are not always identical with those for obtaining the maximum production under these circumstances. Since nutrient requirement for innate immune response is probably greater than for acquired immune response, it appear likely that control of innate immune response following production of pro-inflammatory cytokines such as iterleukin(IL)-1, 6 and tumor necrosis factor (TNF)-α have impact on chicken production during immune stimulation. However, TNF-α, a pro-inflammatory cytokine, is not cloned so far even though this cytokine is important not only in immune regulation, but also in metabolic changes.

## *Immune regulation and Nutrition*

Klasing (1988) and Klasing and Johnstone (1991) have suggested that performance of poultry is adversely affected by immune stimulants and vaccination. Stimulation of selected cells of immune system triggers systemic metabolic changes, which include fever, anorexia, decreased net skeletal muscle protein deposition, and thus growth retradation. Improved sanitary condition and antibiotic therapy reduced the catabolic changes

**Figure 1.** Simple diagram of source and cause regarding pro-inflammatory cytokine (IL-1, 6 and TNF) release and its action in acute phase response



caused by immunological stimulation (Roura *et al.*, 1992). On the other hand, nutritional modulation of specific immune responses or response to immune signals offers a simple method of regulating catabolic effects of immune stimulation. One of the goals for nutritional modulation of immune system is to alleviate loss of performance following immune stimulation without affecting the immune or inflammatory response *per se*. Feeding diets with essential nutrients excess or deficiency causes impairment of immune function and response in many aspects as reviewed by Katous and Klasing (2001). I introduce here how addition of certain nutrients to broiler diet affects immune and metabolic responses during immunological stimulation and some functions of chick TL1A as avian TNF-α.

### *Cysteine (Cys) or sulphur amino acid (SAA)*

Cysteine (Cys) or sulphur amino acid (SAA) is of prime importance in increasing liver glutathione (GSH) in rats treated with TNF-α (Grimble *et al.*,1992; Hunter *et al.*, 1994). Thus dietary sulfur amino acids also have certain influence on inflammatory and immune responses. Chicks fed on a Met sufficient diet had higher IL-1 activity, growth rate and feed intake compared to chicks fed on a Met-deficient diet when they received immunogen injections (Klasing and Barnes, 1994). Tsiagbe *et al.* (1987b) showed that antibody production against sheep red blood cell and delayed hypersensivity against phytohemagglutinin (PHA)-P in chicks fed a SAA-deficient diet was lower than those in chicks fed a SAA-sufficient diet. Although Hunter *et al.*(1994) showed production of several acute phase proteins in rats fed a Met-rich diet did not differ from that in rats fed on a Cys-rich diet when they were injected with TNF-α, it is likely that the function of Cys under stressful conditions is possibly different from that of Met. Tsiagbe et al. (1984a) showed that Cys was 70-84 % as efficient as Met in enhancing immunoglobulin-G (IgG) production and in delaying hypersensitivity to PHA-P stimulation, respectively, indicating that Cys is an essential nutrient for potentiating the immune response in broilers. Feeding L-Cys increases tissue GSH level (Graber and Baker, 1971) and an increase in tissue GSH concentration may be beneficial for growth of chicks reared in conditions with immunological stress by drug administration (Boebel and Baker, 1983). Furthermore, Cys and Cys derivatives have been shown to modulate lymphocyte and macrophage functions in the in vitro studies (Droge *et al.*, 1991). As shown in Fig 2, a high Cys diet enhanced plasma α1-acid glycoprotein concentration, IL-1 like activity when chicks were single injected with *Escherichia coli* lipopolysaccharide (LPS), and lymphocyte proliferation (Takahashi *et al.*, 1999). Our experiment also indicates that a high Cys diet enhanced mitogen-induced proliferation of lymphocytes compared to a high Met diet (Takahashi *et al.*, 1997). Thus, a change in the ratio of Cys and Met in diet has an impact on the immune and inflammatory responses in chickens.

### *Conjugated linoleic acids (CLA)*

Conjugated linoleic acids (CLA) are an isomeric mixture of 18:2 fatty acids that have conjugated

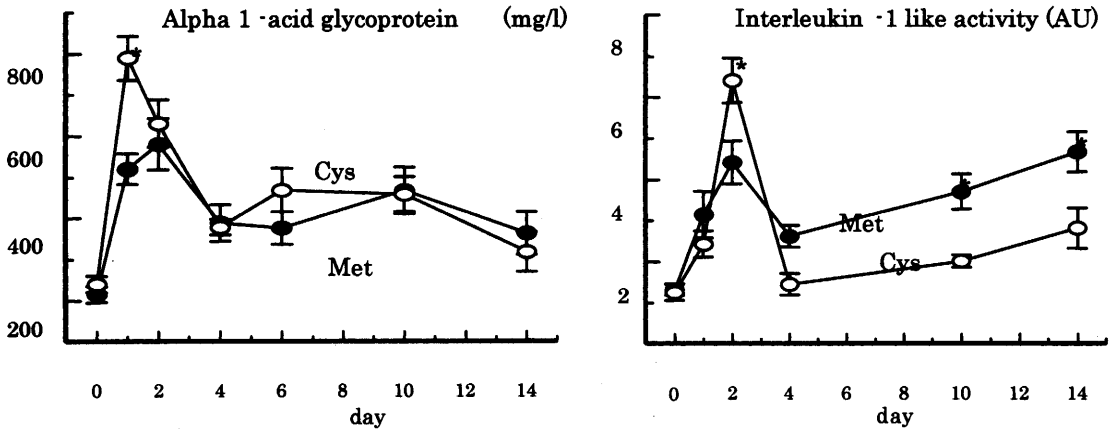



**Figure 2.** Effect of dietary methionine and cystine on plasma alpha-1 acid glycoprotein and interleukin-1 activity in chicks injected with LPS every other day (1 mg/kg body weight gain).

double bonds (Fritsche and Steinhart, 1998). The effects of CLA in animals are well reviewed by Fritsche and Steinhart (1998) and Pariza *et al.* (2000). There is a great interest in these fatty acid isomers because CLA has several unique proprieties that modulate the physiological and metabolic responses including immune response like lymphocyte proliferation in mice (Chew *et al.*, 1997; Wong *et al.*, 1997) and interleukin (IL)-2 production in mice (Hayek *et al.*, 1999) and proinlfammatory cytokine production from macrophages (Turek *et al.*, 1998). The immunomodulatory effects of CLA in animals have been recently reviewed by O'Shea *et al.* (2004). It has been suggested that CLA protected the catabolic responses against endotoxin in chicks and mammals (Cook *et al.*, 1993; Miller *et al.*, 1994). Takahashi *et al.* (2002) demonstrated that anti-inflammatory effect of dietary CLA in male broiler chickens by assessing the several inflammatory parameters, e.g. growth performance, blood heterophil to lymphocyte ratio, and plasma acute phase protein concentrations such as ceruloplasmin and α1 acid glycoprotein during lipopolysaccharide (LPS) and sephadex injections. Some results of this study are summarized in Table 1. CLA probably modulated immune responses in mammals although the effect has not been fully clarified (O'Shea *et al.* (2004). In addition, there are a few reports for effect of CLA on antibody or immunoglobulin (Ig) production. Dietary CLA enhanced Ig production in immunocompetent organs and plasma IgG concentration in rats (Sugano *et al.*, 1998). Yamasaki *et al.* (2000) observed that CLA enhanced Ig production in spleen but did not affect serum Ig level in rats. Cook *et al.*(1993)showed that antibody production to sheep red blood cell (SRBC) was not affected by feeding CLA in chicks. We re-evaluated the effect of dietary CLA on antibody production to SRBC and IgG concentration in plasma using broiler chickens.

As shown in Fig 3, dietary CLA enhanced primarily antibody production to SRBC and IgG concentration in plasma (Takahashi *et al.*, 2003). Our following study regarding effect of CLA on immune response suggests that mitogen-induced proliferation and IL-2-like activity in splenocytes was higher in splenocytes from chicks fed CLA-supplemented diet than those in chicks fed safflower-supplemented diet, which was comparable to those in chicks fed basal diet. Dietary CLA also had the potential to alter the T cell subpopulation in the spleen (submitted). Hence CLA

**Table 1.** Effect of dietary conjugated linoleic acid (CLA) on body weight gain and alpha 1 acid glycoprotein and ceruloplasmin in plasma during immunological stimulation induced by LPS (0.5 mg/kg body weight) and sephadex (250mg/ kg body weight) for 5 days[1]

| | Saline | | LPS and sephadex | |
|---|---|---|---|---|
| | Control | CLA | Control | CLA |
| Body weight gain (g,5days) | 288 ± 18 | 276 ± 11 | 235 ± 17 | 262 ± 16* |
| Alpha 1 acid glycoprotein (mg/l)[2] | 183 ± 17 | 203 ± 27 | 674 ± 51 | 511 ± 37* |
| Ceruloplasmin (mg/l) [2] | 21.1 ± 2.9 | 25.6 ± 3.4 | 60.6 ± 6.9 | 44.1 ± 3.2* |

[1] Male broiler chicks were used at 21 days of age. Mean ± standard error (n=10).
[2] Sample was obtained 24 hours after first LPS injection.
* Significantly different relative to the control ($p<0.05$)

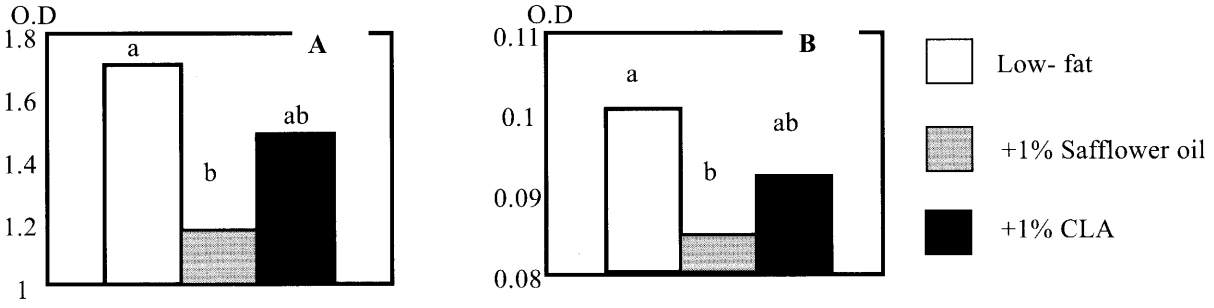



**Figure 3.** Effect of dietary conjugated linoleic acid on splenocytes proliferation to Con A (A) and interleukine-2 like activity of splenocytes induced by Con A (B). a,b<0.05.

may potentially be used as an alternative to feed antibiotics in chick's diet. Dietary CLA in chick also have potential as means of improving responses to vaccination and conferring disease resistance.

### *Xylitol*

Van Heugten *et al.* (1996) showed that increasing energy density of diet did not alter the growth depression following LPS challenge, and that dietary addition of lard improved feed efficiency and efficiency of energy conversion in pigs. On the other hands, Benson *et al.* (1993) showed that increasing energy density by cornstarch, but not oil (corn oil), eliminated the growth depressing effect of immunogen in chicks when dietary energy level was above 13.4kJ/kg diet. It is known that xylitol supplemented parental nutrition has beneficial effects on nitrogen and glucose utilization during stress in mammals. Xylitol is a five-carbon polyol and an intermediate product in the glucuronic acid-xylulose cycle and the pentose phosphate pathway. It exerts a nitrogen-sparing effect without appreciable effects of insulin secretion, and is metabolized primarily in the liver, where it is converted via an insulin-independent pathway to glucose 6-phosphate. Dietary xylitol at the 20% level tended to lower insulin secretion, but did not change the plasma glucagon and corticosterone concentration in rats (Hamalainen & Makinen, 1985). Those observations may lead to an assumption that dietary xylitol has potential to alleviate the reduction in performance during immune stimulation.

Our serial studies (Takahashi *et al.*, 1999, 2000 and 2005) shows that dietary xylitol prevents growth retardation (Table 2) without impairing acute inflammatory responses, e.g. IL-1 like activity, α-1 acid glycoprotein release (Table 2) under stressful conditions induced by LPS and Sephadex injections, but enhances pokeweed mitogen-induced nuclear cell proliferation of lymphocytes and antibody production against sheep red blood cell (Fig 4). In those experiments, metabolizable energy content of the experimental diets and energy intake did not differ among the dietary groups. This result suggests that dietary xylitol is a useful nutrient for controlling

**Table 2.** Effect of dietary xylitol (6%) on body weight gain and alpha 1 acid glycoprotein and interleukin-1 in plasma during immunological stimulation induced by LPS (0.5 mg/kg body weight) and sephadex (250mg/ kg body weight) for 5 days[1]

| | Saline | | LPS and sephadex | |
|---|---|---|---|---|
| | Glucose | Xylitol | Glucose | Xylitol |
| Body weight gain (g,5days) | 254 ± 15 | 243 ± 13 | 181 ± 18 | 216 ± 12* |
| Alpha 1 acid glycoprotein (mg/l)[2] | 247 ± 18 | 227 ± 31 | 1160 ± 79 | 1126 ± 67 |
| Interleukin-1 activity (AU) [2] | 1.24 ± 0.18 | 1.39 ± 0.05 | 1.89 ± 0.12 | 1.78 ± 0.20 |

[1] Male broiler chicks were used at 21 days of age. Mean ± standard error (n=10).
[2] Sample was obtained 24 hours after first LPS injection.
* Significantly different relative to the control ($p<0.05$)

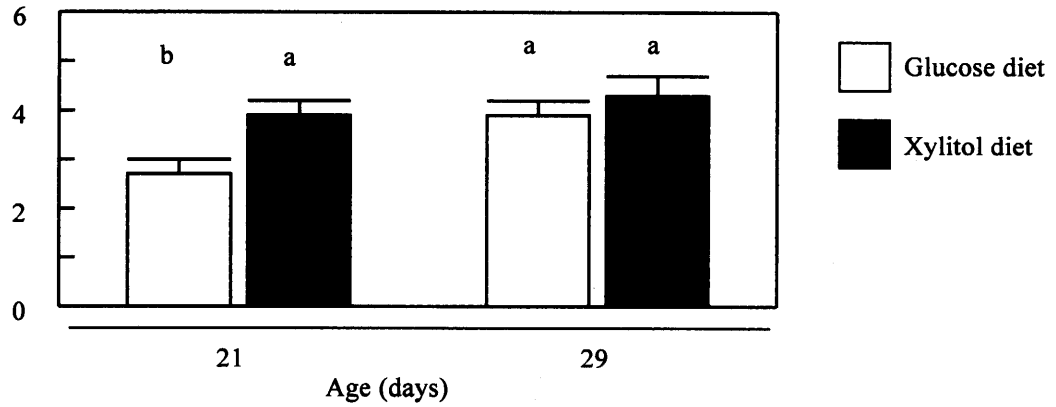



**Figure 4.** Antibody titers (log2) agnist Sheep Red Blood Cell in chicks fed the glucose or xylitol contained-diets. Chicks were challenged at 14 and 21 days of age. a, b <0.05.

growth performance during immune stress, but a mode of action of stress preventing effect of dietary xylitol may not be the same as those of increased energy concentration by glucose as previously reported by Benson *et al* (1993).

### *Tumor necrosis factor like ligand 1A (TL1A) as TNF-alpha substitute in chicks*

TNF-α plays crucial roles in the immunological modulation of inflammation and cellular immune responses. In chickens, although TNF-α like activity has been detected only in the supernatant of chicken macrophages culture medium (Rautenschlein *et al.*, 1999), molecular cloning of TNF ligand superfamily members in avian species has been unsuccessful so far. It is also notable that the possible sequence of a chicken homologue of mammalian TNF-α has not been identified to date in the chicken genome database. Recently, TL1A was cloned as a long form of vascular endothelial cell-growth inhibitor, another member of the TNF ligand super family, in mammals (Migone *et al.*, 2002). Takimoto *et al.* (2005) first time showed the cloning and functional characterization of chicken TL1A. ChTL1A, a TL1A homologue in chickens, comprised of an ORF of 717 nucleotides that translated to produce a putative peptide of 239 amino acids. This is very similar to human pro-TNF-α that codes for 233 amino acids (Wang *et al.*, 1985), whereas the human TL1A sequence encodes for a slightly longer polypeptide of 251 amino acids, translated from a 753 bp ORF (Migone *et al.*, 2002). The phylogenic analysis suggests that molecule of chicken TL1A is closely related to that of human is nearer to TNF-α rather than any other molecules of chicken TNF ligand superfamily although the chicken TL1A was distantly related distant from to mammalians or teleosts TNF-α. The amino acid sequence predicted from ChTL1A revealed a TNF signature sequence similar to the TNF superfamily members reported in other animals and the cDNA sequence existing on chromosome 17 in the chicken.

Takimoto *et al.* (2005) showed that an increase in expression of ChTL1A mRNA following the induction of inflammation by LPS and addition of recombinant chicken TL1A to culture medium of L929 cells and chicken fibroblast cells reduced their survival. They also demonstrated decrease in feed intake, and increase in fever and nitric oxide production in chicks injected with TL1A as shown in Fig 5. Thus, chicken TL1A plays an important role in inflammation in chickens. Taken together these findings and observations, it is likely that chicken TL1A possibly substitutes for a part of function of TNF-α observed in mammals.

### *References*


Benson, B. N., C. C. Calvert, E. Roura and K. C. Klasing, 1993. Dietary energy source and density modulate the expression of immunological stress in chicks. J. Nutr. 123:1714-1723.

Boebel, K.P. and D. H. Baker, 1983. Blood and liver concentrations of glutathione and plasma concentrations of sulfur-containing amino acids in chicks

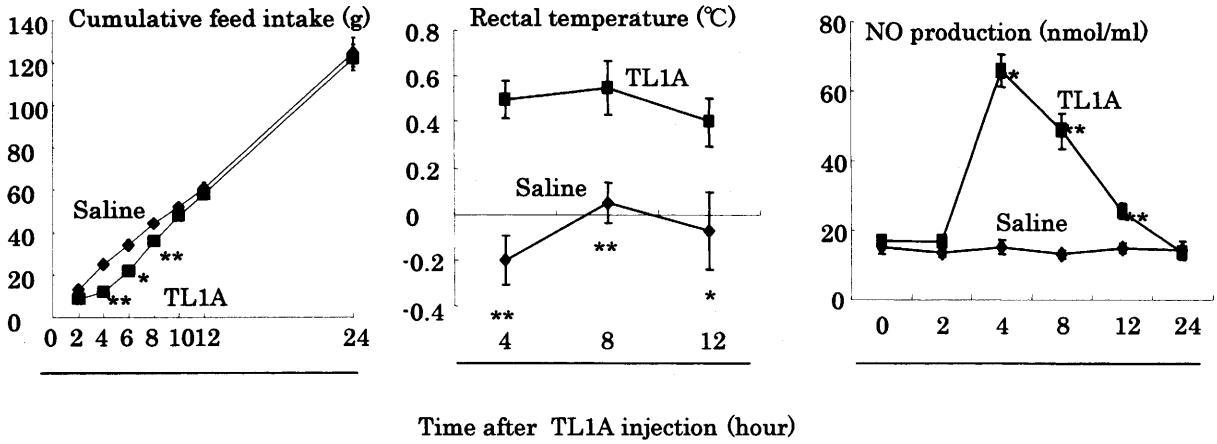




**Figure 5.** Temporal changes in feed intake, rectal temperature and NO production of chicks injected with TL1A.
** $p<0.05$

fed deficient, adequate, or excess levels of dietary cysteine. Pro. Soc.Exp. Biol. Med. 172: 498-501.

Chew, B. P., T. S. Wong, T.D. Shultz and N. Magnuson, 1997. Effects of conjugated dienoic derivatives of linoleic acid and beta-carotene in modulating lymphocyte and macrophage function. Anticancer Res.17:1099-1106.

Cook, M.E., C. C. Miller, Y. Park and M. Pariza, 1993. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. Poultry Sci. 72:1301-1305.

Droge, W., H. P. Eck, H. Gmunder and S. Mihm, 1991. Modulation of lymphocyte functions and immune responses by cysteine and cysteine derivatives. Amer. J. Med. 30: 104S-114S.

Frische, J. and H. Steinhart, 1998. Analysis, occurrence, and physiological properties of trans fatty acids (TFA) with particular emphasis on conjugated linoleic isomers (CLA) – a review. Fett Wiss. Tech.100:190-220.

Graber, G. and D. H. Baker, 1971. Sulfur amino acid nutrition of the growing chick: quantitative aspects concentrating the efficiency of dietary methionine. J. Animal Sci. 33: 1005-1011.

Grimble, R. F., A. A. Jackson, C.Persaud, M. J. Wride, F. Delers and R. Engler, 1992. Cysteine and glycine supplementation modulate the metabolic response to tumor necrosis factor alpha in rats fed a low protein diet. J. Nutr. 122: 2066-2073.

Hamalainen, M. H. and K. K. Makinen, 1985. Metabolic effects in rat of high oral doses of galactitol, mannitol and xylitol. J. Nutr. 115:890-899.

Hayek, M. G., S. N. Han, D. Wu, B. A. Watkins, M. Meydan, J. L. Dorsey, D. E. Smith and S. N. Meydani, 1999. Dietary conjugated linoleic acid influences the immune response of young and old C57BL/6NCrlBR mice. J. Nutr. 129: 32-38.

Hunter, E. A. L. and R. F. Grimble, 1994. Cysteine and methionine supplementation modulate the effect of tumor necrosis factor alpha on protein synthesis, glutathione and zinc concentration of liver and lung in rats fed a low protein diet. J. Nutr. 124: 2319-2338.

Klasing, K. C., 1988. Nutritional aspects of leukocytic cytokines. J. Nutr. 118:1436-1446.

Klasing, K.C and D. M. Barnes, 1994. Decreased amino acid requirements of growing chicks due to immunological stress. J. Nutr. 118: 1158-1164.

Klasing, K. C. and B. J. Johnstone, 1991. Monokines in growth and development. Poultry Sci. 70: 1176-1186.

Koutsos, E. A. and K. C .Klasing, 2001. Interactions between the immune system, nutrition, and productivity of animal. In: In: Recent advances in animal nutrition. (editor: Garnswothy, P. C. and J. Wiseman,) pp. 173-190. Nottingham University Press. UK.

Migone, T. S., J. Zhang, X. Luo, L. Zhuang, C. Chen, B. Hu, J. S. Hong, J. W. Perry, S. F. Chen, J. X. H. Zhou, Y. H. Cho, S. Ullrich, P. Kanakaraj, J. Carrell, E. Boyd, H. S. Olsen, G. Hu, L. Pukac, D. Liu, J. Ni, S. Kim, R. Gentz, P. Feng, P. A. Moore, S. M. Ruben and P. Wei, 2002. TL1A is a TNF-like ligand for DR3 and TR/DcR3 and functions as a T cell costimulator. Immunity 16: 479-492.

Miller, C. C., Y. Park, M. W. Pariza and M. E. Cook, 1994. Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. Biochem. Biophys. Res. Comm. 198: 1107-1112.

O'Shea, M., J. Bassaganya-Riera and I. C. M. Mohede, 2004. Immunomodulatory properties of conjugated linoleic acid. Am. J. Clin. Nutr. 79: 1199S –1206S.

Pariza, M.W., Y. Park and M. E. Cook, 2000. Mechanisms of action of conjugated linoleic acid: evidence and speculation. Pro .Soc. Exp. Biol. Med. 223: 8-13.

Rautenschlein, S., A. Subramanian and J. M. Sharma, 1999. Bioactivities of a tumor necrosis-like factor released by chicken macrophages. Develop. Comp. Immunol. 23: 629-640.

Roura A., E., J. Homedes and K. C. Klasing, 1992. Prevention of immunologic stress contributes to the growth-permitting ability of dietary antibiotics in chicks. J. Nutr. 122: 2383-2390.

Sugano, M., A. Tsujita, M. Yamasaki, M. Noguchi and K. Yamada, 1998. Conjugated linoleic acid modulates tissue levels of chemical mediators and immunoglobulins in rats. Lipid 33: 521-527.

Takahashi, K. and Y. Akiba, 1999. Effect of dietary methionine to cysteine ratio on inflammatory responses in chicks injected with multiple injections of Escherichia coli lipopolysaccharide. Animal Sci. J. 70: 312-318.

Takahashi, K., K. Kawamata and Y. Akiba, 2005. Effect of xylitol feeding on antibody production and inflammatory responses in broiler chicks. J. Poult.

Sci. 42: 245-253.

Takahashi, K., K. Kawamata, Y. Akiba, T. Iwata and M. Kasai, 2002. Influence of dietary conjugated linoleic acid isomers on early inflammatory responses in male broiler chickens. Br. Poult. Sci. 43: 47-53.

Takahashi, K., K. Kawamata, Y. Akiba, T. Iwata and M. Kasai, 2003. Effect of a mixture of conjugated linoleic acid (CLA) isomers on growth performance and antibody production in broiler chicks. Br. J. Nutr. 89: 691-694.

Takahashi, K., T. Mashiko and Y. Akiba, 2000. Effect of dietary concentration of xylitol on growth in male broiler chicks during immunological stress. Poultry Sci. 79: 743-747.

Takahashi, K., K. Onodera and Y. Akiba, 1999. Effect of dietary xylitol on growth and inflammatory responses in immune stimulated chickens. Br. Poult. Sci. 40: 552-554.

Takahashi, K., T. Ohta and Y. Akiba, 1997. Influences of dietary methionine and cysteine on metabolic responses to immunological stress by Escherichia coli lipopolysaccharide injection, and mitogenic response in broiler chickens. Br. J.Nutr. 78: 815-821.

Takimoto, T., K. Takahashi, K. Sato and Y. Akiba, 2005. Molecular cloning and functional characterizations of chicken TL1A. Develop. Comp. Immunol. 29: 895-905.

Tsiagbe, V. K., M. E. Cook, A. E. Harper and M. L. Sunde, 1987a. Efficiency of cysteine in replacing methionine in the immune responses of broiler chicks. Poult. Sci. 58: 1138-1146.

Tsiagbe, V. K., M. E. Cook, A. E. Harper and M. L. Sunde, 1987b. Enhanced immune responses in broiler chicks fed methionine supplemented diets. Poult. Sci 58: 1147-1154.

Turek, J. J., Y. Li, I. A. Schoenlein, K. G. D. Allen and B. A. Watkins, 1998. Modulation of macrophage cytokine production by conjugated linoleic acids is influenced by the dietary n-6:n-3 fatty acid ratio. J. Nutr. Biochemi. 9: 258-266.

Van Heugten, E., M. T. Coffey and J. W. Spears, 1996. Effects of immune challenge, dietary energy density, and source of energy on performance and immunity in weanling pigs. J. Animal Sci. 71: 2431-2440.

Wong, M. W., B. P. Chew, T. S. Wong, H. L. Hosick and T. D. Shultz, 1997. Effects of dietary conjugated linoleic acid on lymphocyte function and growth of mammary tumors in mice. Anticancer Res. 17: 987-993.

Yamasaki, M., K. Kishihara, K. Mansho, Y. Ogino, M. Kasai, M. Sugano, H. Tachibana and K.Yamada, 2000. Dietary effect of conjugated linoleic acid increases immunoglobulin productivity of Sprague-Dawley rat spleen lymphocytes. Biosci. Biotech. Biochem. 64: 2159-2164.