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REVIEW ARTICLE

Molecular nutrition: Interaction of nutrients, gene regulations and performances

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ABSTRACT

Nutrition deals with ingestion of foods, digestion, absorption, transport of nutrients, intermediary metabolism, underlying anabolism and catabolism, and excretion of unabsorbed nutrients and metabolites. In addition, nutrition interacts with gene expressions, which are involved in the regulation of animal performances. Our laboratory is concerned with the improvement of animal productions, such as milks, meats and eggs, with molecular nutritional aspects. The present review shows overviews on the nutritional regulation of metabolism, physiological functions and gene expressions to improve animal production in chickens and dairy cows.

Key words: *chicken, dairy cow, gene expression, metabolism, nutrition.*

INTRODUCTION

Nutrition is the science of investigating interrelationships between the animal's body and its feed. Recent trends in increased accumulation of knowledge and technology in the nutrition, biochemistry and molecular biology of tissues, cells and genes of animal species, nutrition today has evolved toward an integrated science unifying many aspects related to biological science. I and collaborators have studied to develop the novel animal production system which provides high quality products with low cost using molecular nutritional techniques in chickens and cows. Based on these aspects, I introduce, in the present review, the four points of our results; that is, characterization of chicken lipoprotein metabolism, adipocyte and myoblast regulation with the improvement of meat production, improvement of immune function, and the reduction of oxidative stress.

CHARACTERIZATION OF CHICKEN LIPOPROTEIN METABOLISM

Lipogenic activity in the liver is much greater than in adipose tissue in chickens and most fats accumulated in adipose tissues may be accounted for by incorporation of triacylglycerols from plasma lipoproteins which are either synthesized in the liver or provided from dietary fats (Griffin & Hemier 1988). On the other hand, fat synthesizing and accumulation are mainly regulated by adipose tissue in mammals, while in chickens, fat accumulation in adipose tissue is dependent on uptake of triacylglycerols from very low density lipoprotein (VLDL) secreted and transported from liver. In this process, lipoprotein lipase (LPL)-catalyzed hydrolysis of triacylglycerols in adipose tissues is the rate-limiting step. The crucial role of LPL has been evidenced by inhibition of LPL activity by anti-LPL monoclonal antibody causing lipemia and decreasing adipose fat deposition to half that of control chickens (Sato *et al.* 1999a). Thus, LPL has been targeted for nutritional modification in order to reduce the fatness of chickens safely and in the consumer's perception. However, LPL messenger RMA (mRNA) expression in growing chickens is less responsive to aging and nutritional manipulation than in mammals (Sato & Akiba 2002), indicating species-specificity in the regulatory mechanism in fat deposition as well as functionality of nutrients and/or feed ingredients. We have further explored two possibilities to modulate chicken fat deposition, which are the regulation of LPL-catalyzed hydrolysis (Sato *et al.* 1999b) and VLDL secretion from liver (Tachibana *et al.* 2002; Chiba *et al.* 2003; Tachibana *et al.* 2005).

Lipoprotein metabolism in chickens has the species characterization described above. In addition, the developing oocytes of laying hens have been identified as a

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characteristic site for lipid deposition (Schneider *et al.* 1998). These results suggest a species-specific difference between chicken and mammalian lipid metabolism. Based on these speculations, we have further investigated the characterization of lipid metabolism in chickens. Figure 1 shows the brief overview of chicken lipid metabolism described by our research. At first, we identified the molecular characterization of two key enzymes, 3-hydroxy-3-methylglutaryl coenzyme A reductase and cholesterol 7-alpha hydroxylase, that are the regulators of cholesterol metabolism (Sato *et al.* 2003). Further studies on regulatory factors involved in lipoprotein and cholesterol metabolism also provided cholesteryl ester transfer protein (Sato *et al.* 2007), lipase gene family (Sato *et al.* 2010), lipoprotein remnant (Sato *et al.* 2009a) and angiopoietin-like 3 (Sato *et al.* 2008). In addition, the transcriptional factors to regulate these factors were identified (Seol *et al.* 2007; Sato & Kamada 2011). The role of Liver X receptors (LXRs) on lipid metabolism was determined in chicken primary hepatocytes, with results showing that LXR activates not only fatty acid synthesis but also bile acid and cholesterol synthesis in these cells (Sato & Kamada 2011). Then, the novel methods reducing fat deposition in chickens might be developed by new techniques using molecular nutrition, including LXR regulation.

ADIPOCYTE AND MYOBLAST REGULATION WITH THE IMPROVEMENT OF MEAT PRODUCTION

Obesity, a condition in which there is an excessive amount of adipose tissue mass in relation to lean body mass, is a nutritional disorder most prevalent in animals. The increase in adipose tissue mass can result from the multiplication of new fat cells through adipogenesis and/or from increased deposition of cytoplasmic triglycerides (Soukas *et al.* 2001). In studying the development of adipose tissue in chickens, it has previously been shown that increases in the abdominal fat pad mass of broiler chickens mainly depends on hyperplasia of adipocytes until 4 weeks of age and from thereafter on hypertrophic growth (Hood 1982). Peroxisome proliferation-activated receptor gamma (PPARγ) is key regulatory factor in fat deposition in the hypertrophic growth stage of chicken adipose tissues (Sato *et al.* 2004, 2009b) and in the early stages of chicken preadipocyte differentiation (Matsubara *et al.* 2005). Differentiation from pre-adipocyte to mature adipocyte results in cell growth arrest (Ntambi & Young-Cheul 2000). Thus, one might expect that the activation of PPARγ in the hypoplasia stage of fat accumulation causes decreased fat deposition in chickens after growth, so as to induce adipocyte cell growth arrest. We clearly demonstrated that the abdominal adipose tissue weight in chickens given a single intraperitoneal injection of troglitazone, a PPARγ agonist, at 1 day of age was lower than that of control chickens (Sato *et al.* 2008). It is therefore likely that PPARγ plays an important role in the regulation of both the hyperplasia of adipocytes and hypertrophic growth in broiler chickens, and that the activation of PPARγ in the hyperplasia stage of chicken adipose tissue deposition effectively manipulates fatness in broiler chickens (Fig. 2A). In addition, the other regulatory factors of adipocyte differentiation, PPARβ/δ (Sato *et al.* 2009c), ZNF423, KLFs and FGF10

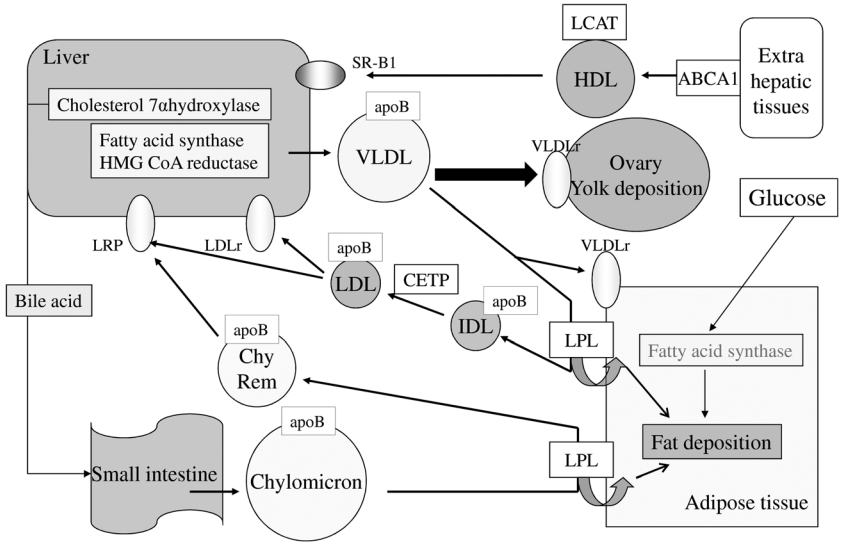

Figure 1 A schematic diagram of lipoprotein metabolism in chickens. VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LPL, lipoprotein lipase; VLDLr, VLDL receptor; LDLr, LDL receptor; LRP, LDL receptor-related protein; LCAT, lecithin-cholesterol acyltransferase; ABCA1, ABC transporter 1; CETP, cholesteryl ester transfer protein; SR-B1, schavenger receptor B1.

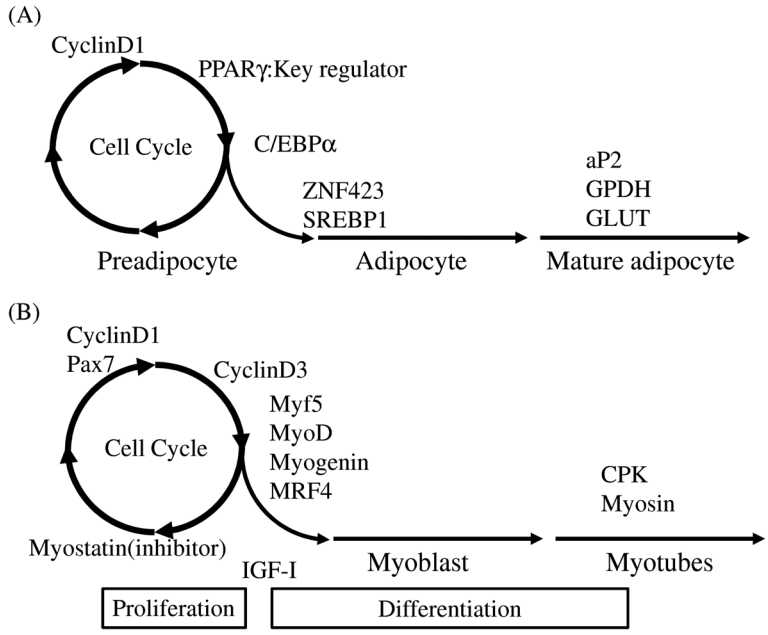

Figure 2 Transcriptional factors involved in cell proliferation and differentiation in adipocyte (A) and myoblast (B).

(Matsubara *et al*. 2013), were identified. These results may provide novel information for improvement of chicken meat production.

Skeletal muscle fibers are formed during embryogenesis and continue to enlarge post-natally until mature size has been reached. In chicks, myoblast proliferation, differentiation and fusing into myofibers continue during the early stage of post-hatch growth until day 3 (Halevy *et al*. 2004). It is therefore likely that this period, that is a few days prior to hatching, may be targeted for the modulation of growth performance and meat weight in chickens. In order to explore novel methods to control chicken performance, it may be beneficial to accumulate knowledge of the expression of regulatory factors that are involved in myoblast differentiation during myogenesis in chickens (Fig. 2B). We reported that insulin and this signaling (insulin/PI3K/Akt) is involved in the process of myogenesis, such as proliferation and differentiation *in vivo* and *in vitro*, and insulin or tolbutamide administration in newly hatched chicks is an efficient manipulation to improve chicken performance after growth (Sato *et al*. 2012a). These results provided not only species difference of insulin action in chick myoblast but also novel basic information for improvement of chicken meat production.

IMPROVEMENT OF IMMUNE FUNCTION

Meat production in the broiler industry needs to decrease or stop the use of antibiotics which are used to prevent disease and thereby promote growth in poultry (Ferket 2004). An alternative way to avoid the use of antibiotics is the control of the immune system that enhances humoral immunity and minimizes immunological stress in chickens (Klasing 1998). Immunomodulators could protect chickens from disease without decreasing growth performance by enhancing the immune system and could be used as a substitute for antibiotics. However, chicken immune systems have species-specific differences compared to mammals. We reported that TL1A plays an important role as a pro-inflammatory cytokine instead of tumor necrosis factor (TNF)-α in chickens (Takimoto *et al*. 2008). Then, it is important to identify new supplements which act as immunomodulators in chickens for efficient meat production without antibiotics. The immune response in broiler chickens has been evaluated by the expression of T-cell-related mRNAs (including cluster of differentiation 3 (CD3), interleukin (IL)-2, interferon (IFN)-γ) and toll-like receptors (TLRs) in the foregut and spleen, as well as phagocytes of blood mononuclear cells (MNC), mitogen (concanavalin A (Con A))-induced proliferation of splenic MNC of growing broiler chickens (Takahashi *et al*. 2008, 2010). Thus, we identified three molecules using these parameters (Fig. 3). The lactobacilli used in the study, particularly *Lactobacillus gasseri* TL2919, enhanced development of the gut immune system in neonatal chicks, suggesting that they could be useful as immunomodulators that minimize immunological stress and therefore protect chicks from disease without decreasing growth performance (Sato *et al*. 2009d). Modified arabinoxylan rice bran (MGN-3), which is a denatured hemicellulose obtained by reacting rice bran hemicellulose with multiple carbohydrate hydrolyzing enzymes from the shiitake mushrooms, enhances the expression of T-cell-related mRNAs and TLRs in the foregut and spleen, as well as phagocytes of blood MNC and mitogen-induced

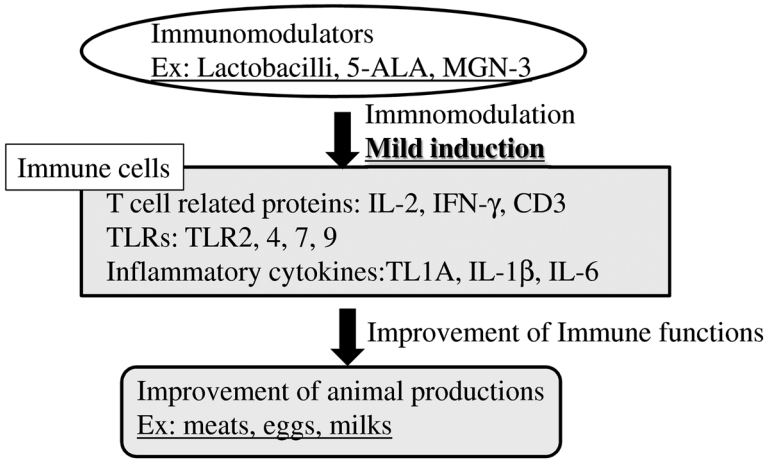

Figure 3 Improvement of animal production by immunomodulators. CD3, cluster of differentiation 3; IL, interleukin; IFN, interferon; TLR, toll-like receptor; TL1A, tumor necrosis factor-like ligand 1A, MGN-3, modified arabinoxylan rice bran; 5-ALA, 5-aminoleevulinic acid.

proliferation of splenic MNC of growing broiler chickens (Sato *et al*. 2012b). In addition, the inflammatory response resulting from *Escherichia coli* lipoploysaccharide-induced immune stimulation was improved in the chickens fed 5-aminoleevulinic acid (5-ALA)-supplemented diets, resulting in improvements in performance (Sato *et al*. 2012c). Further investigation on nutritional modification of immune developments and responses in broiler chicks may improve production efficiency under stressful raising conditions.

THE REDUCTION OF OXIDATIVE STRESS IN DAIRY COWS

Oxidative stress leads to aging and disease in animals, and is caused by an imbalance between the production of reactive oxygen species (ROS) and antioxidant activity. Although ROS play an important role in biological defense against infections, they also injure cells, DNA, RNA, proteins, carbohydrates and lipids, which can in turn give rise to serious health problems (Boots *et al*. 2008; Herrera *et al*. 2009). A serious problem for the dairy industry is the lipid oxidation of milk fats which gives rise to lipocatabolic odor (Lindmark-Månsson & Akesson 2000). These odors result from unsaturated fatty acid oxidation by reactive oxygen during long-term preservation (Al-Mabruk *et al*. 2004). Based on this concept, it has been reported that the supplementation of antioxidative nutrients; that is, vitamin E (Al-Mabruk *et al*. 2004; Sympoura *et al*. 2009), in the diets of dairy cows could result in milk with a low lipid peroxide content and high antioxidant activity. However, milk with a high antioxidant and low lipid peroxide content has not been developed, because ruminants have a specific digestive organ, that is the rumen.

Trehalose is a natural disaccharide composed of two molecules of glucose joined by an α,α-1,1 glucosidic linkage; it is used widely, particularly as a food additive and in cosmetics, as well as for its antioxidant activity (Oku *et al*. 2003). We reported that dietary supplementation with trehalose brings about an improvement of oxidative status in the rumen fluid, blood and milk of cows treated in this manner compared to controls (Aoki *et al*. 2010). These results suggest that trehalose is useful as a supplement for reducing oxidative stress in dairy cows and improving milk quality. In addition, the antioxidant activity associated with trehalose supplementation is not a direct antioxidative effect of trehalose, but due to improvements in ruminal antioxidative conditions, particularly with respect to an increase of relative superoxide

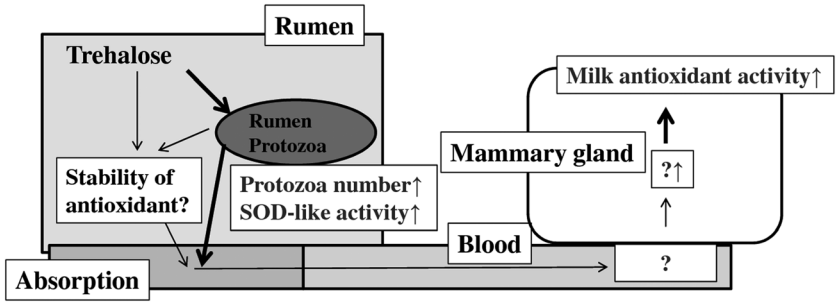

Figure 4 Possible mechanism underlying the antioxidant activity associated with trehalose supplementation of the diet in dairy cows.

dismutase (SOD) activity (Aoki *et al*. 2013). We suggested that the low lipid peroxide and high antioxidant content in the rumen fluid of cows fed trehalose-supplemented diets might be explained by the enhancement of protozoal activities through the activation of rumen fermentation (Fig. 4). Further molecular nutritional approach, that is, a study to determine the relationship between relative SOD activity in rumen fluid and antioxidant activity in milk, as well as alterations to rumen microbes and mammary gland cells in cows fed trehalose-supplemented diets, may help to elucidate the mechanisms underlying the effect of trehalose supplementation.

CONCLUSION

Recent advanced biochemistry and molecular biology has made it possible to provide information on the modification site in genomic DNA, transcriptome, proteome and metabolome against nutrition. The deposition of these data will enhance novel molecular nutritional regulation to improve animal production and animal health, resulting in the development of novel animal production systems.

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