Animal Feed Science and Technology 165 (2011) 230–237

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Tomato powder supplementation activates Nrf-2 via ERK/Akt signaling pathway and attenuates heat stress-related responses in quails

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ARTICLE INFO

Article history:
Received 20 August 2010
Received in revised form 3 March 2011
Accepted 4 March 2011

Keywords:
Lycopene
Transcription factors
Heat stress
Quail

ABSTRACT

Rearing birds at temperatures higher than thermoneutral temperature might impair cellular signal transduction pathways, influencing the expression of genes coding for antioxidant proteins, and affect the laying performance of the bird. This experiment was conducted to evaluate the effects of tomato powder supplementation on the activation of nuclear factor erythroid 2-related factor 2 (Nrf-2), and phosphorylated forms of extracellular signal-regulated kinase (ERK) 1/2 and protein kinase B (Akt) in heat stressed Japanese quails (*Coturnix coturnix Japonica*). A total of 180 five-week-old female Japanese quails were reared either at 22 °C for 24 h/d (thermoneutral, TN) or 34 °C for 8 h/d (heat stress, HS) for 12 weeks. Birds were randomly fed one of the three diets: basal diet or basal diet supplemented with 25 or 50 g/kg tomato powder. Each of the 2 × 3 factorially arranged groups was replicated in 10 cages, each containing 3 quails. Significantly higher level of Nrf-2 ($P<0.001$), ERK 1/2 ($P<0.001$) and Akt ($P<0.001$) were found in HS quails when compared to the TN group. The effect was dose dependent, and counteracted by tomato powder supplementation. The HS group also showed a concomitant increase in the activation of nuclear factor-kappa B (NFκB), a transcription factor regulating the expression of genes involved in inflammation ($P<0.001$). The activation of NFκB decreased dose dependently in HS quail supplemented with tomato powder. The level of hepatic peroxide and superoxide decomposing enzymes measured in tomato powder supplemented group of HS birds also increased ($P<0.001$). In conclusion, tomato powder is likely to augment cellular antioxidant defense in HS quails by causing an increase in the level of phosphorylated forms of Akt and ERK, leading to the activation of Nrf-2, and reduce inflammation by inhibiting NF-κB.

1. Introduction

Temperatures above thermoneutral (TN) values have been reported to cause heat stress (HS) in bird, and influence its laying performance. Heat stress causes the generation of reactive oxygen species (ROS), possibly by disrupting the electron transport assemblies of the membrane (Ando et al., 1997; Mujahid et al., 2005), which might modulate the activities of transcription factors such as the nuclear factor erythroid 2-related factor 2 (Nrf-2) (Na and Surh, 2008) and nuclear factor-κB (NFκB) (Ali and Mann, 2004). Heat and oxidative stresses adversely affect the structure and physiology of the cell, causing impairment of transcription, RNA processing, translation, oxidative metabolism, and altered membrane structure

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doi:10.1016/j.anifeedsci.2011.03.003

and function (Mager and De Kruijff, 1995; Iwagami, 1996). Hormonal and metabolic changes, secretion of inflammatory markers (Etches et al., 1995; Hargreaves et al., 1996), and decrease in the level of vitamins and minerals have also been reported in response to stress (Sahin and Kucuk, 2003; Sahin et al., 2009).

One of the key oxidative stress-related events reported in birds is the activation of Nrf-2, a key transcription factor that controls the cellular antioxidant response against oxidants. Under normal physiological conditions, Keap1 (Kelch-like erythroid CNC homologue (ECH)-associated protein 1), a cytoskeleton binding protein, binds to Nrf-2, and represses its translocation into the nucleus. Covalent modification or oxidation of critical cysteine residues in Keap1 has been hypothesized to facilitate the dissociation of Keap1–Nrf-2 complex or increase the stability of Nrf-2 (Kobayashi et al., 2006; Na and Surh, 2008); the cysteine residues serve as molecular sensor for recognizing the altered intracellular redox-status triggered by electrophiles or reactive oxygen species (ROS) (Dinkova-Kostova et al., 2002; Na and Surh, 2008). Beside this, post-transcriptional changes in Nrf-2 directly modulate the Nrf-2-Keap1 signaling. Phosphorylation of Nrf-2 on its serine and threonine residues by protein kinase C (PKC), phosphoinositol 3-kinase (PI3K), and mitogen activated protein kinases (MAPKs) have been reported to activate Nrf-2 (Yu et al., 2000; Na and Surh, 2008). MAPK and protein kinase B (PKB, Akt) are associated with the modulation of antioxidant response element (ARE)-driven gene expression via Nrf-2 (Li et al., 2007; Yu et al., 2000). Tomato powder contains several antioxidants such as lycopene and ascorbic acid, which can modulate the cellular signal transduction pathways. Lycopene is particularly important because of its ability to quench singlet oxygen (DiMascio et al., 1989). This experiment was therefore conducted to evaluate the effect of dietary supplementation of tomato powder on Nrf-2 and NF-κB, two critical transcription factors, in signal transduction pathways in heat stressed quails.

2. Materials and methods

2.1. Birds, diets and experimental design

Female Japanese quails (*Coturnix coturnix japonica*) ($n = 180$; 5 week old), purchased from Insanay Kanatli Hayvan Uretim Paz. Tic. Inc., Elazig, Turkey, were used in this study. The study was carried out in accordance with guidelines and regulations and ethics for animal experiments at Veterinary Control and Research Institute, Elazig, Turkey. Birds were randomly assigned, according to a 2 (TN; HS) × 3 (tomato powder levels; 0, 25, 50 g/kg) factorial design, to 6 treatment groups consisting of 10 replicates of 3 birds. Birds were kept in 20 cm × 20 cm dimension cages, providing a space of 400 cm^2 per bird in a temperature-controlled room at either 22 °C for 24 h/d (TN) or 34 °C for 8 h (09.00 and 17.00 h) followed by 22 °C for 16 h/d (HS) during the experimental period. At both temperatures, birds were fed either a basal diet or the basal diet supplemented with 25 or 50 g/kg tomato powder (Sahin et al., 2008), which was a sun dried whole tomato. Tomato powder was analyzed and each gram of the powder contained 0.80 mg lycopene, 0.13 mg β-carotene, 0.07 mg α-tocopherol, 1.73 mg vitamin C, 125 mg protein, and 4 mg fat. The experimental diets were fed for a period of 12 weeks. During the period of experiment, quails were exposed to a 17-h daily photoperiod and had free access to feed and water. Composition of the basal diet is shown in Table 1.

2.2. Sample preparation

At the end of the experiment, 10 birds (one bird per replicate) were killed by cervical dislocation. The liver was removed, and stored at −80 °C till analysis. For analysis, the liver was chopped into small pieces on ice, and a 100 mg/mL (w/v) homogenate was prepared in 10 mM phosphate buffer (pH 7.4). The homogenate was centrifuged at 13,000 × *g* for 10 min at 4 °C, and the supernatant was collected and stored at −80 °C for the estimation of malondialdehyde and antioxidant enzymes.

2.3. Laboratory analyses

Malondialdehyde (MDA) was determined in the liver (Barim and Karatepe, 2010) using a fully automatic HPLC System (Shimadzu, Kyoto, Japan). Lycopene was measured as described previously (Stahl and Sies, 1992; Campbell et al., 2007) with minor modifications by the fully automatic HPLC System (Shimadzu, Kyoto, Japan), consisting of a pump (LC-20AD), a UV–visible detector (SPD-20A), a column oven (CTO-10ASVP), an autosampler (SIL-20A), a degasser unit (DGU-20A5), and a computer system with LC solution Software (Shimadzu, Kyoto, Japan). Briefly, liver samples were combined with a KOH/ethanol solution (1:5) containing 4.5 mmol/L butylated hydroxytoluene (BHT). Samples were placed on ice, and deionized water was added. Tissue and serum carotenoids were extracted four times with hexane. Hexane extracts were evaporated under nitrogen in the dark, and the residue was dissolved in 100 μl mobile phase. Extracts were kept on ice and in subdued light. HPLC analyses were performed on a C_{18} column (inertsil ODS-3 C_{18} column (250 mm × 4.6 mm, 5 m), with methanol: acetonitrile: dichloromethane: water (7:7:20:0.16) containing 20 mmol/L ammonium acetate. The flow rate was 1 mL/min and detection was set at 470 nm (Stahl and Sies, 1992; Ishida et al., 2001).

Glutathione peroxidase (GPx, GSH-Px) activity was assayed by the method of Lawrence and Burk (1976) with hydrogen peroxide as substrate. The activity was expressed as U/mg protein; one unit of GSH-Px is the amount of GSH-Px required to oxidise 1 mmol NADPH/min. Total superoxide dismutase (SOD) activity was assayed according to Spitz and Oberley (1989);