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Expression profile of six stress-related genes and productive performances of fast and slow growing broiler strains reared under heat stress conditions

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ABSTRACT

High temperature is one of the prominent environmental factors causing economic losses to the poultry industry as it negatively affects growth and production performance in broiler chickens. We used One Step TaqMan real time RT-PCR (reverse transcription polymerase chain reaction) technology to study the effects of chronic heat stress on the expression of genes codifying for the antioxidative enzymes superoxide dismutase (SOD), and catalase (CAT), as well as for heat shock protein (HSP) 70, HSP90, glucocorticoid receptor (NR3C1), and caspase 6 (CASP6) in the liver of two different broiler genetic strains: Red JA Cou Nu Hubbard (CN) and Ross 508 Aviagen (RO). CN is a naked neck slow growing broiler intended for the free range and/or organic markets, whereas RO is selected for fast growing. We also analysed the effect of chronic heat stress on productive performances, and plasma corticosterone levels as well as the association between transcriptomic response and specific SNPs (single nucleotide polymorphisms) in each genetic strain of broiler chickens. RO and CN broilers, 4 weeks of age, were maintained for 4 weeks at either 34 °C or 22 °C. The results demonstrated that there was a genotype and a temperature main effect on the broilers' growth from the 4th to the 8th week of age, but the interaction effect between genotype and temperature resulted not statistically significant. By considering the genotype effect, fast growing broilers (RO) grew more than the slow growing ones (CN), whereas by considering the temperature effect, broilers in unheated conditions grew more than the heat stressed ones. Corticosterone levels increased significantly in the blood of heat stressed broilers, due to the activation of the HPA (hypothalamic–pituitary–adrenocortical axis). Carcass yield at slaughter was of similar values in the 4 cohorts (genotype/temperature combinations or treatment groups), ranging from 86.5 to 88.6%, whereas carcass weight was negatively influenced by heat stress in both broiler strains. Heat stress affected gene expression by downregulating *CASP6* and upregulating *CAT* transcript levels. HSPs, *SOD* and *NR3C1* mRNA levels remained unaffected by heat stress. The differences found in the mRNA copies of *CASP6* gene could be partly explained by SNPs.

1. Introduction

High environmental temperature is one of the most important stressors associated with economic losses to the poultry industry (Lin et al., 2006; Lu et al., 2007). It causes poor growth performance (Bottje and Harrison, 1985), immunosuppression (Young, 1990), and high mortality (Yahav et al., 1995), contributing thus to a decrease in productivity. Furthermore, heat stress deteriorates meat quality by accelerating post-mortem glycolytic metabolism, resulting in pale and exudative meat characteristics in chicken (Sandercock et al., 2001; Hashizawa et al., 2013).

Although the responses to heat differ between chickens of different genetic backgrounds (Altan et al., 2003; Franco-Jimenez et al., 2007;

Abbreviations: CASP6, caspase 6; CAT, catalase; cDNA, DNA complementary to RNA; CN, Red JA Cou Nu Hubbard; CORT, corticosterone; Ct, cycle threshold; GPX, glutathione peroxidase; HPA, hypothalamic–pituitary–adrenocortical axis; HSP, heat shock protein; kDa, kilodalton(s); NR3C1, glucocorticoid receptor: GR or nuclear receptor subfamily 3, group c, member 1; PCR, polymerase chain reaction; RO, Ross 508 Aviagen; rTH, reverse transcriptase; RT-PCR, reverse transcription PCR; SNP, single nucleotide polymorphism; SOD, superoxide dismutase.

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Star et al., 2008; Felver–Gant et al., 2012), broilers are in general more sensitive to high environmental temperatures than other domestic animals (Geraert et al., 1993). Indeed, domestication and selective breeding are producing individuals that are more susceptible to stress rather than more resistant (Washburn et al., 1980; Cahaner et al., 1995). In particular, the resistance to heat stress of strains selected for rapid growth is significantly lower than that of slow-growing strains and the continuous selection for fast growth seems to be associated with increased susceptibility of broiler chicken to heat stress (Berrong and Washburn, 1998; Tan et al., 2010; Soleimani et al., 2011).

Different studies have demonstrated oxidative injury induced by high ambient temperatures in broiler chickens (Altan et al., 2003; Mujahid et al., 2007). Heat is a major source of oxidative stress since it causes a redox imbalance between the pro- and anti-oxidants in favour of prooxidants. However, several effective antioxidant systems prevent oxidative damage, including various antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) (Halliwell and Gutteridge, 1996). SOD catalyses dismutation of superoxide radicals to hydrogen peroxide and oxygen; CAT catalyses the breakdown of hydrogen peroxide to water and molecular oxygen, and GPX decomposes peroxides through other mechanisms (Halliwell, 2006). Several studies have clearly demonstrated that exposure to high ambient temperatures causes a compensatory increase in the activity of SOD, GSH-Px, and CAT in serum, liver, and muscle of broiler chickens (Tan et al., 2010; Azad et al., 2010).

Most organisms respond to heat stress by inducing the synthesis of a group of highly evolutionarily conserved stress-modulated proteins known as heat shock proteins (HSPs). Analogously to enzymatic oxygen scavengers, the expression of HSPs is upregulated in response to high temperatures, given that one of the most important functions of HSPs is to protect organisms from the toxic effects of heating (Barbe et al., 1988; Ganter et al., 2006; Staib et al., 2007;). Moreover, several HSPs function as intracellular chaperones for other proteins. They play an important role in protein assembling and disassembling (Pelham, 1985), protein folding and unfolding (Randall and Hardy, 1986), and protein translocation (Murakami et al., 1988). Of the many expressed HSPs, those with a molecular weight of approximately 70 kDa, named HSP70, have been extensively studied in chicken because they seem to best correlate with heat tolerance (Gabriel et al., 1996; Soleimani et al., 2011; Hasheimi et al., 2012; Gu et al., 2012;).

Rearing stress conditions and physical agents, like heat, can also activate apoptosis, which is triggered by caspases, a family of structurally related cysteine aspartases. It has been well documented that the expression of caspase genes may be influenced by persistent stress in broiler chicken and other vertebrates such as rainbow trout (Laing et al., 2001; Lin et al., 2004).

In chicken as in other vertebrates, stress activates the hypothalamus-pituitary-adrenocortical (HPA) axis, leading to a rapid release of corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) from the cells located in the hypothalamus and pituitary, respectively. ACTH stimulates the synthesis and release of steroids from the adrenal cortex by promoting the uptake of cholesterol and its enzymatic conversion to the glucocorticoid hormone corticosterone (CORT) (Jones et al., 1988; Fraisse and Cockrem, 2006). CORT released into the circulatory system diffuse across the plasma membrane of the cells and binds to a high affinity cytosolic glucocorticoid receptor (NR3C1), which is mainly found in cytoplasm as a heterocomplex by coordinated associations with molecular chaperones, such as HSP40, HSP70 and HSP90 (Derijk et al., 2002; Marelli et al., 2010; Ramamoorthy and Cidlowski, 2013). Binding of CORT to NR3C1 induce the NR3C1 heterocomplex leading to NR3C1 homodimerization and nuclear translocation. Once inside the nucleus, NR3C1 can regulate gene transcription, through binding to a palindromic response element termed the glucocorticoid response element (GRE), which is located in the promoter regions of target genes (Yudt and Cidlowsky, 2001; Kwok et al., 2007). Binding to GRE induces conformational changes in NR3C1 leading to coordinated recruitment of coactivators and chromatin-remodelling complexes that influence the activity of polymerases and activate gene transcription (Kwok et al., 2007; Ramamoorthy and Cidlowski, 2013). However, according to recent discoveries, many NR3C1-binding sites are located far from the promoter proximal region of target genes and showed an unexpected difference between the activation and repressive functions of the NR3C1. What remains to be established, thus, is the functionality of these distant NR3C1-binding sites in relation to the transcription of genes or other undiscovered functions encoded in the NR3C1 protein (Ramamoorthy and Cidlowski, 2013).

Different authors have carried out studies in poultry utilizing molecular markers (including SNPs) (Sheng et al., 2013; Hoque et al., 2013). Currently, the search for SNP markers represents one of the favourite genotyping approaches because they are very abundant in the genome and amenable to high-throughput analysis (Yang et al., 2013).

Genetic variation influences gene expression as demonstrated by Stranger et al. (2007a, 2007b). The same authors, in a study carried out in human lymphoblastoid cell lines (Stranger et al., 2007a, 2007b), highlighted that SNPs captured 83.6 of the total detected genetic variation in gene expression. For these reasons, many SNPs in candidate genes seem to have an important role in regulating the expression level of these genes (Dixon et al., 2007).

In view of these considerations, the first aim of our study was to investigate the effects of chronic heat exposure on the expression of genes codifying for the antioxidative enzymes SOD and CAT as well as for HSP70, HSP90, NR3C1, and caspase 6 (CASP6) in the liver of two different broiler hybrid strains: Red JA Cou Nu Hubbard (CN) and Ross 508 Aviagen (RO). CN is a naked neck slow growing broiler strain intended for the free range and/or organic markets, whereas RO is a strain selected for fast growing (Castellini et al., 2002). The effect of chronic heat stress on broilers was also studied by checking their productive performance, and plasma corticosterone levels. Another aim was to analyse a possible association between transcriptomic response and specific SNPs in each strain of broiler chickens.

2. Materials and methods

2.1. Animals, experimental design, and sample collection

RO and CN birds (average weight ± SE: RO = 98.48 ± 1.70 g; CN = 94.09 ± 1.67 g), were obtained from commercial hatcheries and raised for 4 weeks under standard conditions. As the chickens grew, the RT was gradually decreased from 35 °C to 22 °C and maintained by controlled ventilation and heating until day 28. At the age of 4 weeks, 120 RO and 120 CN broilers (sex ratio 1/1) were randomly divided into 4 cohorts (genotype/temperature combinations) of 60 animals which were then reared for 4 weeks at two different environmental temperatures: 60 RO and 60 CN were housed in separate pens situated in a room at 34 °C (high temperature, HT) and other 60 RO and 60 CN were housed (in separate pens) in another room and maintained at 22 °C (control temperature, CT). All birds were kept under standard conditions (wood-shaving litter, wire pens), and fed the same standard commercial diet (metabolizable energy: 11.8 MJ/kg; crude protein: 18%). Birds were given ad libitum access to feed and water throughout the experiment and were weighed weekly from the 1st week of age on. During the experiment, birds were under careful veterinarian examination; the underlying health status was good and no mortality was recorded. At the age of 8 weeks (end of the trial), all birds were individually weighted and then placed into coops and transported to an authorized commercial processing plant very close to the experimental farm. At the processing plant, birds were manually removed from coops, hung on shackles, and then electrically stunned by using two-stage electrical stunner (214 V, pulsed direct current at approximately 500 Hz for 18 s, followed by 14 V, 60 Hz alternate current for 9 s). After stunning, birds were jugulated by using a conventional unilateral neck cut to severe the carotid artery and jugular vein, bled for 140 s, and then eviscerated. Liver of 10 randomly selected