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Activation of glucocorticoid receptors is associated with the suppression of antioxidant responses in the liver of goats fed a high-concentrate diet

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ABSTRACT

This study investigated changes in oxidative stress and the relevant mechanisms in the liver of goats fed a high-concentrate (HC) diet for 5 weeks. Twelve goats were randomly assigned to a low-concentrate (concentrate-to-forage = 55:45, LC, $n = 6$) or HC diet (concentrate-to-forage = 90:10, $n = 6$), with dry matter as the base. Enzyme activity assays, real-time polymerase chain reaction and western blotting were used to evaluate antioxidant parameters and gene expression in the liver. Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), total nitric oxide synthase activity and total antioxidant capacity (T-AOC) in the liver declined ($p < .01$) in HC-fed goats compared to those in LC-fed goats. The mRNA levels of *GPX1*, *CAT* and *SOD1* were down-regulated (3.69, 47.37 and 27.61%, respectively) in HC-fed goats compared to those in LC-fed goats. Furthermore, glutathione S-transferase M1 (*GSTM1*) was upregulated (466.35%, $p < .01$) in the liver of HC-fed goats. The mRNA and protein levels of the nuclear factor E2-related factor 2 (NRF2) and total glucocorticoid receptor (GR) declined ($p < .05$) in HC-fed goats (by 28.57, 33.1, 30.85 and 34%, respectively). However, the nuclear translocation of GR increased ($p < .05$; by 44.75%) in HC-fed goats. Negative correlations were detected for hepatic nuclear GR protein expression with hepatic CAT activity and GPx activity. In conclusion, feeding an HC diet to goats for 5 weeks suppressed NRF2-dependent antioxidant responses and enhanced GR nuclear translocation in the liver.

HIGHLIGHTS

- Feeding goats high-concentrate diets suppresses NRF2-dependent antioxidant responses in liver, downregulating NRF2 and altering SOD1, GPx and CAT activity.
- This suppression of NRF2-dependent antioxidant responses is associated with GR activation in the liver of goats.

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Antioxidant response; high-concentrate diet; nuclear factor E2-related factor 2; glucocorticoid receptor

Introduction

Oxidative stress is an imbalance between the production of cell-damaging free radicals and the body's ability to neutralise them, which usually causes cellular and tissue damage (Trevisan et al. 2001; Zhang et al. 2015). Recently, several studies have shown that stress responses arise in ruminants fed high-grain or high-starch diets, resulting in oxidative damage and increased levels of acute-phase proteins (Sgorlon et al. 2008; Jia et al. 2014; Duanmu et al. 2016; Abaker et al. 2017). It is widely accepted that oxidative stress is a significant factor responsible for impaired immune and inflammatory responses, and it is positively correlated to the high-grain or high-starch diets of ruminants (Tao et al. 2015; 2016; Aditya et al. 2018). It has also been reported that feeding high-concentrate (HC) diets to lactating cows reduces pH

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Supplemental data for this article can be accessed here.

Table 1. Ingredients and composition of the experimental diets (% on dry matter).

Item	LC	HC
Rice with shell	33.2	54.3
Soybean meal	9.6	15.7
Wheat bran	6.0	9.8
Fat powder	3.2	5.2
Calcium carbonate	0.5	0.8
Calcium bicarbonate/$CaHPO_4$	1.1	1.8
Sodium chloride/NaCl	0.6	1.0
Premix[a]	1.0	1.4
Rice straw	45	10
Nutrient levels[b], % of DM		
Crude protein	13.46	17.62
Crude ash	9.34	9.12
Crude fat	11.97	11.88
Neutral detergent fibre	49.82	38.35
Acid detergent fibre	36.52	9.51
Non-fibre carbohydrates	5.74	12.47

[a]Provided per kg of premix: $NaHCO_3$ 571.4 g; $FeSO_4 \bullet H_2O$ 2 g; $CuSO_4 \bullet 5H_2O$ 1 g; $CoCl2 \bullet 6H_2O$ 0.01 g; KIO_3 0.1 g; $MnSO_4 \bullet H_2O$ 7.5 g; $ZnSO_4 \bullet H_2O$ 4 g; $NaSeO_3$ 0.0025 g carrier 371.7 g; vitamin E 250 mg; vitamin A 25,000 IU; vitamin D 50,000 IU.
[b]Nutrient levels were estimated currently used goat feed.

and increases the release of lipopolysaccharide (LPS) from the rumen and hindgut (Zebeli and Ametaj 2009). LPS induces hepatic oxidative injury by changing the enzyme activity of glutathione and superoxide dismutase (SOD) (Taira et al. 2008; Abdel-Salam et al. 2012). The HC diet causes oxidative stress but the relevant mechanism is unclear.

Cortisol is the primary glucocorticoid (GC) and is a regulator of physiological responses to stress (Yamaji et al. 2009). Serum cortisol levels are usually elevated after exposure to acute stressors, including oxidative stress in dairy cows (Laven and Peters 1996; Gupta et al. 2005). GC increases in response to stress via the hypothalamic–pituitary–adrenal (HPA) axis, influencing the intracellular glucocorticoid receptor (GR) and modulating the expression of different target genes (stress-response genes) (Prunet et al. 2006; Stahn and Buttgereit 2008; Ayroldi et al. 2012). Previous studies have shown that hepatic GR protein expression is downregulated in lactating goats fed an HC diet for 9 weeks (Dong et al. 2013). Other experiments have demonstrated that the presence of oxidative stress in many chronic inflammatory diseases generally weakens GR function, and leads to GC insensitivity in several cell systems (Chung and Marwick 2010; Hakim et al. 2013). Oxidative stress levels are also elevated in the primary cells of patients with severe asthma, where GC sensitivity is closely linked to reduced GR nuclear import (Matthews et al. 2004). GR activation is associated with the suppression of antioxidant response signalling through the inhibition of nuclear factor E2-related factor 2 (NRF2). This is a pleiotropic transcription factor that contributes to the regulation of the antioxidant cell defense system (Copple et al. 2008; Muthusamy et al. 2012). This factor also upregulates antioxidant enzymes, including SOD, catalase (CAT), and heme oxygenase-1, in hepatic cells (Chen et al. 2017). In contrast, the downregulation of NRF2 signalling is considered to increase the levels of antioxidant enzymes (Kratschmar et al. 2012; Alam et al. 2017).

Existing studies demonstrated that the nuclear translocation of GR is associated with suppressed antioxidant response signalling via NRF2. Yet, knowledge on the underlying mechanism of oxidative stress in response to HC diets in ruminants remains limited. Here, we hypothesised that HC diets would increase the nuclear translocation of GR in the liver of goats. Thus, we aimed to: (1) investigate changes to oxidative stress parameters in the liver of growing goats fed an HC diet for 5 weeks, and (2) explore hepatic GR nuclear translocation and NRF2-mediated antioxidant responses underlying HC diet-induced oxidative stress.

Materials and methods

This experiment was conducted following the recommendations of the Animal Care and Use Guidelines of the Institute of Subtropical Agriculture, Chinese Academy of Sciences, China. The protocol was approved by the Animal Care Committee on the Ethics of Animal Experiments of Institute of Subtropical Agriculture (Permit No. 20170712). All surgeries were performed after euthanasia, and all efforts were made to minimise any suffering of animals.

Animals and experimental procedures

Twelve healthy growing goats with an average initial body weight of 12 ± 1.35 kg were used as the animal model in this study. Animals were housed in individual stalls of a standard animal feeding house at the Institute of Subtropical Agriculture (ISA), Chinese Academy of Sciences (Changsha, China). All animals were randomly allocated to two groups [low-concentrate (LC) group versus HC group, $n = 6$). The experimental period was 5 weeks. The first week was used as an acclimation period to the diet and the last 4 weeks were used to perform the measurements (Wang et al. 2019). The LC group (control) was fed a low-concentrate (concentrate-to-forage = 55:45) diet. The HC group was fed a high-concentrate (concentrate-to-forage = 90:10) diet. Equal quantities of each diet were offered at approximately 08:00 and 18:00. Goats were given free access to fresh water throughout the experimental period. The feed intake of each

Table 2. Primer sequences of the target genes.

Target genes[a]	Primer sequence (5′→3′)	Product length, bp	Accession no.[b]
GPX1	Forward (F): ACATTGAAACCCTGCTGTCC Reverse (R): TCATGAGGAGCTGTGGTCTG	216	XM_005695962.3
CAT	F: TGGGACCCAACTATCTCCAG R: AAGTGGGTCCTGTGTTCCAG	178	XM_005690077.3
SOD1	F: TGCAGGCCCTCACTTTAATC R: CTGCCCAAGTCATCTGGTTT	207	NM_001285550.1
NRF2	F: CCAACTACTCCCAGGTAGCCC R: AGCAGTGGCAACCTGAACG	227	XM_013968675.2
GR	F: AGCAGTGTGCTTGGTCGAGA R: GTGAGAAGCAGCAGCCAGTG	114	XM_005683086
GSTP1	F: AGACCTCACGCTGTACCAGTC R: CCTTCACATAGTCCTCCTTGC	80	AF186248.1
GSTM1	F: GCCATCCTTCGGTACATCG R: GCCAAGCGGACATCCATAA	90	AF249588.1
β-actin	F: CCAACCGTGAGAAGATGACC R: CGCTCCGTGAGAATCTTCAT	247	XM_018039831.1

[a]CAT: catalase; *GPX1*: glutathione peroxidase1; GR: glucocorticoid receptor; *GSTP1*: glutathione S-transferase P1; *GSTM1*: glutathione S-transferase M1; *NRF2*: nuclear factor E2-related factor 2; SOD1: superoxide dismutase1.
[b]The reference sequence number is given for primers for which the source is the National Centre for Biotechnology Information (NCBI) GenBank database (http://www.ncbi.nlm.nih.gov/genbank/).

goat was recorded daily. Dry matter intake was 572 ± 24.12 and 602 ± 62.86 g/day, crude protein intake was 77 ± 1.41 and 106 ± 2.71 g/day and energy intake was 5.77 ± 0.023 and 7.56 ± 0.034 MJ/day for LC and HC goats, respectively (as published in Wang et al. 2019). Goats in the LC and HC groups showed weight gains of 1.88 ± 0.25 and 3.5 ± 0.34 kg (Wang et al. 2019), respectively. The ingredients and nutrient compositions of the experimental diets are presented in Table 1. Feed samples were analysed in triplicate for crude protein (method 984.13; AOAC 2005), crude fat (method 2003.05; AOAC 2005) and crude ash (method 942.05; AOAC, 2005). Neutral detergent fibre content and acid detergent fiber content were determined according to Van Soest et al. (1991), using an Ankom 220 Fiber Analyser (Ankom Co.), and were expressed inclusive of residual ash.

Sample collection

After 4 weeks of feeding, all goats were euthanized by injecting xylazine (0.5 mg/kg; Xylosol; Ogris Pharma, Wels, Austria) and pentobarbital (50 mg/kg WDT, Garbsen, Germany) in the jugular (before overnight fasting and exsanguination). After euthanasia, liver tissue (the medial lobes of the liver) was carefully removed, immediately frozen in liquid nitrogen and stored at −80 °C until RNA isolation and protein extraction.

Preparation of liver homogenate

Antioxidant assays were performed using assay kits purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Frozen liver tissue (1 g) was homogenised in 10-mL physiological saline (0.9% NaCl; 4 °C) on ice using a Polytron-aggregate homogeniser PT-1200E (Lucerne, Switzerland) for 30 s at 12,000×*g*. The homogenate was centrifuged at 3800×*g* for 10 min at 4 °C. The resulting supernatant (10% concentrations) was aliquoted and stored at −20 °C until analysis.

Total antioxidant capacity (T-AOC), antioxidant enzyme activity and lipid peroxidation in the liver

Various liver parameters were analysed using their corresponding reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). These included T-AOC, hepatic SOD, CAT, glutathione peroxidase (GPx) enzyme activity, malondialdehyde (MDA) concentrations and total nitric oxide synthase (NOS). All procedures were performed according to the manufacturer's instructions. SOD (EC1.15.1.1), CAT (EC1.11.1.6), GPx (EC1.11.1.9) and NOS (EC1.14.13.39) activity were expressed as units/mg protein. T-AOC and MDA were expressed as mmol/mg protein.

RNA extraction and cDNA synthesis

Total RNA from the liver tissue of each goat was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocols. The concentration of isolated total RNA was quantified with an ND-1000 UV-vis spectrophotometer (NanoDrop Ltd., Wilmington, DE). The integrity of RNA was inspected using 1% agarose gel electrophoresis (Supplementary Figure S1). Protein contamination was assessed through the spectrophotometric determination of the 260–280 nm absorbance ratio. Only

Table 3. Comparison of liver antioxidant enzyme activity, lipid peroxidation, total antioxidant capacity and NOS activity in goats fed high- (HC) *versus* low-concentrate (LC) grain diets.

Item[a]	LC	HC	*p*-value
SOD (U/mg prot)	286.92 ± 15.14	158.11 ± 40.65	.001
CAT (U/mg prot)	382.10 ± 54.53	210.03 ± 17.85	.006
GPx (U/mg prot)	72.87 ± 10.11	48.29 ± 4.63	.01
NOS (U/mg prot)	1059.93 ± 177.81	505.20 ± 199.94	.001
T-AOC (mmol/mg prot)	0.25 ± 0.035	0.071 ± 0.019	.004
MDA (mmol/mg prot)	0.25 ± 0.088	0.46 ± 0.29	.161

[a]All data are presented as the mean ± SD. CAT: catalase; GPx: glutathione peroxidase; MDA: malondialdehyde; NOS: total nitric oxide synthase; T-AOC: total antioxidant capacity; $n = 6$/group; SOD: superoxide dismutase.

samples with a ratio between 1.8 and 2.1 were used in subsequent experiments. To remove traces of chromosomal DNA, 1 μg of total RNA was treated with 5× g DNA Eraser Buffer, 2.0 μL Eraser Buffer, 1.0 μl g DNA Eraser and RNase-free DNase I (TaKaRa, Japan) for 2 min at 42 °C. Subsequently, first-standard cDNA was synthesised using a PrimeScript™ RT reagent Kit (TaKaRa, Japan) and stored at −20 °C for real-time polymerase chain reaction (RT-PCR).

RT-qPCR analysis

Oligonucleotide primer sequences for *SOD1*, *CAT*, *GPx1*, *GSTM1*, *GSTP1* and *β-actin* were obtained from previously published papers (Yang et al. 2014; Duanmu et al. 2016). *NRF2*- and *GR*-specific primers were designed using Premier 5 software (Premier Biosoft, Palo Alto). The RT-PCR products of *NRF2* and *GR*-specific primers were sequence-validated. The identity of *NRF2* and *GR* was 99 and 100%, respectively, indicating that the RT-PCR products were the target genes. Details on the oligonucleotide primer sequences, primer lengths and accession numbers are listed in Table 2. RT-PCR was performed using SYBR® Premix EX Taq™ (TaKaRa, Japan) on a LightCycler480 system (Roche, Castle Hill, New South Wales, Australia). The cDNA was diluted fourfold before equal amounts were added to duplicate RT-PCR reactions. The thermal profile for all reactions was 30 s at 95 °C, followed by 40 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 20 s and extension at 72 °C for 1 min. The specificity of PCR amplification was confirmed by melting curve analysis and 2% agarose gel electrophoresis of the PCR products. The efficiency of PCR amplification for each gene was checked by diluting the samples. Relative expression levels of target genes were normalised to those of *β-actin* (Jia et al. 2014; Yang et al. 2014). Data were analysed using the $2^{-\Delta\Delta Ct}$ method, following existing procedures (Livak and Schmittgen 2001). All samples were included in the same run of RT-PCR. The experiment was repeated at least three times.

Western blot analysis

Total protein was extracted from frozen liver tissue using 1 mL of ice-cold homogenisation RIPA buffer containing the protease inhibitor cocktail Complete EDTA free (Roche, Penzberg, Germany) and the phosphatase inhibitor Cocktail PhosSTOP (Roche, Penzberg, Germany). Cytosolic proteins, membrane proteins and nuclear proteins were separated with the Nucl-Cyto-Mem Preparation Kit (Applygen Technologies, Beijing, China), according to the manufacturer's protocol. Protein concentration was determined with an enhanced BCA protein assay kit (P0010, Beyotime Biotechnology, Shanghai, China). Protein extracts were subjected to 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Isolated proteins were then transferred to polyvinylidene fluoride membranes (PVDF membranes, Millipore, Danvers, MA). Western blot analysis for GR and NRF2 was performed with specific primary antibodies (Supplementary Table S1) and corresponding secondary antibodies (Supplementary Table S1). Finally, the blot was washed and detected through enhanced chemiluminescence (ECL) using Luminata Classico Western HRP Substrate (WBLUC0100, Millipore, Danvers, MA). ECL signals were recorded using an imaging system (Bio-Rad), and were analysed using Quantity One software (Bio-Rad). GR and NRF2 protein values were presented as fold-change relative to the average value of the LC group.

Statistical analysis

All data are presented as the mean ± SD. Statistical significance was assessed using the independent sample *t*-test in SPSS (SPSS version 21.0 for Windows; SPSS Inc., Chicago, IL) under the conditions of normal distribution and homogeneity of variance. Differences were considered significant at $p < .05$, and $.05 < p < .10$ was considered a tendency towards significance. Graphs were created using GraphPad Prism version 5.01 (GraphPad Software, La Jolla, CA). Pearson correlations were analysed in GraphPad Prism.

Results

Antioxidant enzyme activity, and T-AOC and MDA content

The results showed that SOD, GPx, CAT and NOS enzyme activities, as well as T-AOC, decreased

Table 4. Real-time PCR analysis of relative mRNA expression in the liver of goats fed the high- (HC) *versus* low-concentrate (LC) grain diets.

Item[a]	LC group	HC group	*p* Value
GPX1	0.92 ± 0.048	0.61 ± 0.056	.009
CAT	1.14 ± 0.081	0.65 ± 0.089	.005
SOD1	1.06 ± 0.058	0.7673 ± 0.075	.028
NRF2	1.05 ± 0.077	0.75 ± 0.067	.025
GR	0.94 ± 0.079	0.65 ± 0.069	.049
GSTP1	1.01 ± 0.019	0.81 ± 0.130	.061
GSTM1	1.07 ± 0.045	6.06 ± 0.941	.006

[a]All data are presented as the mean ± SD. β-actin was used as the reference gene for gene expression. *CAT*: catalase; *GPX1*: glutathione peroxidase1; *NRF2*: nuclear factor E2-related factor 2; *GR*: glucocorticoid receptor; *GSTP1*: glutathione S-transferase P1; *GSTM1*: glutathione S-transferase M1; *SOD1*: superoxide dismutase1; $n = 6$ per group.

significantly ($p < .01$) in HC goats compared to those in LC goats. However, there was no significant difference ($p > .01$) in MDA between the two groups (Table 3).

mRNA expression of antioxidant enzymes, encoding genes GR and NRF2

Hepatic *GPX1*, *CAT*, *SOD1*, *NRF2* and *GR* mRNA expression levels decreased significantly ($p < .05$) in HC goats compared to those in LC goats (Table 4). Among the GST family genes, hepatic *GSTP1* mRNA expression tended to decrease more ($p = .061$) in the liver of HC *versus* that in LC goats, whereas *GSTM1* gene expression increased significantly ($p < .01$).

Liver protein expression levels of GR and NRF2

Total liver protein expression of NRF2 decreased significantly ($p < .05$) in HC goats compared to that in LC goats (Figure 1(A)). However, liver GR protein expression in the whole cell and cytoplasm extract also decreased significantly ($p < .05$) in HC goats versus that in LC goats (Figure 1(B)), whereas GR protein expression in the cell nuclear extract significantly increased ($p < .05$; Figure 1(B)). There was no significant difference ($p = .254$) in GR protein expression in the liver cell membrane between the two groups.

Correlation between hepatic GR protein expression and antioxidant enzymes

Hepatic nuclear GR protein expression was negatively correlated with hepatic CAT ($r = -0.56$, $p = 0.048$) and GPx activity ($r = -0.59$, $P = .04$; Figure 2(B,C)). No significant correlations were observed between nuclear GR protein expression and SOD, NOS, T-AOC or MDA levels (Figure 2(A,D–F)).

Discussion

The current study demonstrates that antioxidant activity declines in the liver of goats fed HC diets versus those fed LC diets for 5 weeks. Oxidative stress occurs when oxidant–antioxidant systems become imbalanced, potentially through the generation of more free radicals and the decline in antioxidant activity. Reduced SOD, CAT and GPx activity probably enhances the production of O_2 and H_2O_2, and the dysfunction of the antioxidant defense system (Spolarics 1996; Fischer et al. 2012), as demonstrated previously. For example, feeding an HC diet to lactating goats reduces SOD and GPx activity (Tao et al. 2015; Duanmu et al. 2016). SOD and CAT are the primary antioxidant enzymes that scavenge superoxide anions in the tissue and are generally regulated by oxidative stress (Xiang et al. 2016). The antioxidant capacity in the liver of goats fed an HC diet appeared to decline, based on reduced SOD, GPx and CAT activity, and corresponding changes to the relative mRNA levels of *SOD1*, *GPX1* and *CAT* genes. GPx activity in the brain and liver was previously reported to decline in lactating mice, which exhibited 254% higher gross energy intake than that in non-lactating mice (Zheng et al. 2015). The energy intake of HC goats in the current study was 7.56 ± 0.034 MJ/day, which was 31% higher than that of LC goats. Hence, the reduced antioxidant capacity of goats fed an HC diet might be the result of increased energy intake.

Furthermore, cows fed a high-grain diet exhibited higher levels of LPS, both in rumen fluid and plasma (Abaker et al. 2017). The decreased antioxidant capacity in goats fed an HC diet might also be explained by an increase in LPS concentrations (Tao et al. 2014). Reduced T-AOC levels also explained the decline in antioxidant capacity in this study. Moreover, nitric oxide (NO) strongly contributes towards mediating oxidative stress at a certain physiological concentration. In comparison, excessive NO generated by NOS (inducible NOS and endothelial NOS) forms nitrogen-free radicals, causing oxidative stress (Zhang et al. 2017). However, our results showed that NOS activity in the liver declined under an HC diet. This finding supports the results of Chen et al. (2017), who documented a similar down-regulation in neuronal NOS expression for the hippocampus under acute stress. One possible explanation is the feedback inhibition of hepatic NOS activity via NO (Alderton et al. 2001). Oxidative stress-related genes (*GSTM1* and *GSTP1*) also contribute to the control of cellular redox regulation, providing protection against cellular DNA oxidative damage. The absence of GSTM1 and GSTP1 increases

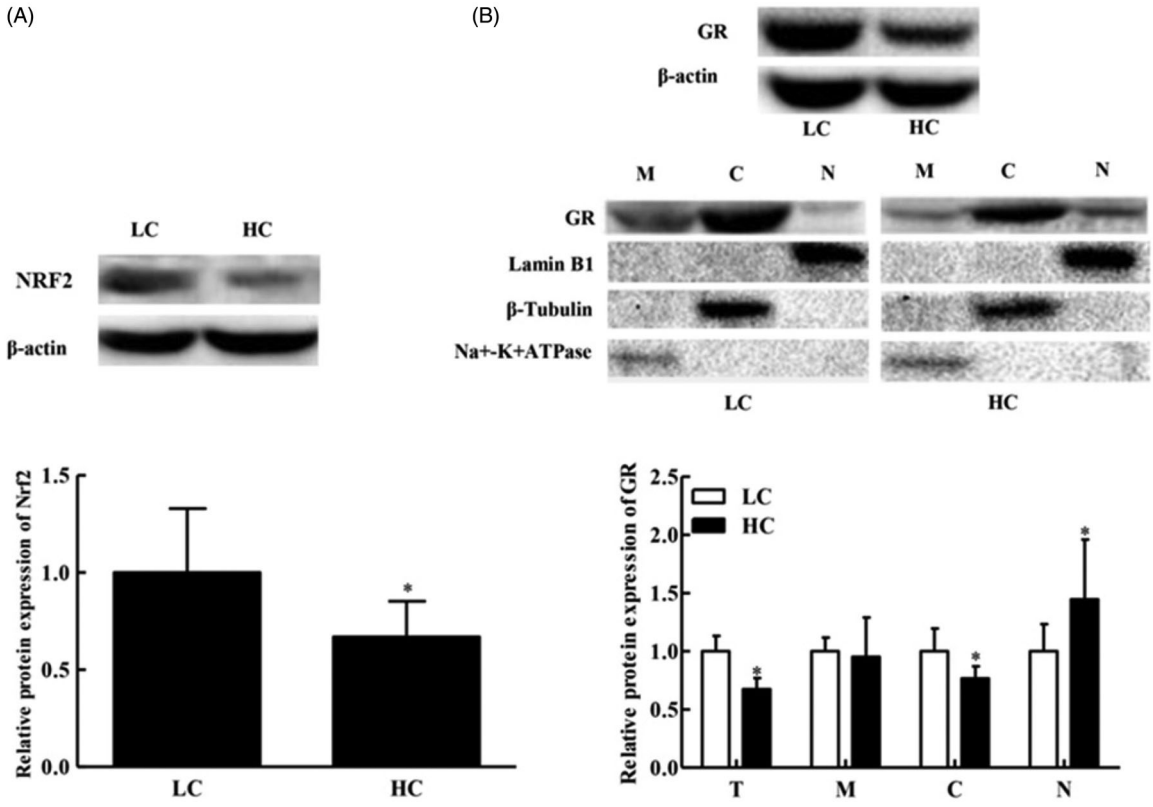

Figure 1. Western blot analysis of the relative expression of liver NRF2, β-actin, GR, Lamin B1, β-tubulin and Na^+/K^+-ATPase protein in goats fed high- (HC) *versus* low-concentrate (LC) grain diets. (A) NRF2 expression in total liver protein; (B) GR expression in the cell membrane (M), cytoplasm (C) and nucleus (N), and hepatic total GR protein expression (T). Cell membrane, cytoplasm and nucleus fractions were obtained by standard subcellular fractionation and were analysed by western blotting. Controls of the cell membrane, cytoplasm and nucleus fractions were performed using an antibody against Na+/K+-ATPase, β-tubulin and Lamin B1, respectively. The Mw of β-actin, GR, NRF2, Lamin B1, β-tubulin and Na^+/K^+-ATPase proteins is 42, 95, 68, 67, 55 and 100 kDa, respectively. These autorads shown from a single goat are representative of six goats. $^*p < .05$ versus LC. All data are presented as the mean ± SD. $n = 6$/group. GR: glucocorticoid receptor; NRF2: nuclear factor E2-related factor 2; Na+/K+-ATPase: sodium-potassium ATPase pump.

the accumulation of oxidative DNA damage (Savic-Radojevic et al. 2013; Mian et al. 2016). In this study, the mRNA expression of *GSTM1* increased, whereas that of *GSTP1* tended to decrease. This result was consistent with a report stating that HC diets mediated decreases in antioxidant capacity, up-regulating *GSTM1* and down-regulating *GSTP1* mRNA levels, in lactating dairy goats (Duanmu et al. 2016). The upregulation of *GSTM1* expression in this study might be an adaptive response to increased peroxidative stress. The reduced expression of *GSTP1* might explain why HC diets suppress antioxidant responses in goats.

Oxidative stress is generally activated in response to challenges that arise when organisms are exposed to external stimuli (Gessner et al. 2013). NRF2 is an important mechanisms used to protect against external stimuli. As an essential transcription factor, NRF2 regulates the expression of antioxidant enzymes by binding to the antioxidant-response element (ARE), thus enhancing cellular defense against oxidative stress (Huang et al. 2014). In contrast, the suppression of the NRF2 antioxidant pathway might give rise to oxidative stress in tissues. Goats fed an HC diet in this study exhibited down-regulation in the expression of *NRF2* and NRF2-regulated antioxidative genes, such as *SOD1*, *CAT*, *GPX1* and *GSTP1*. These results are consistent with reduced SOD, CAT and GPx activity in the liver of HC-fed goats. Thus, the HC diets fed to goats might reduce antioxidant responses in a NRF2-dependent manner. This finding was consistent with an earlier report showing that hepatic *NRF2* expression is inhibited higher in HC-fed dairy cows compared to that in LC-fed dairy cows (Abaker et al. 2017). Furthermore, the decreased expression of *NRF2* might be attributed to GR activation (Ki et al. 2005). The activation of GR usually suppresses the NRF2-dependent antioxidant response (Kratschmar et al. 2012). GR is a low-affinity receptor, contributing to the regulation of

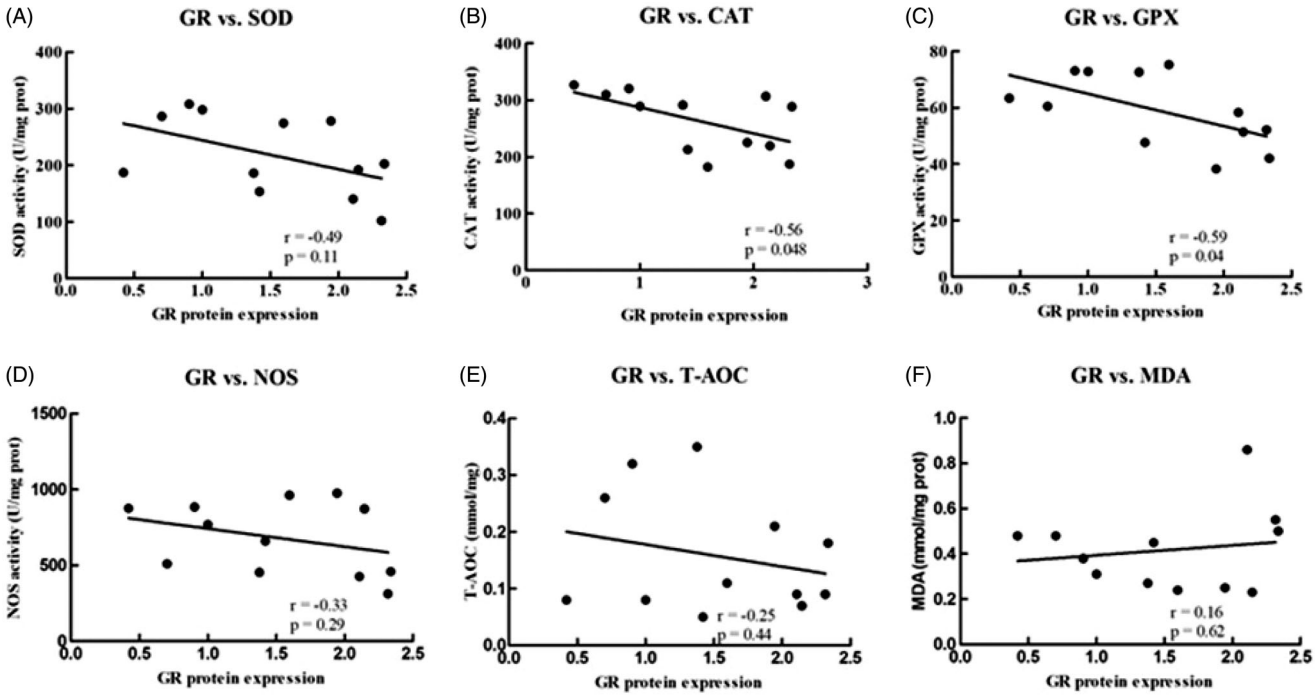

Figure 2. Pearson's correlation of hepatic nuclear GR protein expression with hepatic antioxidant enzyme activity, as well as NOS, T-AOC and MDA level in goats. (A) SOD versus the nuclear expression of GR protein in the liver; (B) CAT versus the nuclear expression of GR protein in the liver; (C) GPx versus the nuclear expression of GR protein in the liver; (D) NOS versus the nuclear expression of GR protein in the liver; (E) T-AOC versus the nuclear expression of GR protein in the liver; (F) MDA versus the nuclear expression of GR protein in the liver. Data were analysed using Pearson's model with SPSS (SPSS version 21.0 for Windows; SPSS Inc., Chicago, IL). All data are presented as the mean ± SD. $n = 6$/group. The results indicate a significant correlation based on the *P*-value at the 0.05 and 0.01 levels. CAT: catalase; GPx: glutathione peroxidase; GR: glucocorticoid receptor; MDA: malondialdehyde; NOS: total nitric oxide synthase; T-AOC: total antioxidant capacity; SOD: superoxide dismutase.

many genes involved in biotransformation reactions, and in adjustments to altered energy demands.

Previous studies have proposed that the HPA is activated by HC-diet challenge or other stressors in domestic farm animals (Minton 1994; Jia et al. 2014). Hepatic *GR* mRNA and protein expression levels were down-regulated in HC goats in this study. HC diets reduce hepatic GR protein expression in goats to modulate the immune response and nutrient metabolism (Dong et al. 2013). Evaluation of the sub-cellular distribution of GR in this study showed that (compared to those in the LC group) GR nuclear translocation was higher, while cytoplasmic retention of GR was lower in the HC group. Thus, HC diets are likely to activate the HPA. The nuclear translocation of GR suppresses NRF2-mediated antioxidant responses. This phenomenon was demonstrated in peripheral blood mononuclear cells. Specifically, reduced GR nuclear translocation and increased cytoplasmic retention of GR under melatonin treatment causes the up-regulation of NRF2 (Kratschmar et al. 2012; Singh and Haldar 2016). Moreover, decreased liver antioxidant enzyme activity (SOD, CAT and GPx) generally contributes towards reducing total GR levels and GR activation (GR nuclear translocation) (Liu et al. 2008; Skuza et al. 2011; Djikic et al. 2012; Wei et al. 2013). In this study, the expression levels of hepatic nuclear GR proteins were negatively correlated with hepatic CAT and GPx activity. Thus, GR likely contributes towards regulating antioxidant responses in goats fed HC diets.

Conclusions

Our data suggest that feeding goats HC diets induces a reduction in the antioxidant response, which was associated with GR activation in goat liver within the 5-week experimental period.

Ethical Approval

The protocol was approved by the Animal Care Committee on the Ethics of Animal Experiments of Institute of Subtropical Agriculture (Permit No. 20170712).

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Disclosure statement

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