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Ghrelin serves as a signal of energy utilization and is involved in maintaining energy homeostasis in broilers

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ABSTRACT

Ghrelin, one of the most important appetite regulating peptides, is involved in the regulation of energy homeostasis. The anorexia effect of ghrelin in chickens is contrary to that of ghrelin in mammals. In the present study, the effects of feeding status and dietary energy level on plasma total ghrelin levels and expression were studied in broilers. The gene expression of ghrelin and its receptor GHS-R1a were measured in the hypothalamus, proventriculus, duodenum, liver, and abdominal fat pad. The results showed that ghrelin mRNA and GHS-R1a mRNA are moderately expressed in liver and abdominal fat. Ghrelin secretion was increased by fasting and refeeding. The gene expression of ghrelin and GHS-R1a in the hypothalamus, proventriculus, liver, and abdominal fat pad were changed by feeding status and dietary energy level. The results suggest that ghrelin is a signal of energy utilization in chickens. The abundant expression of ghrelin and GHS-R1a in liver and abdominal fat pad may be associated with energy balance.

1. Introduction

Ghrelin, a polypeptide with 28 amino acids originally isolated from rat stomach, is the endogenous ligand for the GH secretagogue (GHS) receptor (GHS-R) and is the first known example of a bioactive peptide modified by acylation (Kojima et al., 1999; Date et al., 2000). Chicken ghrelin consists of 26 amino acids and possesses 54% sequence identity with human ghrelin. The serine residue at position 3 (Ser3) is conserved between the chicken and mammalian species, with its acylation by either n-octanoic or n-decanoic acid (Kaiya et al., 2002). The post-translational addition of n-octanoic, n-decanoic acids, or an unsaturated form of these fatty acids by the enzyme ghrelin O-acyltransferase (GOAT) at Ser3 results in acyl-ghrelin, whereas desacyl-ghrelin occurs following enzyme-mediated hydrolysis of the acyl moiety, and O-n-octanoylation is essential for ghrelin to activate GHS-R (van der Lely et al., 2004; Yang et al., 2008; Zaniolo et al., 2011).

Ghrelin functions by binding to the GHS-R and transmits signals by increasing intracellular calcium levels (Reviewed by Kaiya et al., 2013a). In chickens, GHS-R has two isoforms, GHS-R1a and GHS-R1c (Geelissen et al., 2003; Tanaka et al., 2003). GHS-R1a, having high homology with mammalian species, is thought to be the effective receptor of ghrelin in chicken (Geelissen et al., 2003). Similar to mammals, the gene expression of ghrelin and GHS-R in poultry is widely distributed in both central and peripheral organs, while the proventriculus is the main expression site of ghrelin (Kojima et al., 1999; Date et al., 2000; Richards et al., 2006; Kaiya et al., 2013a). Ghrelin can pass through the blood-brain barrier, linking the central and peripheral systems, playing an integral role in regulation and having a protective effect on the blood-brain barrier (Banks et al., 2002; Lopez et al., 2012; Stark et al., 2015; Howick et al., 2017). In mammals, studies have shown that ghrelin is a hormone that promotes appetite, both after central and peripheral ghrelin administration (Nakazato et al., 2001; Date et al., 2002; Abtahi et al., 2017). In poultry, however, the biological functions of ghrelin are not entirely consistent and are even opposite to mammals, especially in the regulation of appetite and energy metabolism (Kaiya et al., 2009; Kaiya et al., 2013b). Intracerebroventricular injection of ghrelin suppresses feed intake in chickens and Japanese quails (Furuse et al., 2001; Saito et al., 2002; Dimaraki and Jaffe, 2006; Khan et al., 2006; Xu et al., 2011; Zendehdel et al., 2013). Surprisingly, the effect of peripheral ghrelin injection on feed intake shows contradictory results; some promote feeding, some inhibit feeding, and some have no effect on feeding (Shousha et al., 2005; Geelissen et al., 2006; Kaiya et al., 2007). Our previous work suggests that ghrelin involved in the modulating network of energy homeostasis in concert with glucocorticoids and insulin (Song et al., 2018). Hence, we hypothesized that the energy state of chicken is associated with ghrelin secretion.

The aim of the present study was to investigate the effect of energy

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status on ghrelin expression and secretion in chickens. Three experiments were conducted to investigate the influence of feeding status (feeding, fasting and refeeding) and dietary energy (high vs. low energy level) on ghrelin secretion in broilers. The total ghrelin in serum and gene expression profiles of ghrelin and GHS-R1a in different organs were measured.

2. Materials and methods

2.1. Animals

One-day-old healthy male broiler chicks (Arbor Acres, *Gallus gallus domesticus*) were obtained from a local hatchery (Dabao Breeding Technology Co., Ltd., Taian, P. R. China). The chicks were reared in an environmentally controlled room. The brooding temperature was maintained at 35 °C (65% relative humidity, RH) for the first 2 days and was then gradually reduced to 21 °C on day 21 and the temperature was maintained until the end of the experiment. Unless otherwise noted, the broilers received a starter diet (metabolic energy: 12.13 MJ/kg; 21% crude protein) until 21 d of age; thereafter, they received a grower diet (metabolic energy: 12.55 MJ/kg; 18% crude protein) until the end of the experiment.

All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of Shandong Agricultural University (No. 2001002) and performed in accordance with the "Guidelines for Experimental Animals" of the Ministry of Science and Technology (Beijing, P. R. China).

2.2. Experiment design

2.2.1. Ghrelin and GHS-R1a genes expression profile in chickens

Ten 42-day-old broilers with similar body weight (BW, 2.22 ± 0.06 kg) were sacrificed by cervical dislocation, followed by exsanguination (Close et al., 1997). The breast muscle, thigh muscle, abdominal fat, liver, glandular stomach, duodenum, and hypothalamus were sampled, snap frozen in liquid nitrogen and then stored at −80 °C for further analysis.

2.2.2. Effect of feeding status on ghrelin mRNA expression

Forty 42-day-old broilers were randomly divided into four groups of 10 birds. Two groups of chickens were subjected randomly to the following two treatments: sacrificed at feeding state (12 h after feeding, Feeding) and at fasting state (after 12 h feed withdrawal, Fasting). The last two groups of chickens were subjected randomly to the following treatments: sacrificed at fasting (after 12 h feed withdrawal, Fasting) or refeeding state (2 h re-fed after 10-h feed withdrawal, Refeeding). Chickens had free access to water during the experimental period. Before being sacrificed, a blood sample was drawn from a wing vein and collected in pre-cooled tubes. Serum samples were obtained after centrifugation at 400 g for 10 min at 4 °C and were stored at −20 °C for further analysis. Tissue samples were obtained by the same method as in Experiment 1.

2.2.3. Effect of dietary energy level on ghrelin mRNA expression

In all, 160 broilers of 21 days of age were randomly divided into 16 groups of ten birds and assigned into two dietary treatments that provided a high-energy diet (14.44 MJ/kg of metabolizable energy, 21.9% crude protein and 14.6% crude fat, HE) or a low-energy diet (11.05 MJ/kg of metabolizable energy, 16.3% crude protein and 6.9% crude fat, LE). During the three-week experiment period, BW and feed intake were recorded weekly. At the end of the third week after treatment, one chicken was randomly selected from each group and sacrificed after fasting 12 h. Blood and tissue samples were collected as described above.

2.3. Blood metabolites, ghrelin, and insulin measurement

Serum concentration of glucose (GLU, F006), triglycerides (TG, F001), and uric acid (UA, C012) were measured with commercial diagnostic kits (Jiancheng Bioengineering Institute, Nanjing, P. R. China). The concentration of total ghrelin was measured with a commercial diagnostic ELISA kit (CSB-E14230C, Wuhan Huamei Bioengineering Co., Ltd, Wuhan, P. R. China), which has been successfully used in the study of chickens (Yu et al., 2016; Höhne et al., 2017; Vizcarra et al., 2018; Song et al., 2018). The sensitivity of the assay was 25 pg/mL and the intraassay coefficient of variation was less than 15%. Insulin was measured using a radioimmunoassay with guinea pig anti-porcine insulin serum (3 V, Bio-Engineering Group Co., Ltd, Weifang, P. R. China). The sensitivity of the assay was 1 μIU/mL and the intraassay coefficient of variation was 6.9%. The kit has been validated in the measurement of chicken insulin by Wang et al. (2012).

2.4. RNA isolation and analysis

The expression levels of the ghrelin mRNA and GHS-R1a mRNA were determined by real-time PCR. Primers used in this study were designed using Primer 5.0 software and synthesized by Sangon Bioteach (Shanghai, P. R. China, Table 1). Total RNA of the collected tissues was extracted using TRIzol (Invitrogen Life Technologies, Carlsbad, CA). The quantity and quality of the isolated RNA were determined using a biophotometer (Eppendorf, Hamburg, Germany) and agarose-gel electrophoresis, respectively. Total RNA (1 μg) was reverse-transcribed into first-strand cDNA using Prime Script™ RT Master Mix (Takara, Dalian, China) following the manufacturer's instructions, and quantitative real-time RT-PCR was performed with a SYBR Green I master mix (Roche, Basel, Switzerland) on ABI 7500 Real-Time PCR System (Q5, Applied biosystems, ABI, USA) using the following parameters: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, and 60 °C for 34 s. The specific products were confirmed by a melting curve generated automatically using SDS analytic software (AVI) and a single-correct product on a 1.2% agarose gel. The relative amount of mRNA of a gene was calculated according to the method of Livak and Schmittgen (2001). The expression levels of those genes were normalized with the expression of chicken GAPDH mRNA. The analyses of the relative gene expression data were performed by the $2^{-\Delta\Delta CT}$ method. All of the samples were run in duplicate, and the primers were designed to span an intron to avoid genomic DNA contamination. Therefore, all gene transcription results are reported as the n-fold difference relative to the calibrator. The specificity of the amplification product was verified by the standard curve and dissolution curve.

2.5. Statistical analysis

All the values were expressed as the means ± standard error (S. E.). All statistical analyses were performed using SAS statistical software (SAS version 8e, SAS Institute, 1998). When the main effect of the treatment was significant, the differences between means were assessed by Student's *t*-test. $P < 0.05$ was considered as statistically significant.

Table 1
Quantitative real-time PCR primer sequences.

Gene	Sequences (5′→3′)	Accession NO.	Product size (bp)
Ghrelin	F: CCTTGGGACAGAAACTGCTC R: CACCAATTTCAAAAGGAACG	NM_001001131.1	203
GHS-R1a	F: TTTTTCCTGCCCGTATTCTG R: GCTTGGTGCTGGAGAGTCTT	NM_204394.1	397
GAPDH	F: ACATGGCATCCAAGGAGTGAG R: GGGGAGACAGAAGGGAACAGA	NM_204305.1	244

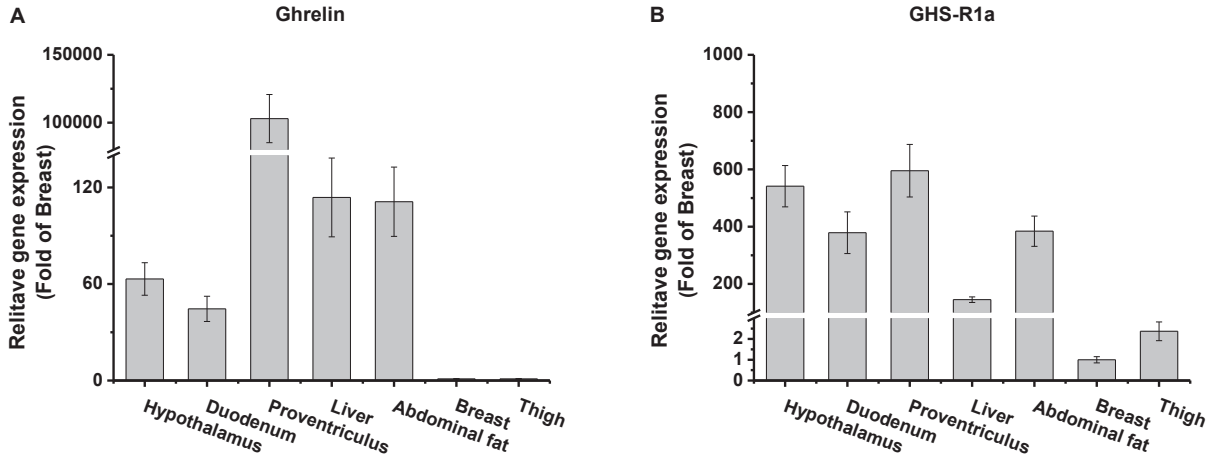

Fig. 1. The gene expression of ghrelin and GHS-R1a in tissues. (A) Ghrelin mRNA, (B) GHS-R1a mRNA. Data were presented as the Means ± SE (n = 10).

3. Results

3.1. The gene expression profile of ghrelin and GHS-R1a in chickens

The result showed that ghrelin mRNA was widely expressed in chickens, and proventriculus exhibited the highest expression, followed by liver, abdominal fat, hypothalamus, and duodenum (Fig. 1A). The proventriculus, hypothalamus, duodenum, abdominal fat, and liver displayed a high GHS-R1a mRNA expression level (Fig. 1B). Ghrelin mRNA and GHS-R1a mRNA expressed at a low level in breast and thigh muscle tissues (Fig. 1A and B).

3.2. Effect of feeding status on the gene expression of ghrelin and GHS-R1a

We first investigated the effect of fasting on the gene expression of ghrelin and its receptor. Serum TG ($P < 0.05$), GLU ($P < 0.001$), and insulin ($P < 0.05$) levels were much lower after food deprivation (Fig. 2 A). Fasting chickens had significantly higher serum ghrelin concentration compared to the feeding chickens ($P < 0.001$, Fig. 2 B). Meanwhile, fasting chickens showed upregulated ghrelin mRNA in the proventriculus ($P < 0.001$) and hypothalamus ($P < 0.05$), down-regulated ghrelin mRNA expression in the liver ($P < 0.05$), and no change in the duodenum and abdominal fat (Fig. 2C). At the same time, fasting promoted the expression of GHS-R1a mRNA in the proventriculus ($P < 0.05$) but inhibited the expression of GHS-R1a mRNA in the liver and abdominal fat ($P < 0.05$); no significant difference was shown in the duodenum and hypothalamus (Fig. 2D).

We thereafter determined the influence of refeeding on ghrelin and GHS-R1a genes expression. Compared with fasting group, refeeding increased serum TG ($P < 0.01$), GLU ($P < 0.05$), insulin ($P < 0.01$), and ghrelin ($P < 0.001$) (Fig. 3A and B). Refeeding increased the expression of ghrelin mRNA in the hypothalamus ($P < 0.01$), liver ($P < 0.05$), and abdominal fat ($P < 0.05$) but had no significant influence on its expression in the proventriculus and duodenum compared to fasting chickens (Fig. 3C). Refeeding increased the expression of GHS-R1a mRNA in the duodenum ($P < 0.01$) and abdominal fat ($P < 0.01$), whereas refeeding had no detectable influence on GHS-R1a mRNA expression in the hypothalamus, proventriculus, and liver (Fig. 3D).

3.3. Effect of dietary energy on ghrelin mRNA expression

After 3 weeks of feeding, HE chickens had lower food intake ($P < 0.05$) and the same energy intake (Table 2), compared with LE-chickens. There was a significantly lower level of serum GLU ($P < 0.001$) in HE-chickens, while TG, insulin and ghrelin concentrations were not changed by dietary treatment (Fig. 4A and B). Compared to LE-chickens, the mRNA level of ghrelin was increased in the hypothalamus ($P < 0.05$) and decreased in the duodenum ($P < 0.01$), liver ($P < 0.05$), and abdominal fat ($P < 0.05$) by HE treatment; whereas the ghrelin mRNA level was not changed in the proventriculus (Fig. 4C). The transcriptional level of GHS-R1a mRNA was increased in the hypothalamus ($P < 0.01$) and proventriculus ($P < 0.01$), decreased in the duodenum ($P < 0.05$) and liver ($P < 0.05$) and were not influenced in the abdominal fat (Fig. 4D) in HE treatment, compared to LE birds.

4. Discussion

4.1. Ghrelin is a signal of both starvation and energy utilization

With few exceptions, ghrelin concentration increases significantly in response to food deprivation in mammals, aves, amphibian and fish (Toshinai et al., 2001; Tups et al., 2004; Perry and Wang, 2012; Jönsson, 2013; Kaiya et al., 2013c). In Japanese quails and layer type chicken, plasma acyl-ghrelin concentration is increased after 24-h or 12 h feed deprivation (Shousha et al., 2005; Kaiya et al., 2007). In contrast, the total ghrelin shows a trend toward to be increased following 48-h feed deprivation (Richards et al., 2006). In order to investigate the effect of feeding status on ghrelin secretion, we used the total ghrelin to reflect the effect of experimental treatment on ghrelin secretion without distinguishing between acylated and unacylated forms. In the present study, the decreased circulating GLU, TG, and insulin levels in fasting chickens indicated that the model was successfully established. In line with the previous studies, 12-h fasting significantly elevated serum total ghrelin concentration, suggesting that starvation stimulates the release of ghrelin in chickens. We further compared the fasting chickens with fasting/refeeding chickens. Compared to the fasting broilers, 2-h refeeding significantly elevated the serum content of GLU, TG, and insulin, indicating a positive energy status in refeeding chickens. The circulating total ghrelin, however, was even further elevated by refeeding. This result was in accordance with the work in quails by Shousha et al. (2005), who reported that fasting and refeeding groups displayed elevated circulating ghrelin compared to feeding groups. Furthermore, food intake was stimulated by peripheral (i.p.) but not i.c.v. injections of small doses of ghrelin, whereas both i.p. and i.c.v. injections of higher doses inhibited feeding in the Japanese quail (Shousha et al., 2005). The increased ghrelin secretion by fasting and refeeding suggests that ghrelin is involved in the energy mobilization and deposition.

In the present study, the gene expression of ghrelin and GHS-R1a increased in the proventriculus after fasting, in line with previous works

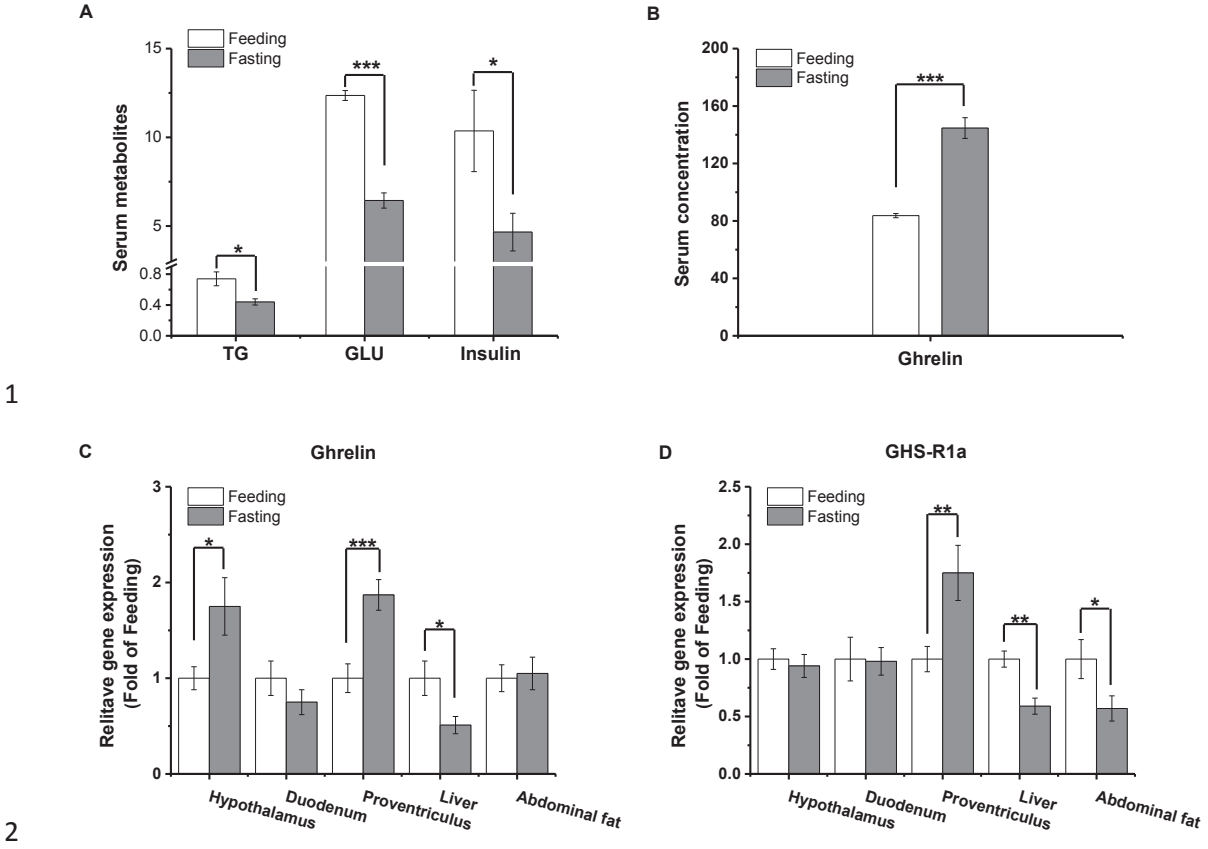

Fig. 2. Effects of fasting and feeding status on blood metabolites and the gene expression of ghrelin and GHS-R1a in tissues. (A) Triglycerides (TG, mmol/L), glucose (GLU, mmol/L), and insulin (μIU/mL), (B) serum ghrelin (pg/mL), (C) ghrelin mRNA, and (D) GHS-R1a mRNA. Data were presented as the Means ± SE (n = 8). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

in chickens (Kim et al., 2003; Kaiya et al., 2007; Richards et al., 2006). We further observed that 2-h refeeding after 10-h fasting, however, had no detectable influence on ghrelin and GHS-R1a expression in the proventriculus. The result indicated that the increased ghrelin mRNA expression in the proventriculus by fasting is maintained after refeeding in broilers. This result was in accordance with the result of Richards et al. (2006), who reported that ghrelin expression in the proventriculus was increased by fasting (24 or 48 h) but not reduced by subsequent 24-h refeeding. Similarly, the increased ghrelin mRNA level in proventriculus by fasting remains high after 6-h refeeding in layer chicks (Kaiya et al., 2007). Chen et al. (2007) reported that ghrelin mRNA level in chicken proventriculus was increased in response to either 12-h or 36-h fasting but not decreased after subsequent 12-h refeeding. The results supported the speculation that ghrelin serves not only as a signal of starvation but also a signal of energy utilization. Furthermore, in hypothalamus, ghrelin expression was upregulated by fasting and was further upregulated by refeeding. It is well known that ghrelin promotes appetite in mammals and most fish (goldfsh and tilapia) (Wren et al., 2000; Nakazato et al., 2001; Kaiya et al., 2008; Kaiya et al., 2013c). Central administration of ghrelin suppresses feed intake of chicken (Saito et al., 2002; Shousha et al., 2005; Zendehdel et al., 2013). Hence, the result suggests that ghrelin serves not only as a starvation signal but also as a signal of energy utilization to keep energy homeostasis in broilers.

In the present study, chickens fed the HE or LE diet differed in energy and fat levels. Although the circulating ghrelin level was not significantly altered by dietary treatment, the gene expression of ghrelin in the hypothalamus and GHS-R1a in the proventriculus was increased in HE-chickens compared to LE group, suggesting that dietary energy level has an effect on the expression of ghrelin. The LE-chickens consumed more feed and had a similar caloric intake as the HE-group, suggesting that dietary fat level, but not the energy level, is associated with the regulation of ghrelin mRNA and its receptor mRNA. This speculation was in line with the observation in mammals. In humans, the post-prandial decline of ghrelin induced by high-carbohydrate feeding is reversed by a high-fat meal (Greenman et al., 2004; Erdmann et al., 2004). In rats, long-term intake of a high-fat diet causes hypoghrelinemia (Handjieva-Darlenska and Boyadjieva, 2009; Gomez et al., 2012). After consumption of a high-fat diet, the acylated ghrelin level loses its correlation with body weight and food intake (Sugiishi et al., 2013). Hence, the result implies that ghrelin is associated with lipid metabolism.

4.2. The abundant expression of ghrelin mRNA and GHS-R1a mRNA in the liver and abdominal fat pad is associated with energy deposition

The gene expression of ghrelin and GHS-R1a was detected in the hypothalamus, gastrointestinal tract, liver, fat, and skeletal muscles of broiler chickens. The present result indicated that ghrelin mRNA and GHS-R1a mRNA expressed widely in central and peripheral organs, in line with the results in mammals and chickens (van der Lely et al., 2004; Kojima et al., 1999; Date et al., 2000; Richards et al., 2006; Kaiya et al., 2013a). In line with the previous results in chickens (Kaiya et al., 2002; Ma et al., 2015; Richards et al., 2006), the present result demonstrated that ghrelin mainly expressed in the proventriculus. Moreover, the results indicated that ghrelin was highly expressed in the liver, abdominal fat, hypothalamus, and duodenum, with less expression in both the breast and thigh muscles. Except the proventriculus, ghrelin is highly expressed in the pancreas, brain, duodenum, and lower small intestine in 3-week-old broilers (Richards et al., 2006). In the

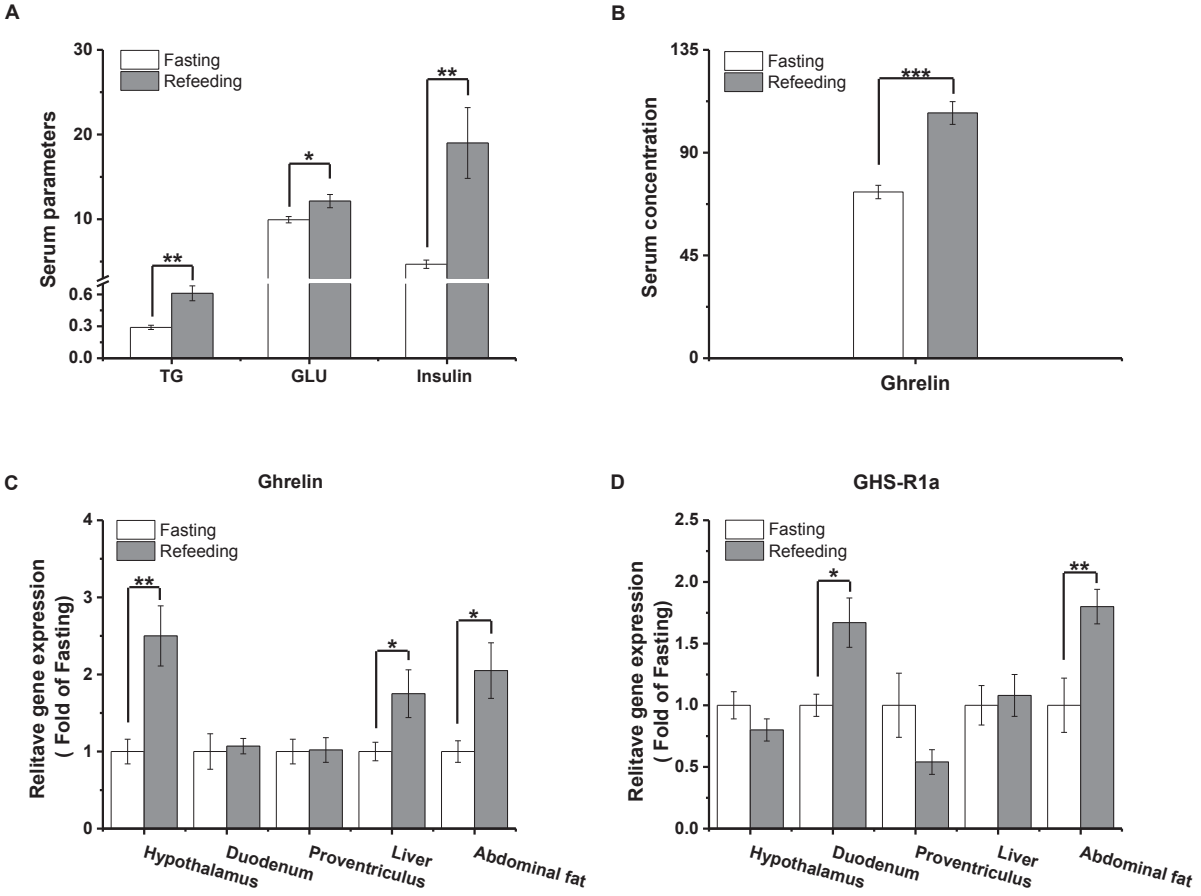

Fig. 3. Effects of fasting and refeeding on blood metabolites and the gene expression of ghrelin and GHS-R1a in tissues. (A) Triglycerides (TG, mmol/L), glucose (GLU, mmol/L), insulin (μIU/ml), (B) serum ghrelin (pg/mL), (C) Ghrelin mRNA, (D) GHS-R1a mRNA. Data were presented as the Means ± SE (n = 8). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

Table 2
Production performance.

	LE	HE	P value
Food intake (g/day)	177.39 ± 9.44	138.88 ± 6.91	0.0302
Energy intake (MJ/day)	2.02 ± 0.07	1.91 ± 0.06	0.3056

Values are means ± SEM.

present study, however, the liver, abdominal fat, hypothalamus, and duodenum displayed relatively high levels of ghrelin in broilers of 6 weeks of age. The different observations of ghrelin expression in organs other than proventriculus may be related to the developing stage of chickens. This speculation was supported by the work of Ma et al. (2015), who reported that the spleen, duodenum, and leg and breast muscles have a medium expression level in chicken embryos. Moreover, ghrelin-induced gastrointestinal contractions decrease in the proventriculus along with ages from hatching to 100 d of age, which is probably due to the age-related decrease in GHS-R1a expression (Kitazawa et al., 2013).

In line with the result of Richards (Richards et al., 2006), GHS-R1a mRNA was highly expressed in the hypothalamus, gastrointestinal tract, and liver. In contrast, however, we found that GHS-R1a mRNA was highly expressed in abdominal fat, which disagreed with the result of Richards, who reported that there was a relatively lower expression in the abdominal fat pad. As both the liver and the abdominal fat pad increase quickly with body weight in modern line broilers (van der Lely et al., 2004; Zuidhof et al., 2014), the relatively high expression of ghrelin mRNA and GHSR-1a mRNA in the liver and abdominal fat suggests that ghrelin may be involved in the lipid metabolism of broiler chickens. In line with the result, Buyse et al. (2009) reported that ghrelin had central anorectic effect and peripheral anti-lipogenic effect in chickens.

We further evaluated the effect of energy state on ghrelin mRNA and GHS-R1a mRNA expression in the liver and the abdominal fat pad. Fasting decreased ghrelin mRNA and GHS-R1a mRNA in the liver and GHS-R1a mRNA in abdominal fat. Conversely, refeeding recovered ghrelin mRNA expression in the liver and ghrelin mRNA together with GHS-R1a mRNA expression in abdominal fat. Hence, this result indicated that the expression of ghrelin and its receptor GHS-R1a in the liver and abdominal fat changed with energy status. Collectively, the results suggest that ghrelin acts as an energy signal to regulate energy balance. Unlike in mammals, the liver in chickens is the main site of lipid synthesis, and adipose tissues mainly function as fat storage (Leveille, 1969; Saadoun and Leclercq, 1987). The effect of ghrelin on lipid metabolism in liver and adipose tissue needs to be studied further.

Compared with chickens fed a LE diet, the downregulated gene expression of ghrelin and GHS-R1a in the liver and ghrelin mRNA in abdominal fat indicated that dietary energy level plays a role on ghrelin mRNA and GHS-R1a mRNA expression. In the present study, LE chickens consumed more feed than HE birds while keeping a similar calorie intake (HE: 2.02 MJ/d vs. LE: 1.91 MJ/d) with HE-birds. In the present study, HE was formulated with a proportion of fat and had a high fat level compared to the LE diet (HE, 14.6% crude fat vs. LE, 6.9% crude fat). Those results imply that the different macronutrient or diet macronutrion composition or proportion of carbohydrates/fat in the diet, not the energy intake, changed the expression of ghrelin and its

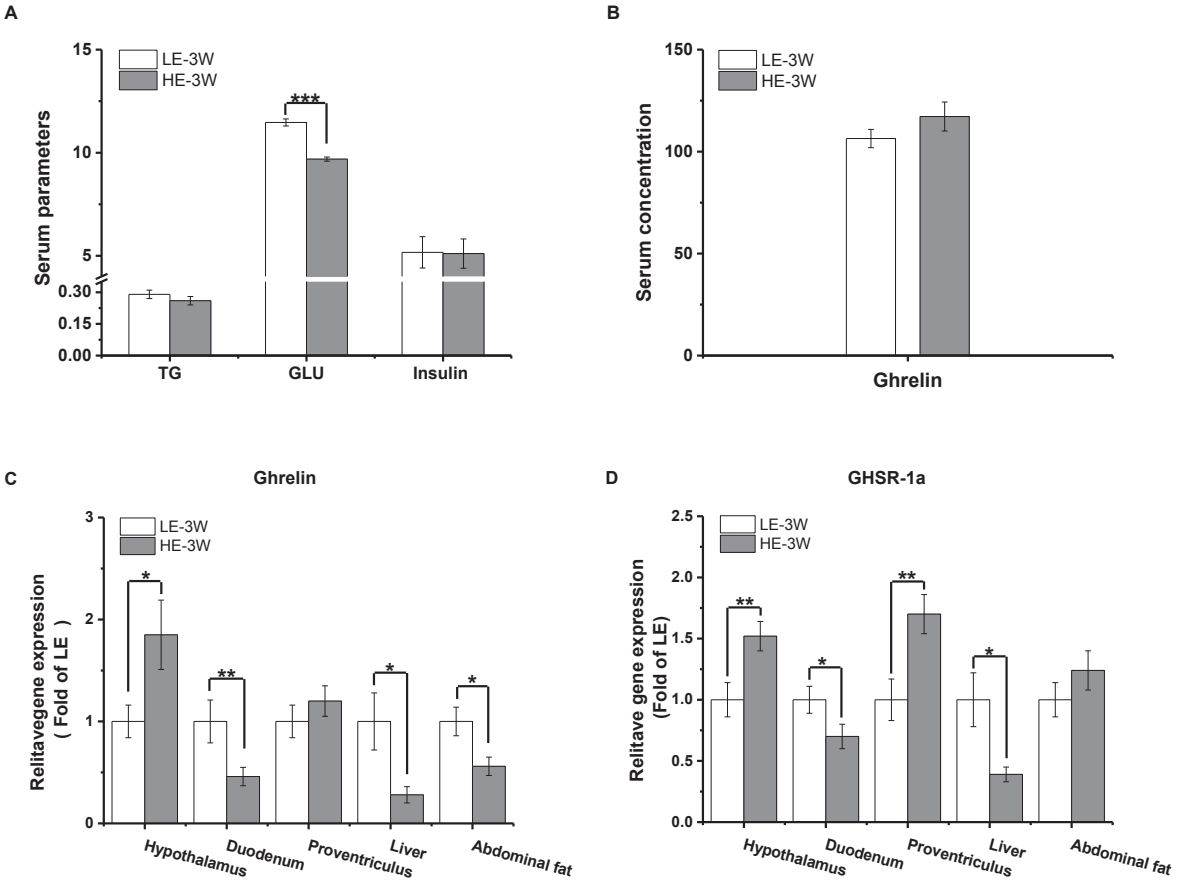

Fig. 4. Effects of dietary energy levels (HE, 14.44 MJ/kg of metabolizable energy; LE, 11.05 MJ/kg of metabolizable energy) on serum parameters and gene expression in different tissues of broiler chickens. (A) Triglycerides (mmol/L), glucose (mmol/L), and insulin (μIU/mL), (B) serum ghrelin (pg/mL), (C) Ghrelin mRNA, (D) GHS-R1a mRNA. Data were represented as the Means ± SE (n = 8). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

receptor.

In summary, the present result indicated that GHS-R1a mRNA is highly expressed in the proventriculus while moderately expressed in the liver, abdominal fat, and hypothalamus in broilers. Ghrelin secretion is stimulated by fasting and maintained a high level after refeeding. The result suggests that ghrelin acts as a regulation signal of energy homeostasis. The abundant expression of ghrelin mRNA and GHS-R1a mRNA in the liver and abdominal fat pad imply that ghrelin is associated with energy storage.

Acknowledgements

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The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2018.11.017.

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