Effects of Stress and Dietary Energy Level on Hepatic Lipid Synthesis Capacity in Broiler Chickens: Postprint

Authors: Cai Yuanli, Lin Hai

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Abstract

This study was designed to investigate the effects of stress and dietary energy level on hepatic fatty acid synthase (FAS) activity and the mRNA expression levels of AMP-activated protein kinase (AMPK) and sterol-regulatory element binding protein-1 (SREBP-1) in broiler chickens, thereby elucidating the mechanisms by which stress induces fatty liver in broilers and identifying methods to alleviate stress. The study consisted of four experiments. The first two experiments focused on broiler chickens aged 3-9 days and 28-34 days, respectively, which were subjected to corticosterone treatment and high- or low-energy diets; liver samples were collected at the end of the experiments to determine FAS activity. In the third experiment, a total of 108 seven-day-old Arbor Acres male broiler chickens with similar body weights were randomly allocated into three groups: a stress group (injected with dexamethasone), a control group (injected with saline), and a pair-fed group (injected with saline, with feed intake matching that of the stress group on the previous day). Injections were administered for 7 consecutive days. At 14 days of age, liver samples were collected to determine hepatic triglyceride content and the mRNA expression levels of AMPK and SREBP-1. In the final experiment, 3-9-day-old broiler chickens were treated with corticosterone and glucose in drinking water, and hepatic FAS activity was measured at the end of the experiment. The results showed that: 1) Corticosterone treatment extremely significantly increased hepatic FAS activity in 3-9-day-old broiler chickens ($P<0.01$), and showed a similar trend in 28-34-day-old broiler chickens ($P=0.0512$). 2) Compared with the pair-fed group, dexamethasone treatment significantly increased hepatic triglyceride content ($P<0.05$) and significantly elevated hepatic AMPK mRNA expression ($P<0.05$); moreover, dexamethasone treatment significantly increased hepatic SREBP-1 mRNA expression compared to both the control and pair-fed groups ($P<0.05$). 3) Supplementing glucose in the drinking water for the stress group significantly reduced hepatic FAS activity ($P<0.05$). These results indicated

that corticosterone treatment increased hepatic FAS activity in broiler chickens, dexamethasone treatment significantly increased hepatic SREBP-1 mRNA expression and activated AMPK, while glucose exhibited a stress-alleviating effect.

Full Text

Effects of Stress and Dietary Energy Level on Hepatic Fatty Acid Synthesis Capacity in Broiler Chickens

CAI Yuanli[1], LIN Hai[2]* (1. College of Life Sciences, Qilu Normal University, Jinan 250013, China; 2. College of Animal Science and Technology, Shandong Agricultural University, Taian 271018, China)

Abstract: This study investigated the effects of stress and dietary energy level on fatty acid synthase (FAS) activity and the mRNA expression levels of AMP-activated protein kinase (AMPK) and sterol-regulatory element binding protein-1 (SREBP-1) in the liver of broiler chickens, aiming to elucidate the mechanisms underlying stress-induced fatty liver and identify potential mitigation strategies.

The study comprised four experiments. In the first two experiments, broiler chickens aged 3–9 days and 28–34 days were administered corticosterone and fed diets with high or low energy levels. Liver samples were collected at the end of each experiment to measure FAS activity. In the third experiment, 108 seven-day-old male Arbor Acres broiler chickens with similar body weights were randomly assigned to three groups: a stress group (injected with dexamethasone), a control group (injected with saline), and a pair-fed group (injected with saline but with feed intake matched to the stress group from the previous day). Injections were administered daily for seven days. At 14 days of age, liver samples were collected to determine triglyceride content and mRNA expression levels of AMPK and SREBP-1. The final experiment involved treating 3–9-day-old broiler chickens with corticosterone and glucose-supplemented drinking water, with liver FAS activity measured at the conclusion.

Key words: broiler chickens; stress; lipid deposition; FAS; AMPK; SREBP-1

Introduction

Under intensive production systems, broiler chickens face various stressors including high temperature, overcrowding, vaccination, and transportation, which lead to reduced growth performance, compromised immunity, decreased reproductive performance, and deteriorated meat quality. Numerous stress models have been employed in animal research, including direct administration of adrenocorticotropic hormone to modulate adrenal secretion and treatment with exogenous glucocorticoids such as corticosterone, cortisone, cortisol, and dexamethasone. Additionally, environmental conditions like cold and heat stress have

been used to study stress responses.

Exogenous glucocorticoids promote fat deposition in the mesenteric region of rats, redirecting energy storage toward internal deposition [1-3]. Corticosterone-treated broiler chickens exhibit impaired skeletal muscle growth and increased fat deposition, indicating that stress reallocates energy storage to favor fat accumulation [4-6]. Previous studies have also demonstrated that dexamethasone treatment increases lipid deposition in both liver and adipose tissue of broiler chickens [7]. Research in mice has confirmed that high-carbohydrate and high-energy diets not only enhance energy intake but also elevate hepatic triglyceride content [8]. While these studies show that both stress and high-energy diets promote fat deposition, the underlying mechanisms remain unclear. Therefore, this study investigated the effects of corticosterone, dexamethasone, and dietary energy levels on broiler chickens by analyzing hepatic FAS activity and mRNA expression of AMPK and SREBP-1, aiming to clarify the mechanisms of stress-induced fat deposition and explore whether glucose supplementation could alleviate stress.

1.1.1 Experiment 1: Effects of Dietary Energy Level and Corticosterone Treatment on Hepatic FAS Activity in 3–9-Day-Old Broiler Chickens

One hundred forty-four one-day-old male Arbor Acres (AA) broiler chickens with similar body weights were randomly divided into a control group and a stress group, each further subdivided into two dietary treatments (high-energy and low-energy diets). Each treatment had three replicates with 12 chickens per replicate, totaling 12 pens. From 3 to 9 days of age, the stress group received dietary corticosterone supplementation at 30 mg/kg (purchased from Sigma, 92% purity). Chickens had ad libitum access to feed and water. Housing temperature, humidity, lighting, and sanitary conditions complied with the Laboratory Animal Environment and Facilities standard (GB 14925–1994). During the first two days, chickens were fed a commercial diet containing 21.5% crude protein and 12.6 MJ/kg metabolizable energy. The composition and nutrient levels of the experimental diets are shown in Table 1 . Except for energy content, all dietary nutrient levels, vitamins, and trace elements met the NRC (1994) recommendations for broiler nutrition.

Sample Collection: At 08:00 on day 10, four chickens from each replicate were selected, weighed, and slaughtered to collect liver samples, which were snap-frozen in liquid nitrogen and stored in a freezer.

1.1.2 Experiment 2: Effects of Dietary Energy Level and Corticosterone Treatment on Hepatic FAS Activity in 28–34-Day-Old Broiler Chickens

One hundred forty-four one-day-old male AA broiler chickens with similar body weights were randomly divided into a control group and a stress group, each further subdivided into two dietary treatments (high-energy and low-energy diets). Each treatment had three replicates with 12 chickens per replicate, totaling 12 pens. The experimental period was from 28 to 34 days of age. From 1 to 21 days and 22 to 27 days, chickens were fed commercial diets containing 21.5% and 20.0% crude protein with 12.6 and 12.8 MJ/kg metabolizable energy, respectively. From 28 to 34 days, chickens received the experimental diets, with the stress group supplemented with 30 mg/kg corticosterone. Chickens had ad libitum access to feed and water. Housing conditions complied with the Laboratory Animal Environment and Facilities standard (GB 14925–1994). Diet composition and nutrient levels are presented in Table 2 . Except for energy content, all dietary nutrient levels, vitamins, and trace elements met NRC (1994) recommendations.

Sample Collection: At 08:00 on day 35, four chickens from each replicate were selected, weighed, and slaughtered to collect liver samples, which were snap-frozen in liquid nitrogen and stored in a freezer.

1.1.3 Experiment 3: Effects of Dexamethasone Treatment on Hepatic Triglyceride Content and mRNA Expression of AMPK and SREBP-1 in Broiler Chickens

One hundred eight one-day-old male AA broiler chickens with similar body weights were randomly assigned to three groups (stress, control, and pair-fed), each with three replicates of 12 chickens. Chickens had ad libitum access to feed and water until 08:00 on day 7 when the experiment began. The stress group received daily subcutaneous injections of dexamethasone (1 mg/mL) at a dose of 2.0 mg/kg body weight at 08:00 each day, with ad libitum feed and water access. The control group received saline injections of equal volume with ad libitum feed and water. The pair-fed group received saline injections of equal volume but were fed according to the stress group' s feed intake from the previous day. Injections continued for seven consecutive days. Body weight and feed intake were recorded daily.

Sample Collection: At 08:00 on day 14, eight chickens from each group were selected after a 12-hour fast (water not restricted). Liver samples were snap-frozen in liquid nitrogen for analysis of triglyceride content and mRNA expression of AMPK and SREBP-1.

1.1.4 Experiment 4: Effects of Corticosterone Treatment and Glucose-Supplemented Drinking Water on Hepatic FAS Activity in Broiler Chickens

Ninety-six broiler chickens were divided into a control group and a stress group, each with two treatments: glucose-supplemented water and saccharin-supplemented water as a control. Each treatment had three replicates with eight chickens per replicate. From 3 to 9 days of age, the stress group received dietary corticosterone at 30 mg/kg. Glucose was supplemented in drinking water at 80 g/L, with saccharin at 2 g/L as a taste-matched control.

Sample Collection: Same as described in Experiment 1.

1.2.1 Determination of Hepatic FAS Activity

Hepatic FAS activity was measured according to the method of Halestrap et al. [9]. **Enzyme Preparation:** Liver tissue was homogenized in ice-cold homogenization buffer at a 1:2 tissue-to-buffer ratio, then centrifuged at 100,000 × g for 1 hour at 4 °C. The supernatant was used for FAS activity analysis. The reaction mixture was prepared in 0.1 mol/L phosphate buffer (pH 6.5) containing 0.1 mmol/L NADPH and 25 mol/L acetyl-CoA. One milliliter of reaction mixture was pre-warmed at 37 °C for 4 minutes, followed by addition of 100 L supernatant enzyme solution and rapid addition of 50 L of 1.38 mmol/L malonyl-CoA. The change in absorbance at 340 nm was measured over 1 minute in a 0.5 cm quartz cuvette.

1.2.2 Determination of Hepatic Triglyceride Content

One gram of liver tissue was dissolved in 10 mL isopropanol to extract triglycerides. Hepatic triglyceride content was determined using an enzymatic method with a commercial kit from Nanjing Jiancheng Bioengineering Institute.

1.2.3 Extraction and Analysis of Total RNA from Liver

1.2.3.1 Total RNA Extraction: Total RNA was extracted from liver tissue using the Trizol method.

1.2.3.2 Reverse Transcription: Reverse transcription was performed using the TaKaRa RNA PCR Kit (AMV) Ver. 3.0 (Code: DRR019A) according to the manufacturer' s instructions in a 10 L reaction system. Conditions were: 42 °C for 20 minutes, 99 °C for 5 minutes, and 5 °C for 5 minutes in one cycle.

1.2.3.3 Real-Time Fluorescent Quantitative PCR: Real-time relative quantitative PCR was performed using the SYBR Green I method with the

SYBR Premix Ex TaqTM (Perfect Real Time) kit (TaKaRa Code: DRR041A) on an ABI 7500 Real-Time PCR System. The 20 L reaction system used primers synthesized by Shanghai Sangon Biotech. Primer sequences are listed in Table 3 . The two-step PCR conditions were: 95 °C pre-denaturation for 10 seconds, followed by 40 cycles of 95 °C for 5 seconds and 60 °C for 34 seconds.

1.3 Statistical Analysis

Data are presented as means ± standard error (±SE). Statistical analysis was performed using SAS 8.0 software with the ANOVA procedure. Experiments 1, 2, and 4 were analyzed using a two-factor model, while Experiment 3 used a one-factor model. Differences were considered significant at $P < 0.05$ and highly significant at $P < 0.01$.

2.1 Effects of Dietary Energy Level and Corticosterone Treatment on Hepatic FAS Activity in 3–9-Day-Old Broiler Chickens

As shown in Table 4 , corticosterone treatment significantly increased hepatic FAS activity in 3–9-day-old broiler chickens under both high- and low-energy dietary conditions ($P < 0.01$). Dietary energy level alone did not significantly affect hepatic FAS activity ($P > 0.05$), and no significant interaction was observed between corticosterone treatment and dietary energy level ($P > 0.05$).

2.2 Effects of Dietary Energy Level and Corticosterone Treatment on Hepatic FAS Activity in 28–34-Day-Old Broiler Chickens

As shown in Table 5 , corticosterone treatment tended to increase hepatic FAS activity in 28–34-day-old broiler chickens ($P = 0.0512$). Dietary energy level did not significantly affect hepatic FAS activity ($P > 0.05$), and no significant interaction was observed between corticosterone treatment and dietary energy level ($P > 0.05$).

2.3 Effects of Dexamethasone Treatment on Hepatic Triglyceride Content and mRNA Expression of AMPK and SREBP-1 in Broiler Chickens

As shown in Table 6 , dexamethasone treatment significantly increased hepatic triglyceride content compared with the pair-fed group ($P < 0.05$), but showed no significant difference compared with the control group ($P > 0.05$), indicating that the increased triglyceride content was induced by dexamethasone rather than by higher feed intake.

As shown in Table 7 , dexamethasone treatment significantly increased hepatic AMPK mRNA expression compared with the pair-fed group ($P < 0.05$). Hepatic SREBP-1 mRNA expression in the dexamethasone-treated group was significantly higher than in both the control and pair-fed groups ($P < 0.05$).

2.4 Effects of Corticosterone Treatment and Glucose-Supplemented Drinking Water on Hepatic FAS Activity in Broiler Chickens

As shown in Table 8 , neither corticosterone treatment nor glucose supplementation alone significantly affected hepatic FAS activity ($P > 0.05$). However, a significant interaction was observed between corticosterone treatment and glucose supplementation ($P < 0.05$), suggesting that glucose supplementation can alleviate the stress-induced increase in fatty acid synthesis.

Discussion

The stress response represents a process of physiological homeostasis disruption and recovery, dependent on activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis. Glucocorticoids released from the adrenal cortex are essential for maintaining internal stability, with corticosterone being the primary glucocorticoid in poultry. Dexamethasone is a synthetic glucocorticoid with high affinity for glucocorticoid receptors and prolonged tissue retention due to slower plasma clearance [10]. Previous studies have demonstrated that corticosterone and dexamethasone treatments significantly reduce average daily gain and feed conversion ratio while elevating plasma uric acid and glucose levels, confirming the induction of stress responses in broiler chickens [4,7]. The current study was built upon these findings.

The liver plays a crucial role in animal lipid metabolism and is the primary site of fatty acid synthesis in avian species. Hepatic steatosis occurs when lipid metabolism becomes imbalanced—when the rates of lipid uptake and de novo synthesis exceed oxidation and re-esterification, resulting in excess fat accumulation within hepatocytes and systemic metabolic disorders. In this study, corticosterone treatment significantly increased hepatic FAS activity in 3–9-day-old broiler chickens and showed a similar trend in 28–34-day-old birds. Numerous studies have demonstrated that glucocorticoid treatment significantly increases the proportions of abdominal, neck, and leg fat relative to body weight [5-6]. As FAS is the key enzyme for fatty acid synthesis in the liver, its increased activity indicates that corticosterone promotes hepatic de novo fatty acid synthesis, thereby increasing overall fat deposition. Dexamethasone treatment significantly increased hepatic triglyceride content in this study, consistent with previous findings [11-14]. This increase in hepatic triglycerides is directly attributable to elevated FAS activity. Although dietary energy level did not significantly affect hepatic FAS activity in this study, Jiang et al. [5] reported that

high-energy diets increase fat deposition in broiler chickens, possibly through enhanced plasma triglyceride and VLDL levels that promote lipid storage.

Glucose supplementation in drinking water significantly reduced hepatic FAS activity in stressed broiler chickens, demonstrating its stress-alleviating effect. Previous research indicates that glucose treatment does not significantly affect daily gain or body weight but reduces daily feed intake and feed conversion ratio [6], suggesting that glucose supplementation can improve production performance under stress conditions. This may be attributed to glucose serving as an energy source that more effectively meets the body' s energy demands, thereby mitigating stress-induced damage.

To further investigate the mechanisms underlying stress-induced fat deposition, we examined the effects on upstream regulators of lipid synthesis enzymes. Hepatic fatty acid synthesis is regulated by several nuclear transcription factors, among which sterol regulatory element binding proteins (SREBPs) modulate lipid metabolism by controlling the expression of enzymes required for cholesterol, fatty acid, triglyceride, and phospholipid synthesis. The SREBP family includes three members: SREBP-1a, SREBP-1c, and SREBP-2. Hepatic overexpression of SREBP-1c in mice promotes expression of lipogenic genes without affecting cholesterol synthesis-related genes [15]. Foretz et al. [16] found that SREBP-1c overexpression in isolated hepatocytes enhances expression of both fatty acid synthesis genes and glucokinase. This study demonstrated that dexamethasone treatment significantly increased hepatic SREBP-1 mRNA expression, consistent with previous findings that dexamethasone upregulates expression of genes related to fatty acid synthesis [7]. These results suggest that stress-induced increases in fatty acid synthesis are mediated through SREBP-1, which directly or indirectly regulates expression of de novo lipogenic genes, thereby promoting fatty acid synthesis and increasing hepatic triglyceride content.

AMPK is a heterotrimeric complex composed of , , and subunits. AMPK activation inhibits energy-consuming pathways such as protein and fatty acid synthesis while activating energy-producing pathways including glycolysis and fatty acid -oxidation [17]. AMPK activation suppresses acetyl-CoA carboxylase (ACC), the rate-limiting enzyme in fatty acid synthesis, thereby inhibiting lipid synthesis. However, dexamethasone treatment has been shown to increase both ACC activity and mRNA expression [7]. The current study found that dexamethasone activated AMPK expression, which would theoretically reduce fat deposition, yet observed increased lipid accumulation. This discrepancy suggests that other factors, such as SREBP-1, may regulate ACC and FAS activity more dominantly, ultimately promoting fatty acid synthesis. Oxidative stress induced by reactive oxygen species such as H O or NO can activate AMPK [18], and this study demonstrated that dexamethasone treatment increased AMPK expression. This aligns with findings that heat stress increases AMPK activity in cultured hepatocytes after 60 and 120 minutes [19]. Stress-induced AMPK activation may redirect energy toward essential cellular and tissue functions,

thereby reducing stress-related damage.

Conclusions

1. Dietary energy level did not significantly affect hepatic FAS activity in broiler chickens, whereas glucocorticoids increased hepatic FAS activity, promoting triglyceride deposition in the liver.
2. The increase in FAS activity was regulated by the nuclear transcription factor SREBP-1. Stress-induced AMPK activation may redirect energy toward more critical cells and tissues, thereby mitigating stress-induced damage.
3. Glucose supplementation in drinking water significantly reduced hepatic FAS activity in stressed broiler chickens, demonstrating its stress-alleviating effect.

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Note: Figure translations are in progress. See original paper for figures.