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Original Article

Effects of Methionine Deficiency on Nutrient Composition, No, Nos Activity and Mrna Expression in Nf-κb Signal Pathway of the Liver and Kidney in Broiler

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ABSTRACT

The study aimed to investigate the effect of methionine deficiency on conventional nutrient composition, NO and NOS activity in the liver and kidney, and expression of genes related to the NF-κB signal pathway. One hundred 1-day-old broilers were divided into control and methionine deficiency group, and provided with a standard diet. The methionine-deficient group was fed with a methionine-deficient diet for 42 days. Moisture content, protein content, and crude lipid content were determined by freeze drying, the Kjeldahl method, and the ether extraction method. The concentration of NO was measured using the nitrate reductase method, and the activity of NOS was measured using colorimetry. mRNA expression of related genes in the NF-κB signal pathway was detected by reverse transcription-polymerase chain reaction (RT-PCR). The results showed the contents of crude protein and crude lipid in the liver and kidney of the methionine-deficient group were significantly higher than those in the control group ($p<0.05$ or $p<0.01$). There was no significant change in dry matter ($p>0.05$). Methionine deficiency resulted in significantly increased NO concentration and total nitric oxide synthase (TNOS) activities in the liver and kidney ($p<0.01$). In contrast, cNOS and inducible iNOS activities significantly decreased ($p<0.01$). The expression of NF-κB, TNF-α, IFN-γ, IL-1, and IL-6 mRNA, which are related to the NF-κB signal pathway of the methionine-deficient group, were significantly higher than those in the control group ($p<0.05$ or $p<0.01$). It is concluded that long-term feeding diets lacking methionine will lead to changes in nutrient composition and inflammatory injury of the liver and kidney.

INTRODUCTION

Methionine is known as the first limiting amino acid and is also an essential functional amino acid for poultry. In addition to participating in the body's protein synthesis, methionine is also related to cell proliferation, differentiation, apoptosis, and oxidative stress, as well as the regulation of intestinal immune function and the elimination of free radicals (Wu *et al.,* 2017; Ruan *et al.,* 2018; Séité *et al.,* 2018). Methionine side chains are located on the surface of proteins and act as endogenous antioxidants in proteins (Levine *et al.*, 1996). Methionine, which is exposed on the cell surface, could protect other amino acids and act as an antioxidant to prevent cell damage (Salmon *et al.,* 2016). Methionine can react with reactive oxygen species and produce two types of methionine sulfoxide isomers: S-methionine sulfoxide and R-methionine sulfoxide (Moskovitz, 2005). These two isomers can be reduced to methionine under the action of methionine sulfoxide reductase. The decrease of methionine sulfoxide reductase activity is closely related to oxidative stress diseases, and its overexpression is

beneficial to protect the body from oxidative stress damage and maintain the stability of protein spatial conformation (Cabreiro *et al.,* 2006).

The content of nutrients in the body is closely related to life activities. Conventional nutrients include water, crude protein, fat, and dry matter. Among them, water is an important part of the animal body, being the carrier of various nutrients and metabolites, and also an indispensable substance for temperature regulation (Häussinger, 1996; Montain *el al*., 1999). Protein is essential for repairing body tissues and can replace the thermogenic effects of carbohydrates and fats. When the heat supply is insufficient, proteins can decompose in the body and oxidize to release heat energy (Hayamizu, 2017). Excess protein can be stored in the liver, blood, and muscles or be converted into fat by deamination; it will be re-decomposed for heat supply when nutrients are insufficient. Fat is the main source of animal heat energy, the best form of chemical energy storage in the body and an important component of animal tissues. Fat is used as the solvent of liposoluble vitamins in feed to ensure the digestion, absorption, and utilization of liposoluble vitamins by animals (Wei *et al.,* 2020; Vanqa, 2022).

Oxidative stress is a state in which the organism produces more free radicals, or the ability to eliminate them decreases, and the imbalance of oxidation and antioxidant system leads to body injury. A free radical is an atom or group of atoms created in this process that can exist independently and contain unpaired electrons (Laederach *et al.,* 2007). Active nitrogen radicals are free radicals that cause oxidative stress, including nitric oxide (NO), nitrogen dioxide (NO_2), and nitrite peroxide (ONOO-), which are called active nitrogen intermediates (MacMicking *et al.,* 1997). NO is produced by cytokines in the pathological state of the body, which are new messenger molecules that can affect transcription factors and serve as cytotoxic molecules with dual protective and toxic effects. While NO is involved in physiological processes, its catalytic production of nitric oxide synthase (NOS) also plays an important role in many disease processes (Ohshima & Bartsch, 1994; Abd-Ellatef *et al.,* 2017). Active nitrogen intermediates are directly related to NOS. Therefore, the amount of NO is closely related to the activity of NOS, and they also have a certain impact on the oxidative stress state of the body. At present, the expression characteristics of NOS in cerebral ischemia, cerebral oedema, and colitis are widely discussed in important studies (Krieglstein *et al.,* 2001; Zhou *et al.,* 2010), but the influence of methionine deficiency on its activity is rarely studied. As a common signaling pathway, the NF-κB signaling pathway controls many genes related to inflammation and plays an important regulatory role in inflammatory response and apoptosis. The function of the cell during inflammation depends on its signaling response to activation with neighboring cells and a combination of hormones, which particularly has an effect on cytokines through specific receptors (Alharbi *et al.,* 2021; Chaithongyot *et al.,* 2021; Zinatizadeh *et al.,* 2021).

The liver is the main organ of metabolism and synthesis in the body. It plays a vital role in immunity, heat production, and the metabolism of fat, sugar, protein, and other substances (Hoekstra *et al.,* 2013). The kidney is the main place for the excretion of biological metabolites, which can filter blood impurities and maintain the metabolic balance of the body (Kim *et al.,* 2019). As the main organs of body metabolism, they are closely related to the oxygen content in the body, thus affecting the free radicals produced by the oxidative metabolism. At present, the immunological effects of the liver have been deeply studied, and the links between antigen-presenting cells, KC cells, and NK cells in the liver and liver disease have been systematically explained (Gao *et al.,* 2009). At the same time, the research direction on kidney disease mainly focuses on the pathogenesis and treatment of acute kidney injury, chronic kidney disease, and glomerular disease (Liu, 2011). The relationship between NO content and NOS activity in liver and kidney functions remains to be studied for broiler chickens in a methionine-deficient state.

Therefore, this study used broiler chickens to explore the effects of changes in conventional nutrients, NO content, NOS activity, and gene expression related to the NF-κB signaling pathway in the liver and kidney during methionine deficiency. This study aimed to investigate the role of methionine in liver and kidney metabolism, oxidative stress, and inflammatory damage and to provide a theoretical basis for the preparation of feed for the breeding industry.

MATERIALS AND METHODS

Experimental animals and groups

100 1-day-old AA broiler chickens (39±3g) were purchased from Chengdu Wenjiang Zhengda Breeding Co., LTD. The broilers were randomly divided into two groups (control and methionine deficiency groups), with 50 birds in each group. Each group was raised in an experimental cage, and the management method

was consistent with conventional brooding. The experiment lasted for 42 days, as shown in Table 1.

Both the control group and methionine deficiency diets were prepared from corn, soybeans, and wheat purchased from Jining, Shandong, and the feed formula was based on (Wu *et al.,* 2018). The rest of the protein content, energy, vitamin, and trace element additions are added based on the nutritional standards of NRC (1994) for chickens. The control group was feed with the conventional diet, and the methionine deficiency groups were fed with a methionine-deficient diet.

Table 1 – Composition of the diet.

Ingredients[1] (%)	Methionine-deficient diet		Conventional diet	
	Starting stage (1-21 days)	Growth stage (21-42 days)	Starting stage (1-21 days)	Growth stage (21-42 days)
Corn	56.00	59.50	56.00	59.50
Bean pulp	37.00	32.85	37.00	32.85
Plant oil	3.66	4.70	3.66	4.70
$CaCO_3$	0.57	0.50	0.57	0.50
CaHPO4	1.80	1.60	1.80	1.60
NaCl	0.30	0.30	0.30	0.30
$C_5H_{14}clNO$	0.10	0.10	0.10	0.10
DL-Met	0.00	0.00	0.24	0.12
Bentonite	0.24	0.12	0.00	0.00
Microelement[2]	0.33	0.33	0.33	0.33
Nutritional level (%)				
ME, MJ/kg	12.39	12.79	12.39	12.79
P	21.17	19.72	21.17	19.72
Lys	1.19	1.08	1.19	1.08
Met	0.26	0.28	0.50	0.40
Cys	0.62	0.50	0.74	0.62
Ca	0.85	0.77	0.85	0.77
NPP	0.44	0.40	0.44	0.40

[1]Feed and nutritional composition were prepared according to the basic level.

[2]Per kilogram of feed added: vitamin A (all-trans-retinol acetate), 12,500 IU; cholecalciferol, 2,500 IU; vitamin E (all-rac-α-tocopherol acetate), 18.75 IU; vitamin K (menadione Na bisulfate), 5.0 mg; thiamin (thiamin mononitrate), 2.5 mg; riboflavin, 7.5 mg; vitamin B6, 5.0 mg; vitamin B12, 0.0025 mg; pantothenate, 15 mg; niacin, 50 mg; folic acid, 1.25 mg; biotin, 0.12 mg; Cu ($CuSO_4 \times 5H_2O$), 10 mg; Mn ($MnSO_4 \times H_2O$), 100 mg; Zn ($ZnSO_4 \times 7H_2O$), 100 mg; Fe ($FeSO_4 \times 7H_2O$), 100 mg; I (KI), 0.4 mg; Se (Na_2SeO_3), 0.2 mg.

Sample preparation

At 14, 28, and 42 days, 15 chickens were randomly selected in the C group and MD group. They were killed, liver and kidney tissues were taken out, isolated in a sterile environment, and washed with normal saline. Part of the liver and kidney tissue was used for nutrient determination, and part was made into 10% liver and kidney tissue homogenate, which was used to detect NO and NOS indicators. The other tissue was stored in liquid nitrogen and used for mRNA detection.

Detection of the conventional nutrient content

The dry matter, crude protein, and crude fat contents of liver and kidney tissue samples were determined. The freeze-drying method determined the moisture content (GB/T6435-1986). The content of crude protein was determined by the Kjeldahl method (GB/T6432-1994). The ether extraction method (GB/T6433-1994) determined the crude fat content.

Determination of NO concentration: nitrate reductase method

NO is chemically active, and metabolism in the body quickly changes it into NO^{2-} and NO^{3-}, and NO^{2-} is further converted to NO^{3-}. In this method, NO^{3-} was specifically reduced to NO^{2-} by nitrate reductase, and its concentration was determined by color. The NO concentration was determined according to the manufacturer's instructions (Nitric Oxide assay kit, A012-1, purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China), as shown in Table 2.

The experimental results were calculated according to the formula of NO concentration, NO content (μmol/L) = (measured OD value - blank OD value) / (Standard OD value - blank OD value) × Standard concentration (100 μmol/L) × dilution ratio.

Table 2 – Determination of the NO concentration by Nitric reductase method.

Reagent	Blank tube	Standard tube	Measuring tube
Double distilled water (mL)	0.5	0.4	/
100 mol/L standard (mL)	/	0.1	/
Sample (mL)	/	/	0.5
Mixed reagent (mL)	0.4	0.4	0.4
Reagent 3 (mL)	0.2	0.2	0.2
Reagent 4 (mL)	0.1	0.1	0.1
Supernatant (mL)	0.8	0.8	0.8
Chromogenic agent (mL)	0.6	0.6	0.6

Determination of NOS activity: colorimetric method

NOS catalyzes the reaction of L-Arg with oxygen to produce NO, which then forms colored compounds with nucleophilic substances. The absorbance was measured at 530nm wavelength, and the NOS activity could be calculated according to the absorbance. There are two main types of NOS: cNOS and iNOS. The method was conducted according to the Nitric Oxide Synthase (NOS) typed assay kit instructions (A014-1, purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China), and the protocols are shown in Table 3.

The subsequent steps were measuring the corresponding absorption value, using the standard product concentration as the horizontal coordinate and the corresponding OD value as the longitudinal coordinate, drawing the linear regression curve of the standard product, and finally calculating the concentration value of each sample according to the curve equation.

Table 3 – NOS activity was determined by the colorimetric method.

Experimental reagent	TNOS Blank tube	TNOS Measuring tube	iNOS Blank tube	iNOS Measuring tube
Double distilled water (μL)	50+100	100	50	/
Sample (μL)	/	50	/	50
Reagent 6 (μL)	/	/	100	100
Reagent 1 (μL)	200	200	200	200
Reagent 2 (μL)	10	10	10	10
Reagent 3 (μL)	100	100	100	100
Reagent 4 (μL)	100	100	100	100
Reagent 5 (μL)	2000	2000	2000	2000

Detection of NF-κB signaling pathway-related gene mRNA expression

The extraction of total RNA was carried out according to the manufacturer's instructions. After the concentration and purity were determined, the RNA was reverse-transcribed into cDNA using the PrimeScript RTreagent kit (RR037A, Takara) with gDNA Eraser. The gene sequence was obtained from GenBank, and the GAPDH gene was selected as the internal reference gene. The primer sequence was designed using Primer Express 6.0 software and synthesized by Shanghai Bioengineering Co., LTD. The primers are shown in Table 4. The qRT-PCR reactions (25 μl total volume) included 12.5 μl of SYBRR Premix Ex TaqTM II (Takara Bio, Japan), 1μl of forward and 1 μl of reverse primers, 8.5 μl of RNase-free water (Tiangen Biotech, Co., Ltd., Beijing, China), and 1 μl of cDNA. A C1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA, USA) was used to perform qRT-PCR. The thermal cycling conditions were as follows: 95°C for 3 min, followed by 44 cycles of 95°C for 10 s, Tm of the specific primer pair for 30 s, and 95°C for 10 s, followed by 72°C for 10 s. Melting curve analysis displayed only one peak for each PCR product. β-actin was used as the internal reference housekeeping gene. Expression fold changes were calculated using the $2^{-\Delta\Delta CT}$ method.

Table 4 – The primer of NF-κB signaling pathway-related gene.

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
NF-κB	TCAACGCAGGACCTAAAGACAT	GCAGATAGCCAAGTTCAGGATC
TNF-α	ACTCCCAGGTCCTCTTCAAG	TGATGCAGAGAGGAGGTTG
IFN-γ	GCTCCCGATGAACGACTTGA	TGTAAGATGCTGAAGATTCATTCG
IL-1	CAGCCTCAGCGAAGAGACCTT	ACTGTGGTGTGCTCAGAATCC
IL-6	TGGTGATAAATCCCGATGAAG	GGCACTGAAACTCCTGGTCT
GAPDH	GCCATCACAGCCACACAGAAGA	CGGCAGGTCAGGTCAACAACAG

Statistical analysis

The data obtained in this experiment were all collected using the SPSS 20.0 software and analyzed using the sample T-test. The results were expressed as mean ± standard deviation, $p<0.05$ meant significant difference, and $p<0.01$ meant extremely significant difference.

RESULT

Content of routine nutrients in the liver and kidney

As shown in Table 5, the crude protein of the MD group was significantly or extremely significantly lower than that of the C group (p<0.05 or p<0.01), and the fat content in the liver and kidney of the MD group was extremely significantly higher than that of the C group (p<0.01). At the same time, there was no significant change in dry matter.

Results were expressed as mean ± standard deviation, * indicated a significant difference compared with the C group (p<0.05), ** indicated an extremely significant difference compared with the C group (p<0.01). The following tables are represented in the same way.

Table 5 – Content of routine nutrients.

routine nutrients	liver		kidney	
	C group	MD group	C group	MD group
dry matter (DM)	1.19±0.14	0.99±0.11	0.67±0.06	0.68±0.04
crude protein (CP)	24.13±2.57	19.27±1.54**	13.65±3.21	11.65±2.44*
crude fat (EE)	4.01±0.41	6.47±0.29**	5.34±0.34	7.45±0.39**

Changes in NO concentration in the liver and kidney

Compared with the C group, NO concentration in the liver and kidneys of the MD group increased. The concentration of NO in the liver increased significantly at 28 days (p<0.05) and was extremely significant at 42 days (p<0.01). The concentration of NO in the kidney increased extremely significantly at 42 days (p<0.01) (Figure 1).

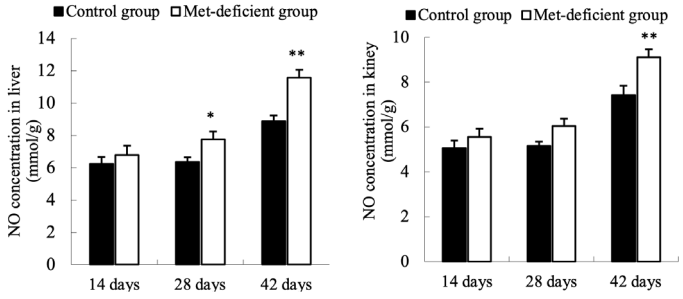

Figure 1 – Changes in NO concentration in the liver and kidney.

Results were expressed as mean ± standard deviation, and * indicated a significant difference compared with the C group (p<0.05). ** indicated an extremely significant difference compared with the C group (p<0.01), the following figures are the same.

Changes in TNOS activity in the liver and kidney

As shown in Figure 2, TNOS activity in the liver and kidney of the MD group was overall increased when compared with the C group. The TNOS activity in the liver increased significantly at 14 days (p<0.05), and was extremely significant at 28 days and 42 days (p<0.01). The TNOS activity in the kidney increased significantly at 28 days and 42 days (p<0.05).

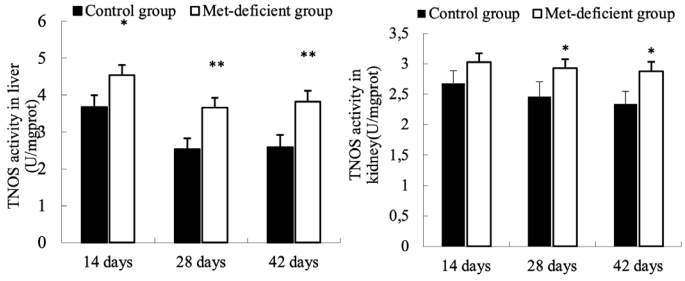

Figure 2 – Changes in TNOS activity in the liver and kidney.

Changes in cNOS activity in the liver and kidney

cNOS activity in the liver and kidneys of the MD group decreased when compared with the C group. The cNOS activity in the liver decreased extremely significantly at 28 days and 42 days (p<0.01). The cNOS activity in the kidney decreased significantly at 28 days (p<0.05) and extremely significantly at 42 days (p<0.01), which is shown in Figure 3.

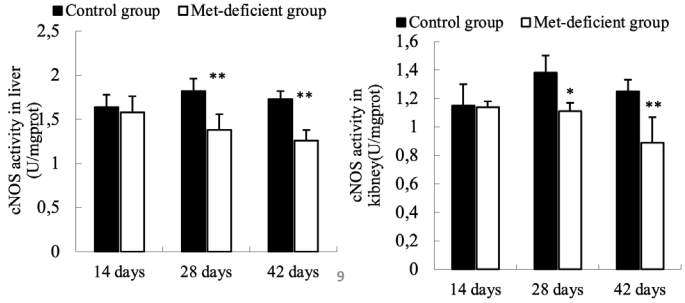

Figure 3 – Changes in cNOS activity in the liver and kidney.

Changes in iNOS activity in the liver and kidney

As shown in Figure 4, compared with the C group, iNOS activity in the liver decreased significantly at 28

days (p<0.05), and extremely significant at 42 days (p<0.01). The iNOS activity in the kidney decreased extremely significantly at 28 days and 42 days (p<0.01).

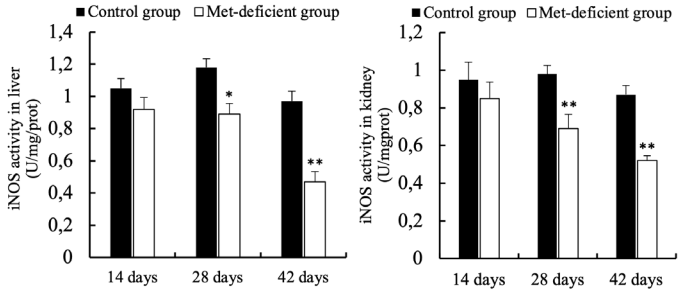

Figure 4 – Changes in iNOS activity in the liver and kidney.

Gene expression associated with the NF-κB signaling pathway in the liver and kidney

The mRNA expressions of NF-κB signaling pathway-related genes in the liver and kidney (such as NF-κB, TNF-α, IFN-γ, IL-1, and IL-6) in the MD group were significantly or extremely significantly higher than those of the C group (p< 0.05 or p<0.01) (Table 6).

Table 6 – Gene expression associated with the NF-κB signaling pathway.

related genes	liver		kidney	
	C group	MD group	C group	MD group
NF-κB	1.00±0.00	1.65±0.43**	1.00±0.00	2.05±0.36**
TNF-α	1.00±0.00	2.13±0.35**	1.00±0.00	1.83±0.23*
IFN-γ	1.00±0.00	1.98±0.26*	1.00±0.00	1.78±0.29*
IL-1	1.00±0.00	1.68±0.33*	1.00±0.00	2.18±0.37**
IL-6	1.00±0.00	2.15±0.44**	1.00±0.00	1.95±0.36**

DISCUSSION

This study determined the dry matter, crude fat and crude protein, NO concentration, TNOS activity (including iNOS and cNOS), and gene expression associated with the NF-κB signaling pathway in the liver and kidney. The results showed that the content of crude fat and crude protein, NO concentration, and TNOS activity all increased in the liver and kidney when broilers were treated with a methionine deficiency diet. Meanwhile, the mRNA expressions of NF-κB signaling pathway-related genes such as NF-κB, TNF-α, IFN-γ, IL-1, and IL-6 were all significantly or extremely significantly higher than that of the control group. These results indicate that metabolic disorders occurred in the liver and kidney, with varying degrees of injuries.

In detecting the conventional nutrient content, we found that the crude fat and protein contents in the liver and kidney of the met-deficient group were significantly higher than those of the control group. It was speculated that the methionine deficiency changed the intensity of fat metabolism in the liver and kidney. Methionine can provide methyl through its metabolite s-adenosine methionine, which can work as a methyl donor to promote the synthesis of carnitine, betaine, and choline (Wang *et al.,* 2021). These substances can promote the synthesis of apolipoprotein, accelerate the outward transport of fat, promote the oxidation of fat, and reduce the activity of fat synthase, thus reducing fat accumulation (James *et al.,* 2002). Methionine can also regulate the concentration of related enzymes in fat metabolism by up-regulating the expression levels of lipolysis-related genes and down-regulating the expression levels of fat synthesis-related genes to regulate the lipid metabolic response (Wang *et al.,* 2022). On the contrary, methionine deficiency can significantly down-regulate the expression of lipolysis enzyme, up-regulate the expression of fatty acid synthase, promote fat synthesis, and reduce lipolysis (Aissa *et al.,* 2014). In addition, methionine deficiency can induce the synthesis of homocysteine (Tang *et al.,* 2010) and increase the level of homocysteine, leading to oxidative stress of the endoplasmic reticulum and promoting the conversion of glucose into lipid substances. Elevated homocysteine levels also affect vascular permeability and inhibit fat transfer in the liver and kidney, thus leading to fat accumulation (Hansrani & Stansby, 2008). Moreover, methionine is one of the raw materials for protein synthesis (Asante *et al.,* 2019), which can initiate protein translation and stabilize protein structure (Aledo, 2019). Therefore, a lack of methionine can inhibit mRNA translation and lead to protein synthesis obstruction, thus affecting protein stability and reducing protein levels in the liver and kidney.

NOS is an important enzyme that regulates NO synthesis, and the stronger its activity, the faster the rate of NO synthesis (Carmignani *et al.,* 2000). NO is a cytokine with dual effects under physiological and pathological conditions in the body, which is unstable and easily oxidized. In small amounts, it can serve as an antioxidant and anti-apoptotic substance (Nagahawatta *et al.,* 2022). On the contrary, if the synthesis of NO significantly increases beyond the range of controllable levels in the body, it will lead to injuries to the body. It has been shown that in pathological conditions, cNOS level would increase, iNOS would be activated and expressed in large quantities, and then continuously catalyze the production of a large number of NO, resulting in the production of various active nitrogen groups (RNS) (McCall *et al.,* 2009; Ascherio *et al.,* 2010). Therefore, due to a lack of methionine,

the body does not clear excess free radicals promptly, which stimulates oxidative stress and disrupts the balance of the body, ultimately leading to an increase in the activity of NOS and an acceleration of NO synthesis. The long-term high NO content in the liver and kidney has a significant toxic effect on these two metabolic organs. These results suggest that adding an appropriate amount of methionine to control NOS activity and NO synthesis can protect the liver and kidney. In the studies of NOS or its related disease, it has been pointed out that only a very small amount of NOS expression is expressed in normal liver and kidney tissues, while in some diseases the cNOS and iNOS expression are increased, which are then related to the occurrence of cancer tissues (Gardner *et al.,* 1998 Marchetti *et al.,* 2005; Cook, 2006; Kawanishi *et al.,* 2006). In the present study, we found that the activities of cNOS and iNOS in both the liver and kidney show a decreasing trend, which is contrary to the conclusion above. According to the pertinent studies (Chang *et al.,* 2004; Vo *et al.,* 2005), under high concentrations of NO, the binding activity of NF-κB and DNA is inhibited, thus down-regulating the expression of iNOS, which prevents the occurrence of inflammation to a certain extent. In our study, when methionine was lacking, the concentration of NO increased significantly, and the level of NF-κB and the activity of iNOS decreased. It is speculated that the organism may try to maintain the homeostasis of its internal environment and resist the inflammatory response, which leads to a high concentration of NO in the body. The level of NF-κB is also adjusted by negative feedback, and the expression of iNOS is inhibited. In addition, (Scicinski *et al.,* 2015) found that cNOS played a small role in the synthesis of NO, and the expression of cNOS could protect the body under normal circumstances. However, in this study, the synthesis of cNOS was lower than the normal value, and the activity of cNOS is related to Ca^{2+}, so it is speculated that methionine deficiency may have caused the decrease in the activity of Ca^{2+} and calmodulin (CAM), thus reducing the activity of cNOS. If cNOS is at a low level for a long time, its protective function in the body will be reduced, and tissues or organs will be injured.

NF-κB signaling pathway plays an important role in inflammatory response, regulating the transcription process of a variety of cellular inflammatory factors such as NF-κB, TNF-α, IFN-γ, IL-1, IL-6 genes. An abnormal NF-κB signaling pathway will bring adverse effects on the body. The inflammatory factors in the methionine-deficient group were higher than those in the control group, suggesting that methionine could regulate the NF-κB signaling pathway. Methionine can regulate the expression balance of NF-κB/IκBα through its metabolite s-adenosine methionine (SAM). In normal cells, NF-κB binds to its inhibitor IκBα protein in an inactive state. When stimulated by external signals, IκBα is degraded, activating the expression of NF-κB, thereby promoting the release of inflammatory factors, and activating the expression of IκBα gene synthesis, inhibiting NF-κB activity (Irrera *et al.,* 2020). Methionine deficiency can lead to an imbalance of NF-κB/IκBα, resulting in an abnormal NF-κB signaling pathway and liver cell damage. Methionine also regulates the expression of inflammatory cytokines through SAM, which, as an important methyl donor, is an important source of methylated DNA (Martínez *et al.,* 2017). SAM can increase the methylation degree of inflammatory factors such as IL-6 and TNF-α and inhibit the expression of inflammatory factor genes, thus inhibiting the inflammatory response (Shen *et al.,* 2017). Lack of methionine reduces SAM levels and leads to reduced methylation degree of inflammatory factor genes, thus promoting the expression of inflammatory factors, aggravating inflammatory response, inducing cell damage, destroying tissues and organs of the body, and seriously affecting body performance.

In conclusion, methionine deficiency can hurt liver and kidney function in broilers. The results in this study demonstrated that, in a state of methionine deficiency, crude protein and crude fat contents, NO concentration, TNOS, iNOS and cNOS activities in the liver and kidney were all changed through the NF-κB signaling pathway.

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