Chapter

Occurrence of Hyperhomocysteinemia in Broilers and Reduction of Its Harmful Effects with Betaine- and Berberine-Supplemented Diets

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Abstract

Homocysteine is a metabolic intermediate in the methionine-cysteine conversion. High level of homocysteine in blood leads to changes in methylation pathways and consequently in transcriptional activation; therefore, it can disrupt gene expression. This chapter presents the biochemical pathways of the transformation of homocysteine in broilers and demonstrates the beneficial effects of certain bioactive feed additives (betaine and berberine) to health-related and production problems caused by the accumulation of homocysteine. Based on recent scientific findings, the following conclusions have been drawn: Hyperhomocysteinosis has received little attention in the field of avian physiology research. Currently used feed additives, such as betaine, potentially decrease circulating homocysteine, but support only one of the pathways responsible for homocysteine decomposition. Various phytonutrients may be suitable owing to their pleiotropic bioactive components, such as berberine. It can potentially maintain redox homeostasis in animals and modulate immune responses and therefore may be able to provide for liver protective functions. Additionally, it can encourage healthy tissue to express enzymes that are responsible for the degradation of homocysteine. Further studies are recommended to investigate how effectively berberine can reduce the incidence of hyperhomocysteinemia in broilers and whether it is necessary to use feed supplements throughout the life cycles of birds.

Keywords: broiler, hyperhomocysteinemia, metabolic disease, performance, betaine, berberine

1. Introduction

Broiler performance and meat quality are influenced by several factors, such as genotype, chick quality, feed consumption and nutrient supply, water supply, vaccination and health status, as well as conditions associated with housing, including stocking density, temperature and ventilation, lighting, litter quality and biosecurity [1]. All these factors also have important roles to play in profitable broiler meat production. Of these, the health status of birds has recently come to the focus of growing professional attention.

According to the calculations of Oxford Analytica (2023), in 2018 the global poultry production dropped by 2.8 million tons due to diseases [2]. During the same time, global egg production decreased by 3 million tons as a result of diseases, adding up to a loss of $5.6 billion in revenues.

Diseases occurring in poultry farming can be categorized in several ways [3]. One way of categorization is when diseases are classified by their common, such as genetic, mechanical, toxic and nutritional, causes. In another form of categorization, diseases are grouped as infectious and non-infectious diseases. Infectious diseases are caused by bacteria, viruses or fungi, whereas parasitic diseases are induced by external parasites, such as protozoa, worms, mites and lice. Unlike infectious diseases, non-infectious diseases originate from non-pathogenic organisms, and consequently they cannot be transferred from one animal to another.

Non-infectious diseases are caused by factors, such as genetics, malnutrition, environment, housing, etc. This group of diseases encompasses numerous illnesses, with the most common metabolic diseases in poultry being avian gout, dysbacteriosis, cage layer fatigue, fatty liver syndrome (FLS), fatty liver and kidney syndrome (FLKS), toxic fat syndrome (chick edema disease), ascites (AS), sudden death syndrome and spiking mortality syndrome [4]. In the poultry industry, metabolic problems have intensified in the past few decades, as the genetic potential of poultry for growth and feed efficiency has rapidly improved. The associated data further underpin that metabolic diseases are particularly common in broilers [4, 5].

In general, metabolic disorders can be chronic, impacting a relatively small percentage of the flock, or acute, meaning they affect a larger proportion of birds, while their incidence is often sporadic [6]. Metabolic diseases occurring in the cardiovascular system are responsible for a significant part of mortality in poultry stocks. The metabolic illnesses of the musculoskeletal system account for lower mortality rates, still they generally lead to slower growth and lameness [4, 7].

It has been revealed in human patients that with vascular diseases, plasma homocysteine (Hcy) level tends to be much higher than normal [8]. When homocysteine concentration in the blood is over the normal value, the resulting condition is called hyperhomocysteinemia. According to a review by Jakubowski and Son and Lewis, hyperhomocysteinemia in humans has been associated with cardiovascular, cerebrovascular and thromboembolic conditions, as well as hip fracture, osteoporosis, chronic kidney disease (CDK), hypothyroidism and mental problems, such as cognitive decline and Alzheimer's disease (AD) [9, 10]. It should be noted that hyperhomocysteinemia is also known in broilers, but this disorder is less documented and explored than in humans.

The central role of the transsulfuration pathway (TSP) in the development of non-infectious metabolic diseases has been demonstrated by research in recent years [11]. While *in vivo* experiments have been mostly performed with rodents (mice, rats),

genomic studies have shown that it works through a conserved pathway that is likely to be present in all vertebrates, consequently for poultry, too. The key enzymes (cystathionine-γ-lyase (CSE) [EC 4.4.1.1] and cystathionine-β-synthase (CBS) [EC 4.2.1.22]) have been identified not only in rodents, but also in pigs and humans. The processes catalyzed by enzymes involved in the degradation of homocysteine are the only pathway for endogenous hydrogen sulfide (H_2S) production. The absence of these processes leads to the emergence of abiotic stress. The accumulation of homocysteine reduces the SAM- (S-adenosylmethionine) dependent transmethylase activity, leading to hypomethylation, deoxyribonucleic acid (DNA) methylation and histone acetylation. These processes can disrupt (suppress or activate) gene expression, and thus compromise basic metabolic functions. Increased plasma homocysteine levels are negatively correlated with the H_2S concentrations in cells or tissues. As a consequence, inflammatory processes are triggered, which can result in the inflammation of the muscles, as well as plaque formation in the cardiovascular system or the emergence of respiratory diseases. Even before the symptoms of metabolic diseases can be observed, metabolic changes may adversely affect the performance of birds (broilers) and the profitability of meat production.

To support the transsulfuration pathway, natural feed additives can be used for their potential to bring down pathologically high homocysteine levels in the plasma. However, these additives can be used efficiently in broiler nutrition only if the biochemical pathway of homocysteine formation is precisely known, and it is also clear how to modify the pathway of transformation of homocysteine into cysteine (Cys) or methionine (Met) with the use of the particular feed additives in the broilers [12].

By way of this review, we want to provide guidance to nutrition professionals in this intricate field of expertise by systemizing and evaluating the latest scientific findings. Therefore, the purpose of this chapter is to describe the biochemical pathways of the transformation of homocysteine into methionine or cysteine in broilers alongside the health-related and production problems that are caused by the large-scale accumulation of homocysteine. A further goal is to demonstrate the beneficial effects of some bioactive feed additives (betaine and berberine (BBR)) on the incidence of hyperhomocysteinemia and the performance of birds.

2. Systematic review methodology

The keywords used to research and collect the literature for this critical review included: "homocysteine," "transsulfuration pathway," "broiler," "methionine," "cysteine," "betaine" and "berberine," either individually or in a combination thereof. Databases searched included PubMed, ScienceDirect, Google Scholar, Scopus and Web of Science. The publication period beginning from 2001 was chosen as a starting point, with the recency of research as the prime focus for the inclusion of the majority of the studies. Subsequently, searching was enlarged from 1990 in order to study the wider perspective. Close to 200 journal articles satisfied the criteria, and after review, 109 were shortlisted for inclusion in this review.

3. Vital roles of methionine and cysteine in broiler nutrition

Homocysteine is a metabolic intermediate in the methionine-cysteine conversion. Therefore, prior to discussing the problem associated with hyperhomocysteinemia,

it seems to be useful to overview the role of the sulfur-containing amino acids concerned in poultry feeding.

3.1 Methionine

An essential amino acid, methionine (Met) plays several important roles in bird metabolism, such as protein synthesis and feather development; furthermore, it serves as a methyl group and sulfur donor for methylation and transsulfuration reactions, respectively. Moreover, it is a precursor of some key intermediates (cysteine [Cys], carnitine, S-adenosylmethionine, glutathione (GSH), taurine, etc.) in metabolic pathways [13–15]. Methionine is the principal donor of methyl radical in the body [16].

This amino acid is also considered as the first limiting amino acid for optimal growth of poultry on corn-soy diets, and therefore has particular significance [14]. Methionine has an essential role in energy production and boosts the livability, performance and feed efficiency in poultry. Beyond its fundamental importance in protein synthesis, methionine also exerts functional roles through its antioxidant capacities [17, 18]. It is also well known that methionine supplementation improves immune response through its direct effects on protein synthesis and breakdown, as well as owing to its indirect effects on the various derivatives of methionine [17, 19]. Methionine deficiency directly and negatively impacts broiler production. In such cases, weight gain, feed efficiency and protein content in the carcass are all reduced. When methionine deficiency is not drastic, feed intake slightly increases, and it contributes to the generation of extra energy in body. However, it results in more massive accumulation of body fat [20].

Amino acids, including methionine and cysteine, are mainly absorbed through the small intestine. In association with the amino acid demand of broilers, the amino acid content of feed ingredients and compound feeds are expressed as digestible amino acids. The amino acid content of diets should be determined in relation to the lysine (Lys) content (100%) with reliance on the ideal protein concept for broilers. That ensures the precise supply of amino acids and the maximum efficiency of their utilization for the smallest possible metabolic load.

The methionine content of diets is usually supplemented with industrially produced methionine. The common sources of methionine in broiler diets are DL-Met (a racemic mixture of the D- and L-isomers of Met in equal proportions), the hydroxy analogue of methionine calcium salt and the hydroxy analogue of methionine to bring the sulfur-containing amino acid content of diets into equilibrium so as to meet the needs of birds [21, 22]. Methionine sources are used in two distinct forms: powder and liquid. The latter one is a DL-Methionine hydroxy analogue-free acid (MHA-FA, containing 88% of the active substance) [23]. In general, conventional methionine products contain 50% of both D- and L-Met, but due to the fact that the animal body can utilize only L-amino acids in protein synthesis, a specific methionine product containing only L-Met has been developed. According to the product specifications, the methionine produced by the industry contains 98.5% L-Met, 0.5% water (loss on drying) and 0.1% ash [17]. However, it seems that the pure L-Met is not that important in practical feeding. In fact, normal metabolism features an efficient process of conversion of D-Methionine into L-Methionine. This two-step reaction encompasses the action of amino acid oxidase to remove the amine group from D-Met, which results in the generation of α-keto-methionine, to which transaminase attaches an amine group to form L-Methionine in the second step [24].

The methionine demand of broilers is influenced by various factors, such as growing phase, the type of production, sex and breed [17]. In their outstanding review, Rehman et al. conclude that different levels of methionine in poultry diets have been reported by researchers, ranging from 0.3% to 1.2% during the initial period and 0.3% to 0.9% in the growth period of poultry. Results also showed that more edible meat yield could be obtained by supplementing Met + Cyst at the rate of 80% of the digestible lysine [25].

Based on the results of Rehman et al., it can be concluded that if DL-Met and L-Met are included in diets at standard levels, they are equally effective as sources of methionine for broilers [25].

A study by Çenesiz et al. demonstrated that with methionine-deficient diet, the addition of this amino acid significantly improved the growth performance and carcass yield of broilers. Similarly to Rehman et al., this study suggested that no significant differences in growth performance and carcass quality parameters could be anticipated when broiler diets were supplemented with DL-Met or L-Met [22, 25].

Another study by Macelline et al. indicated an optimum methionine-to-lysine ratio of 50.3, which was somewhat higher than standard recommendations [26].

3.2 Cysteine

Even though there are four common sulfur-containing amino acids (methionine, cysteine, homocysteine and taurine), only methionine and cysteine are incorporated into proteins [27].

Cysteine is a semi-essential sulfur-containing amino acid. If the methionine-to-cysteine ratio is imbalanced, it causes depression in the growth of birds [28]. Methionine can be converted irreversibly to cysteine by transsulfuration. Therefore, in the feed tables demands for these amino acids are usually added up as methionine and cysteine requirements [20].

Cysteine plays an important role in a number of physiological processes. In addition to methionine, cysteine can improve intestinal histomorphometric indices of broilers [29], leading to an increase in the absorption of nutrients. Cysteine can prevent oxidative damage [30].

Baker points out that consuming more L-isomer of Cysteine (L-Cysteine) than necessary triggers acute metabolic acidosis in chickens [31]. In another study, Baker suggests that even at much larger doses, none of the known amino acids produce the same degree of lethality as excess L-Cysteine [32].

Cysteine, like other amino acids, is primarily absorbed through the small intestine.

Even if the physiological concentration of cysteine is adequate, lots of cells cover at least 47% of their cysteine demand via the transsulfuration pathway [33].

The ideal amino acid ratio relative to Lys was calculated to be 75% Met + Cys on a true fecal digestible basis [34]. Nearly a similar value was estimated by Baker and Han.

In most cases, broiler feed needs to be supplemented with methionine to ensure an adequate supply of Met + Cys to birds [16].

Compound feeds for broilers are formulated to meet methionine + cystine demands based on the assumption that dietary methionine is converted into cysteine [35].

In the literature, values for the Met + Cys demands of broilers vary broadly.

This can be attributed to several reasons, including differences in breeds, diet compositions, circumstances of animal studies, as well as differences in the bioavailability of methionine products used in the study; also, in many cases to the small

number of animals per treatment and consequently, the large standard deviation of the mean values of treatments, etc.

Goulart et al. recommended 0.873, 0.755, 0.748 and 0.661% of digestible methionine + cystine in the diet for the pre-initial, initial, growing and final phases, respectively [20]. On the other hand, Millecam et al. found that the optimal methionine + cysteine levels for broilers are 0.69, 0.66 and 0.62% in the starter, grower and finisher phases, respectively. At present, it appears that more extensive research is needed to clarify the Met + Cys requirement for broilers [36].

In the light of the foregoing, it can be concluded that the metabolism of Met and Cys is closely interrelated, and both amino acids play important roles in the protein metabolism of broilers.

4. Methionine, cysteine and Hcy metabolism

Homocysteine is a sulfur-containing amino acid, a metabolic intermediate in the Met-Cys conversion. Based on stoichiometry, its circulating concentration is regulated by two key pathways: remethylation and transsulfuration (**Figure 1**). There is a third pathway, because Hcy can be converted to homocysteine thiolactone (HTL), but that conversion is active only when Hcy concentration is high. The following section discusses these different pathways.

4.1 Homocysteine transformation in the remethylation pathway

In the methionine cycle, homocysteine is produced from methionine in two steps. Briefly, after methionine adenosylation to S-adenosyl-L-Methionine (SAM/AdoMet) methyltransferase takes over the methyl group (DNA, ribonucleic acid (RNA), protein, phospholipid) to different acceptor molecules yielding S-adenosylhomocysteine (SAH/AdoHyc) as a by-product. The S-adenosylhomocysteine hydrolase cuts off the adenosine part and forms homocysteine. On the other hand, homocysteine

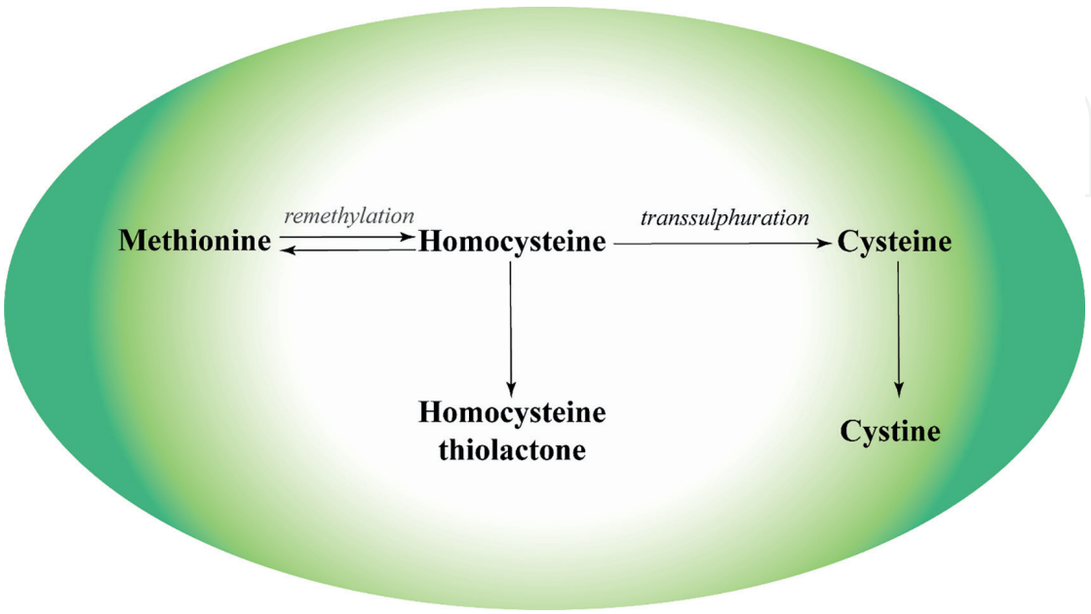

Figure 1.
Formation of homocysteine in the methionine-cystine pathway.

transforms into methionine with the methyl group from 5-*N*-methyl-tetrahydrofolate in a reaction catalyzed by vitamin B_{12}-dependent methionine synthase (**Figure 2**) [37]. As discussed by Vizzardi et al., high-level Hcy is accompanied by a reduced methylation potential, and therefore it compromises the Hcy → Met conversion, whereas folate and vitamin B_{12} tend to increase this potential. Although in the methyl group the main source of homocysteine remethylation into methionine is 5-*N*-methyl-tetrahydrofolate, betaine and choline also act as methyl donor molecules. The betaine pathway mostly occurs in the liver and is catalyzed by Hcy-methyltransferase [38].

4.2 Homocysteine transformation in the transsulfuration pathway

The transsulfuration pathway (TSP) accounts for the transformation of homocysteine into cysteine through cystathionine. It has a key role in sulfur metabolism and the redox environment of cells. TSP is the only way of cysteine biosynthesis in mammals and birds [39]. The first step is catalyzed by the vitamin B_6-dependent cystathionine-β-synthase (CBS) enzyme; homocysteine and serine are involved as substrates in the condensation reaction that produce cystathionine. The second step is a hydrolysis reaction that is catalyzed by the vitamin B_6-dependent cystathionine-γ-lyase (CSE). The substrate here is cystathionine, with cysteine and α-ketobutyrate (αKB) forming during the last step [37].

It should be noted, however, that in addition to remethylation and transsulfuration pathways homocysteine can undergo cyclization to form homocysteine thiolactone. This thioester is the toxic intermediate of homocysteine, as shown in **Figure 3**.

The role of the transsulfuration pathway in metabolic progresses is underlined by the fact that the essential H_2S signaling molecule is synthesized in this pathway. It has come in the focus of the scientific interests during recent years, because it has a principal physiological role, though inorganic H_2S smells like addled egg, and in

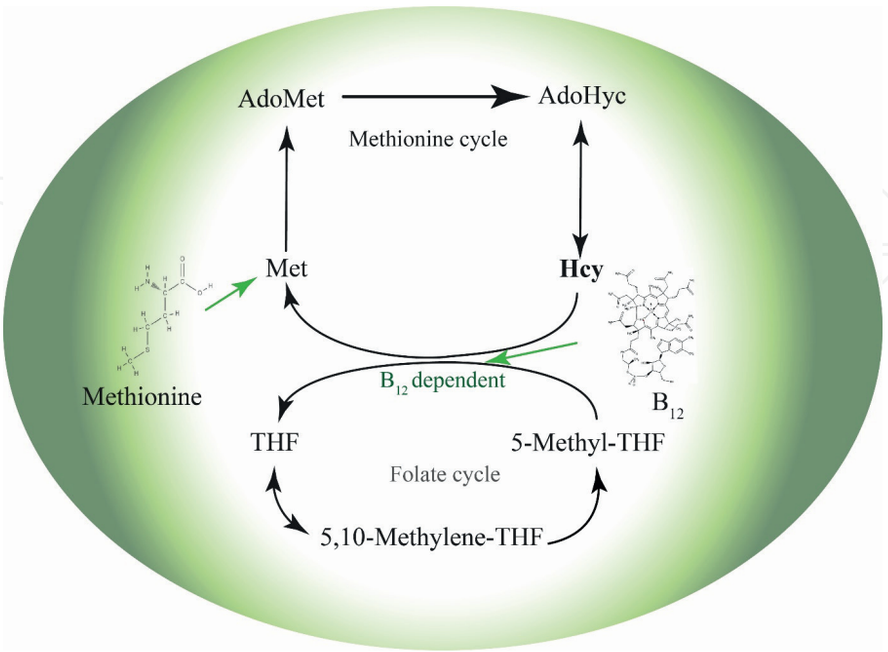

Figure 2.
Remethylation pathway of homocysteine and its dependency on the folate cycle. Abbreviations in the figure: tetrahydrofolate (THF); 5,10 methylene-tetrahydrofolate (5,10 methylene-THF); methionine (Met); S-adenosylmethionine (AdoMet); S-adenosylhomocysteine (AdoHcy); homocysteine (Hcy).

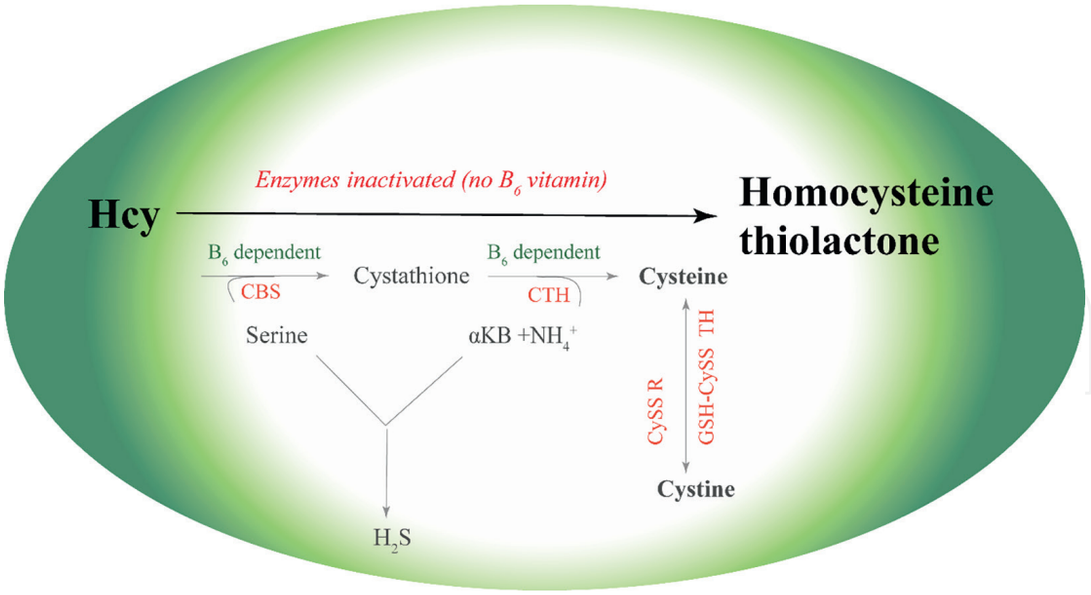

Figure 3.
Transsulfuration pathway and the formation of thiolactone in the absence or presence of vitamin B_6. Abbreviations in the figure: cystathionine-β-synthase (CBS); cystathione-γ-lyase (CSE); α-ketobutarate (αKB); ammonium ion (NH_4^+); cystine reductase (Cyss R); glutathione-cystine transhydrogenase (GSH-Cyss TH).

larger concentrations it is a toxic gas. The endogenous H_2S molecule was proven to be a vasoactive, cytoprotective, anti-inflammatory and antioxidant component. As a gas transmitter, it is able to diffuse through the cell membrane. Endogenous H_2S forms through enzymatic and non-enzymatic pathways in vertebrates. The former process is cytosolic and calls for mitochondrial enzymes: cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), 3-mercaptopyruvate sulfurtransferase (3-MST) and cysteine aminotransferase (CAT), by using L-Cysteine or homocysteine.

Hydrogen sulfide is produced by the enzymatic effect of CBS during the metabolism of Hcy-Cys disulfide into cystathionine. As an alternative way, H_2S also forms from cystine (disulfide of cysteine) as a result of the enzymatic effect of the CSE with ammonia (NH_3) release, while thiocysteine and pyruvate are produced. Thiocysteine splits into H_2S and Cys. Similarly to NO and carbon monoxide (CO), H_2S is a gaseous, fat-soluble messenger molecule. These three gas molecules constitute an unstable biological mediator family called gas transmitters. These findings point out that these molecules are enzymatically controlled and endogenously produced under normal physiological conditions in mammals, and therefore the biological roles of H_2S, NO and CO should be re-evaluated [37].

4.3 The importance of thiolactone

As mentioned above, the accumulated Hcy can easily transform into thiolactone, which is the reactive anhydride of homocysteine. Thioester homocysteine thiolactone (HTL) forms as a by-product of protein biosynthesis. The outcome of the process is that due to the structural similarities between Hcy and Met, during protein biosynthesis, methionyl-transfer ribonucleic acid (tRNA) synthetase builds into Hcy, instead of Met. Owing to repair mechanisms, homocysteine thiolactone is created. HTL forms isopeptide bonds with the residues of lysine (Lys). These isopeptide bonds lead to damaged or altered protein functions, and bring about pathophysiological effects, including autoimmune and intensified thrombosis activity. The HTL

reaction with serum proteins induces the production of new protein antigens and autoimmune antibodies, which escalates inflammatory processes in the human and animal body.

Autoantibodies against the Nε-Hcy-Lys-protein complex can be found in the human plasma, and they positively correlate with the plasma total Hcy. Protein modulation mediated by HTL changes the protein sequence, which probably compromises the protein folding. Changes in the protein structure result in new interactions that influence cell physiology. Furthermore, HTL interacts with low-density lipoproteins (LDLs), which results in aggregation, increased density and vascular macrophage uptake, and also creates foam cells. Mammals, including humans, are able to eliminate the production of thiolactone through two distinct mechanisms. A high-density lipoprotein (HDL)-associated enzyme, Hcy-thiolactonase/paraoxonase-1 is capable of hydrolyzing Hcy-thiolactone both in the serum and intracellularly. Due to another mechanism, Hcy-thiolactone is decomposed by clearance in the kidney [9, 40].

In summary, homocysteine induces predisposition to metabolic dysfunctions, especially when its metabolite, homocysteine thiolactone, is formed at high levels. It has been reported in human studies that other N-homocysteinylated proteins also cause alteration in cell metabolism and trigger other mild or severe dysfunctions. N-homocysteinylated proteins can be cytotoxic and activate immune functions by forming immunoglobulin G (IgG) antibodies against N-homocysteinylated proteins to fight atherothrombosis [41, 42]. Homocysteine counteracts antioxidant enzymes and reduces their activity [43], and therefore hyperhomocysteinemia may deteriorate meat quality traits, too. The compromised antioxidant capacity predisposes to higher drip loss and may as well influence the color of meat.

5. Some metabolic diseases caused by high plasma homocysteine concentration (hyperhomocysteinemia)

5.1 Pathological conditions associated with hyperhomocysteinosis

The concentration of homocysteine, one of the cysteine metabolites, can potentially rise abnormally in the animal body, which may cause metabolic disorders; in severe cases, it may even lead to the death of the animal. In humans, the elevated concentration of circulating homocysteine may cause a number of cardiovascular diseases, such as heart attack, stroke, atherosclerosis and atherothrombosis [44, 45]. Therefore, the maintenance of plasma homocysteine balance in human patients can play an important role in the prevention of morbidity and mortality caused by cardiovascular diseases [46]. The normal level of Hcy in human adult plasma ranges from 5 to 15 μmol/L. In clinical routine, three hyperhomocysteinemia categories are distinguished: mild (15–30 μmol/L), moderate (30–100 μmol/L) and severe (>100 μmol/L). Hcy can be found in the circulation in its free form (approximately 1%), in disulfide, in a mixed form in disulfide and as bound to proteins, and therefore any assessment of real Hcy calls for the proper consideration of all these forms of Hys. The expression "total Hcy" (tHcy) is used for free Hcy, i.e., the quantity of Hcy from the reduction of disulfides together with the released, protein-bound Hcy from protein hydrolysis [47].

Despite the fact that elevated plasma homocysteine levels in humans have long been considered as a risk factor of cardiovascular diseases [44], this metabolic condition still has not received sufficient attention in poultry production. Unlike in the case

of humans, the underlying reason is that in relation to livestock and poultry there is very limited information on the normal and pathological levels of Hcy. Nevertheless, it should be noted that in a few studies high levels of homocysteine have been revealed in specific metabolic disorders, such as sudden death syndrome, ascites, tibial dyschondroplasia and some myopathies that significantly compromise the profitability of poultry production. However, the number of poultry-related studies where plasma Hcy has been measured is extremely limited. For this reason, we consider it important to give a brief overview of some of the diseases that are caused by hyperhomocysteinemia and can also occur in the broiler industry.

5.2 Ascites and vascular diseases

In the chronic phase of ascites (AS), non-inflammatory transudate accumulates in one or more peritoneal cavities. The most common cause is the elevated hydraulic pressure originating right ventricular failure. In different phases, hepatic fibrosis is accompanied by these medical conditions. In the past, this health problem was noted only in the case of birds kept in high mountains, but nowadays it is a regular consequence of oxygen supply failure during rapid growth [48]. Wang et al. showed that broilers with cold-induced ascites suffered from severe liver failure, too [49].

In the course of AS, right ventricular failure triggers ventricular tachycardia and subsequently ventricular fibrillation, which are common consequences of coronary artery calcification. In atherosclerosis, vessel walls thicken, and fibrous caps emerge. If they turn into ulcers, endothelium gives rise to a coagulation cascade. The blood clot gets stuck in the vascular system or in the heart. The associated cause can be hyperlipidemia, which can induce liver failure and non-alcoholic fatty liver disease (NAFLD). Samuels found that the homocysteine concentration was three times higher in broilers with ascites than in healthy animals [50].

5.3 Skeletal disorders and myopathies

The tibial dyschondroplasia is a very common problem in intensively farmed, meat-producing poultry flocks. It is generally caused by the low mineralization of the tibia, the elevated calcium-phosphorus (Ca-P) ratio in the feed, fast primary osteon formation on the periosteal surface, as well as the insufficient filling of channels with osteoblasts [51]. Waqas et al. showed that during the development of the disease, alkaline phosphatase (ALP) and alanine aminotransferase (ALT) concentrations were rising in the plasma [52]. Orth et al. studied cysteine and homocysteine concentrations, and found that the homocysteine concentration of the plasma significantly rose in this musculoskeletal disorder [53].

Wooden breast syndrome (myopathy) is a systemic disease. It affects *pectoralis major* muscle on which pale, rib-like bulges and hemorrhages appear. Its development is associated with the abnormal accumulation of endomysial and perimysial connective tissues, with its consequences including fibrosis, hypoxia, oxidative stress and inflammatory responses to pathological conditions. There exists a well-known, close connection between wooden breast syndrome and hepatocyte injuries. In the plasma, aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) concentrations rise with the elevated inflammatory cytokine profile. Maharjan et al. compared homocysteine concentrations in the plasma of healthy animals and animals with myopathy and found that Hcy concentration was very high in birds suffering from wooden breast syndrome [54]. Greene et al. also reported a 11-fold increase

in the S-adenosylhomocysteine of breast meat categorized as wooden breast when compared to unaffected tissues [55].

6. Biochemical background of the homocysteine-lowering effects of some bioactive additives

6.1 Betaine

The consequences of hyperhomocysteinosis are disturbed methionine supply, low levels of available methionine on the cellular level and pathological conditions in response to high Hcy. Choline and betaine play a vital role in the remethylation of Hcy to Met. Choline is the parent compound of the class of cholines, consisting of ethanolamine residues with three methyl groups attached to the same nitrogen atom (**Figure 4**). It can be produced endogenously, but it is also often added in the form of choline chloride as a dietary supplement. Betaine is a generic name for a class of zwitterion compounds, but in nutritional science it is almost exclusively used to refer to glycine betaine or trimethyl glycine. The compound was first isolated from sugar beet, *Beta vulgaris*, hence the name. Betaine is the trimethyl derivative of glycine, the substrate of betaine-homocysteine S-methyltransferase (BHMT) in the liver and kidney. In the body, it can be found in anhydride, monohydrate, hydrochloride forms, which poultry can take advantage of. As an additive, the recommended concentration of betaine largely depends on the concentration of the methyl groups, environmental circumstances and the health status of birds. Similarly to choline, betaine is methyl donor in transmethylation processes, and consequently it can potentially decrease

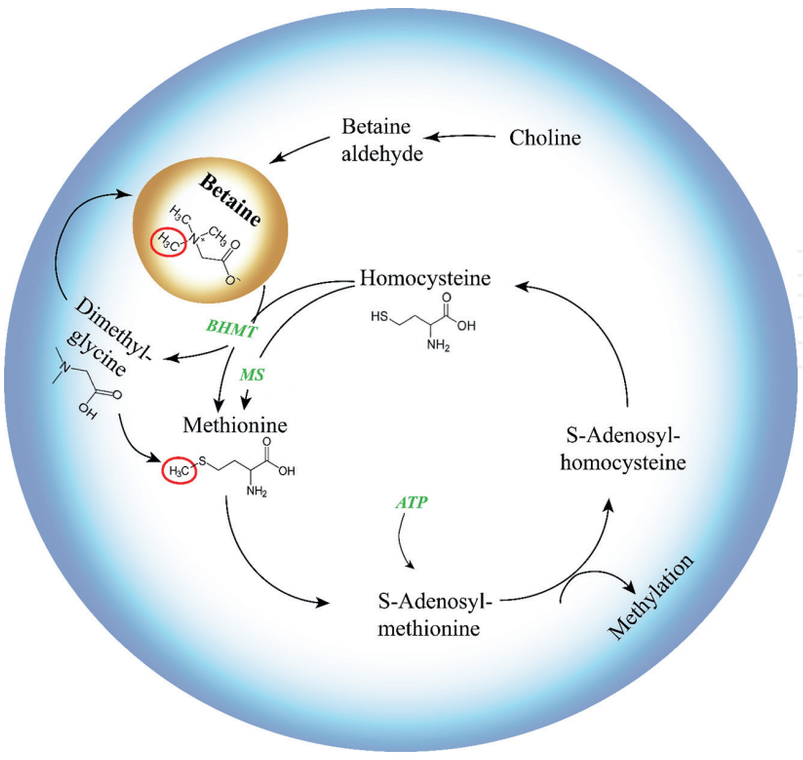

Figure 4.
Role of betaine in homocysteine transformation. Abbreviations in the figure: betaine-homocysteine S-methyltransferase (BHMT); methionine synthetase (MS), adenosine triphosphate (ATP).

creatine, methionine and choline demands. During the remethylation of homocysteine, betaine-homocysteine S-methyltransferase enzyme catalyzes the transfer of the methyl group from betaine into homocysteine, resulting in methionine and dimethyl-glycine. Therefore, for broilers dietary betaine is an alternative for methionine. Data presented in scientific papers are not clear-cut about the use of methionine in substitution for betaine. Studies also found that in the transsulfuration pathway the substitution of cysteine as an additive for betaine had more positive effects on the feed conversion ratio (FCR) in broilers than when given alone [56].

6.2 Berberine

Berberine is an isoquinoline alkaloid that can be found in a number of important herbs, such as *Berberis aristata* and *Berberis aquifolium*. Berberine features numerous pharmacological properties, including antibacterial, antihypertensive, anti-inflammatory, antidiabetic and liver protective effects [57–62].

It is evidenced in human patients that elevated plasma homocysteine levels are indicative of the increased risk of thrombotic and atherosclerotic vascular diseases [38] and steatosis, but berberine may be an effective substance to mitigate the risk of cardiac and metabolic diseases (**Figure 5**) [63]. It has been reported that hyperhomocysteinemia is associated with development of congestive heart failure in individuals who free from myocardial infarction. It induces systolic and diastolic dysfunction, arrhythmia, results in the accumulation of interstitial and perivascular collagen in the cardiac system and increases the risk of stroke [64–66]. In an experiment conducted with mice on homocysteine thiolactone-containing diet, the protective effects of berberine on the vascular function were revealed [67]. In another study,

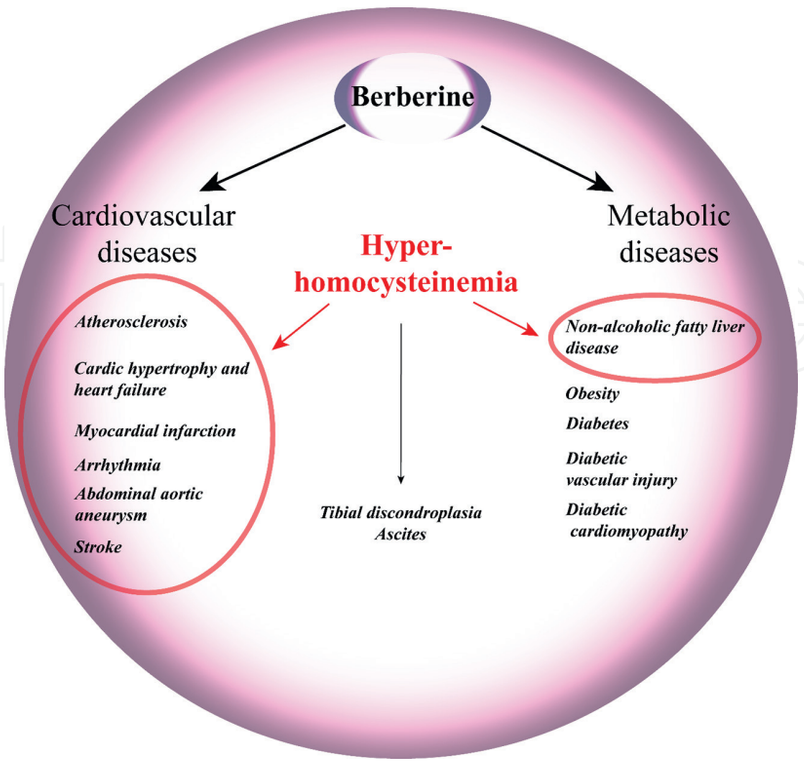

Figure 5.
Therapeutic potentials of berberine in different cardiometabolic diseases, some in association with hyperhomocysteinemia (adapted from Feng et al. [63]).

the area of atherosclerotic plaques could be reduced in mice that received berberine in the daily dose of 150 mg/kg BW per os [63]. Feng et al. have given a detailed overview of the mode of action by which berberine works in cardiac and other metabolic diseases [63].

One of the pathological conditions caused by hyperhomocysteinosis is the dysregulation of lipid metabolism and lipid accumulation in the liver, as a result of which the expression of CBS and CSE becomes damaged in liver tissues. The elimination pathways of homocysteine, such as remethylation and transsulfuration, are impaired in the course of hepatic steatosis. The biological activity of the natural phytochemical substance, berberine, has been studied in several animal experiments.

Homocysteine and cholesterol levels in the plasma of humans and animals with hyperhomocysteinemia have been found to be positively correlated [68–70]. The results of the relevant studies have confirmed a link between hyperhomocysteinemia and steatosis (fatty liver) [69–71]. Woo et al. found that homocysteine enhanced cholesterol secretion in the liver. The results suggested that hyperhomocysteinemia induced intensified cholesterol biosynthesis by regulating the corresponding transcriptomes; in fact, increased β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) reductase gene expression was achieved in the liver [70]. In conclusion, both the liver and the serum cholesterol levels increase in hyperhomocysteinemia.

Wu et al. identified a mechanism by which berberine exerts a protective effect against cholesterol biosynthesis and liver dysfunction induced by homocysteine. Cholesterol synthesis was effectively limited by dietary berberine in rats with hyperhomocysteinemia. This inhibitory effect is mediated through the posttranslational modification of HMG-CoA reductase. Dietary berberine reduced cholesterol levels in the liver and improved liver function due to direct inhibition of HMG-CoA reductase (**Figure 5**) [11]. In line with the foregoing, Chang et al. also found in rats that berberine could counteract hyperhomocysteinemia and hyperlipidemia induced by high-fat diets, in part by upregulating low-density lipoprotein (LDL) receptor and apolipoprotein E (apoE) messenger ribonucleic acid (mRNA) levels, as well as by suppressing 3-hydroxy-3-methylglutaryl-CoA reductase gene expression. There is no direct correlation between HMG-CoA gene expression and changes in homocysteine levels. Although the results are not explanative of the direct cause of the decreasing homocysteine concentration in the plasma, experiments suggest that decreased lipid levels are associated with intact liver tissues and result in normal expression profiles [72].

Berberine is capable of decreasing oxidative stress; by supplementing feeds with berberine, the level of oxidative stress markers, such as malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), becomes altered. Berberine inhibits reactive oxygen species (ROS) formation, improves mitochondrial function to boost the membrane potential and protects against oxidative damage. It moderates the activity of biomarkers mentioned above and increases the activity of antioxidant enzymes that help to bind free radicals and decrease oxidative stress [73]. Therefore, it may at least partly compensate for the compromised antioxidative defense mechanisms induced by hyperhomocysteinemia.

7. Some dietary means to reduce plasma homocysteine levels in broilers

As mentioned above, heart failure, i.e., one of the most frequently reported human diseases in the context of hyperhomocysteinemia, also tends to be a weighty problem in poultry production. Sudden death syndrome occurs typically with

high-producing broilers and turkeys at the end of the fattening phase, shortly before slaughtering, and is accompanied by elevated plasma Hcy [50].

Several papers adopt the hypothesis of morbidity being the potential consequence of increased plasma homocysteine, but there are very few publications reporting measured homocysteine concentrations in birds. In a study with ducks, Xie et al. found increased plasma homocysteine concentration accompanied by decreased feed intake and compromised average daily gain (ADG) in response to an increase in dietary DL-Methionine from 0.285% to 0.685%. In line with that the foregoing, available data for broilers suggest that dietary methionine levels correlate with homocysteine levels in the plasma (**Figure 6**), while the oversupply of methionine results in elevated homocysteine levels in the blood [74]. According to Orth et al., tibial dyschondroplasia was observed to be more severe and frequent, with growth performance remaining poor when the diet was supplemented with homocysteine [53]. Authors, however, noted that bone deformation is probably not due to homocysteine, but may be attributed to the metabolite of homocysteine.

The metabolism of homocysteine requires B vitamins, particularly riboflavin, pyridoxal 5′-phosphate, cobalamin and folate. According to Lu et al., the primary pathway to maintain Hcy levels in the body is Hcy remethylation (61%), still a significant quantity of Hcy is catabolized into cysteine via transsulfuration (39%). The conversion of Hcy into cysteine is supported by betaine [75]. The results obtained by Ganson et al. also indicated that the folate-dependent remethylation of Hcy predominated over betaine-dependent remethylation, whereas betaine-dependent remethylation seemed to be more extensively influenced by dietary sulfur-containing amino acids [76]. Samuels confirmed that plasma Hcy levels decreased when broilers received diets supplemented with mixtures of pyridoxal, cobalamin, folic acid and betaine. Although there was 18% reduction in mortality from ascites and sudden death syndrome in the supplemented group, the difference was statistically not confirmed [50].

There are numerous studies shedding light on the effects of dietary trimethyl glycine (called betaine) on the performance of broilers. Most of these studies come to the conclusion that betaine is beneficial in the case of heat stress due to its role as a methyl donor and function as an intestinal and metabolic osmolyte [77]. It is known as an osmoregulatory substance that controls intracellular biochemical events, thus playing a key role in water balance during heat stress. Since it features three methyl groups, it serves as a methyl donor and can substitute choline or methionine in that

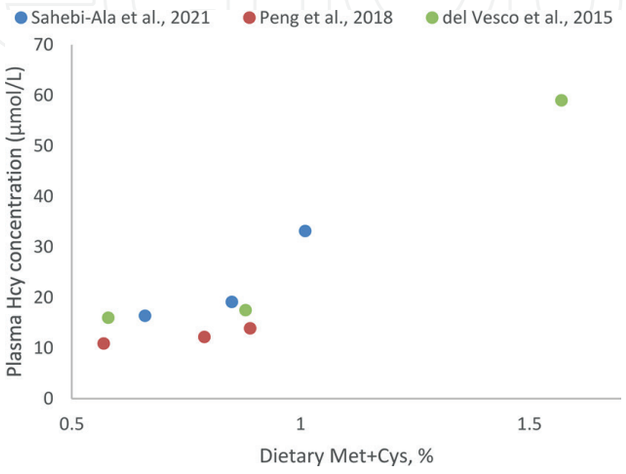

Figure 6.
Effect of dietary methionine plus cystine supply on plasma Hcy levels in broilers.

particular conversion. Sahebi-Ala et al. confirmed that during heat stress it was advisable to replace Met supplementation at least partly with betaine. In that study, 30% of the supplemental Met was replaced with betaine, which resulted in lower plasma homocysteine concentration [78]. Earlier studies also stressed the methionine-sparing effects of betaine [79–81].

In an outstanding review on the nutritional role of betaine, Abd El-Ghany and Babazadeh described a broad range of betaine supplementation that could be efficient in broiler chickens. Although in the cited literature, the dosage of supplementation ranged from 0.05 to 4 g/kg, with the most frequently applied levels of supplementation falling into the 1–2 g/kg range [82]. However, there are a very few studies performed with a focus on how betaine supplementation impacts Hcy levels. **Table 1** summarizes the broiler studies where plasma homocysteine levels were measured. These data demonstrate consistent reduction in plasma homocysteine concentrations in response to betaine supplementation, and the impact tends to be stronger in times of heat stress. The results presented by Mostashari-Mohases et al. show evidence that the regulation of plasma Hcy can be supported through the transsulfuration pathway, since betaine supplementation intensified betaine-homocysteine S-methyltransferase gene expression. In this broiler study, betaine supplementation has improved growth performance and feed efficiency, particularly in the finisher phase [83]. Recently, Maidin et al. have found that betaine decreased plasma homocysteine concentrations in the blood and improved the bone strength in laying hens [84]. Numerous publications have reported positive effects of betaine supplementation on growth performance in intensively farmed broilers [82, 85–87] and in slow-growing or indigenous broilers, too [88–90]. It has been revealed that through its role in methyl supply, betaine is able to support synthesis and increase the activities of enzymes that are responsible for antioxidant defense (glutathione peroxidase (GSH-Px), superoxide dismutase and glutathione). Despite its key role in metabolism, dietary betaine supplementation does not invariably result in better growth performance. There are a few studies that have not been able to confirm the improvement of body weight (BW) or growth rate with supplemental betaine, either in heat stress or in thermoneutral conditions [77, 78, 91, 92]. The ability of betaine to provide for the methyl group is beneficial in the Met→Cys conversion by supporting both the remethylation of Hcy to Met and the transsulfuration of Hcy to Cys. In this context, it plays a key role in maintaining Hcy levels and the reduction in the incidence of hyperhomocysteinemia in poultry. However, as mentioned above, Hcy levels have not been measured in most of the studies.

The metabolic load resulting from the limited ability to decompose homocysteine is likely to appear at later ages, and therefore—due to the short life cycle of broilers—growth performance may not be compromised on the flock level. Nevertheless, hyperhomocysteinemia is a metabolic challenge that predisposes to the sudden death syndrome and bone failure, as discussed above, and consequently can potentially decrease the economic efficiency of poultry farming.

According to relevant literature, among potential feed additives to reduce the incidence of hyperhomocysteinemia, berberine is one of the most promising candidates. While no direct evidence has been obtained for poultry, rat studies have confirmed that dietary berberine supplementation results in lower Hcy [11, 72]. Recently, berberine has come into the focus of interest for nutrition scientists, and numerous studies have been published discussing the health benefits related to its potential to mitigate oxidative stress, its anti-inflammatory and hepatoprotective potentials, as well as antimicrobial and antiviral activities. An excellent review by Imanshahidi and Hosseinzadeh pointed out that berberine exhibited multispectrum pharmacological

Reference	Feeding phase	Treatment	BW (g)	ADG (g/d)	ADFI (g/d)	CumFI (g)	FCR (g/g)	Mortality (%)	Effect	Plasma Hcy
Mostashari-Mohases et al. [83]	Phase 1	No betaine	1122			1711	1.59		Relative gene expression was 28-fold higher in the betaine-supplemented group	
		2 g/kg betaine suppl.	1180			1693	1.53			
	Phase 2	No betaine	2170[a]			2254	1.88[a]			
		2 g/kg betaine suppl.	2295[b]			2240	1.73[b]			
Kettunen et al. [77]	W0–3, ♀ (in heat stress)	Basal diet	Reported (but not confirmed) that BW was unaffected by betaine suppl.						Betaine supplementation reduced plasma Hcy	40.1 ± 2.5[a] nmol/g
		1 g/kg betaine suppl.								30.6 ± 1.4[b] nmol/g
	W0–3, ♂ (in heat stress)	Basal diet								47.8 ± 4.0[a] nmol/g
		1 g/kg betaine suppl.								30.7 ± 1.5[b] nmol/g
Samuels [50]	Phase 1 (W1–3)	Basal diet	892.8				1.328		Betaine supplementation reduced plasma Hcy	
		Supplemented diet[1]	875.1				1.306			
	Phase 2 (W4–6)	Basal diet	2723.8				2.02	9.7		35.9 µM/L
		Supplemented diet[1]	2666.6				2.01	8		29.7 µM/L
Maidin et al. [84]		No betaine	Tibia breaking strength and tibia density was improved by betaine						Betaine supplementation reduced plasma Hcy	20.3[a] µM/L
		1 g/kg betaine suppl.								19.9[b] µM/L

Reference	Feeding phase	Treatment	BW (g)	ADG (g/d)	ADFI (g/d)	CumFI (g)	FCR (g/g)	Mortality (%)	Effect	Plasma Hcy
Sahebi-Ala et al. [78]	Phase 1 (in heat stress)	Basal diet		53.57	78.56		1.487	1.22	Betaine supplementation reduced plasma Hcy	
		Supplemented diet[2]		54.48	78.77		1.463	1.70		
	Phase 2 (in heat stress)	Basal diet		75.54	151.77		2.056	3.09		23.49[a] μmol/L
		Supplemented diet[2]		75.15	151.63		2.035	1.53		22.26[b] μmol/L

BW, body weight; ADG, average daily gain; CumFI, cumulative feed intake; FCR, feed conversion ratio (gain/feed); Hcy, homocysteine.[1]*Supplementation was 1 g/kg betaine, vitamin B_6 and B_{12}, as well as folate.*
[2]*Betaine supplementation was applied as substitution of 30% of methionine.*
Differing superscripts in the same study indicate the differences of treatments ($P < 0.05$).

Table 1.
Effects of betaine supplementation on growth performance and plasma homocysteine concentrations in broiler studies.

action, ranging from cardiovascular conditions through anticancer effects to the modulation of antioxidants, neurotransmitters, enzymes, molecular targets and immune substances [93]. Moreover, berberine has been reported to influence energy, glucose and lipid metabolism [94]. These biological effects have made berberine an attractive natural compound that can be employed in sciences related to human and animal health [95].

As discussed above, homocysteine induces predisposition to metabolic dysfunctions. Homocysteine thiolactone results in protein damage, the aggregation and inactivation of functional proteins, such as enzymes and immune cells, and consequently enhances cell apoptosis [96]. Due to its property to support antioxidant defense mechanisms (**Table 2**), berberine may directly counteract Hcy and probably homocysteine thiolactone formation, or at least mitigate their negative impacts. Homocysteine counteracts antioxidant enzymes and reduces their activities [43], which is why hyperhomocysteinemia can potentially deteriorate the meat quality traits, too. It has been shown in broiler studies that the activities of antioxidant enzymes [97] and the characteristics of meat quality, particularly the water-holding capacity of meat in relation to oxidative damage to cells as induced by mycotoxin exposure, could be improved if the feed was supplemented with berberine [98, 99].

It has been reported in human studies that other N-homocysteinylated proteins also cause alteration in cell metabolism and result in various mild or severe dysfunctions. N-homocysteinylated proteins can be cytotoxic and activate immune functions by forming IgG antibodies against anti-N-homocysteinylated proteins [41, 42]. Berberine is a potential candidate to alleviate inflammatory mechanisms. Studies with broilers have confirmed reduction in ileal pro-inflammatory cytokines (interleukin-1beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α)) and lower intestinal necrosis indices [100, 101]. Fernandez et al. reported lower interleukin-17A (IL-17A), interleukin-17F (IL-17F), IL-6 and IL-1β in duck liver and spleen, when feed was supplemented with 200 mg/kg berberine [102]. As mentioned

Animal model	Dose of BBR	Treatment period (weeks)	Specimen used	Highlighted findings
Broilers	100 mg/kg/d	6	Serum	TAC↑, SOD↑, GSH-Px↑, MDA↓
Broilers	200, 400 and 600 mg/kg	6	Serum	SOD↑, GSH-Px↑, MDA↓
Broilers	200, 400 and 600 mg/kg	6	Meat	SOD↑, GSH-Px↑, MDA↓
Mice	200 mg/kg/d	2	Liver	SOD↑
Sprague-Dawley rats	80, 120 and 160 mg/kg/d	7	Serum	SOD↑
Sprague-Dawley rats	100 and 200 mg/kg/d	8	Kidney	SOD↑, MDA↓
Wistar rats	200 mg/kg/d	12	Serum	SOD↑, MDA↓
Wistar rats	75, 150 and 300 mg/kg/d	16	Serum and liver	SOD↑, GSH-Px↑, MDA↓

Abbreviations: TAC, total antioxidant capacity; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase.

Table 2.
Effects of dietary berberine (BBR) supplementation on antioxidant defense in broilers and laboratory rodents (reviewed by Ghavipanje et al. [95]).

before, transmethylation and transsulfuration are parts of the basic metabolism and are known as conservative pathways. Consequently, we are convinced that it is worth using a trans-species approach to identify the effective feed additive that can reduce the emergence of hyperhomocysteinemia. To this end, in addition to betaine supplementation, berberine supplementation is one of the most potential candidates.

8. Conclusions

The following main conclusions can be drawn from the latest research findings:

- In poultry industry, metabolic diseases have intensified in the past few decades, as the genetic potential of poultry for growth and feed efficiency has improved rapidly.

- Even before the development of symptoms of metabolic diseases, metabolic changes may adversely affect the performance of birds (broilers) and the profitability of meat production.

- Homocysteine is a metabolic intermediate in the methionine-cysteine conversion. When homocysteine concentration in the blood is higher than normal, a condition called hyperhomocysteinemia occurs.

- Hyperhomocysteinosis is a well-known disorder in humans, but in the field of avian physiology it has received little attention in research. It may be useful to introduce the monitoring of homocysteine in poultry in order to understand and reveal the role of this metabolite in a number of systemic diseases.

- Currently used feed additives, such as betaine, are able to decrease plasma homocysteine concentrations, but they support only one of the pathways (transmethylation) responsible for homocysteine decomposition. However, other feed additives may as well be applied to activate the transsulfuration pathway, too. Various phytonutrients may be suitable owing to their pleiotropic bioactive components, such as berberine. This latter phytogenic feed additive may be capable of maintaining the redox homeostasis in animals by typically modulating inflammatory immune responses and may therefore be able to provide for liver protective functions. Furthermore, it can potentially encourage healthy tissues to express enzymes that are responsible for the degradation of homocysteine, such as cystathionine-β-synthase and cystathionine-γ-lyase.

- However, further studies are recommended to investigate how effectively berberine can reduce the incidence of hyperhomocysteinemia in broilers, and whether it is necessary to use feed supplements during the entire life cycle of the birds.

9. Conclusion for practice

In the case of fast-growing broilers, the prevalence of metabolic diseases may be on the rise. One of these frequently occurring metabolic diseases is hyperhomocysteinemia that can cause various conditions, such as dyschondroplasia, ascites and

sudden death syndrome. In order to recognize these conditions on time, it is important to check the blood plasma homocysteine content of the flock. If the homocysteine content is higher than normal, it is advisable to add betaine to diets.

The concentration of betaine strongly depends on feeding, housing and health conditions. Therefore, it is recommended for farmers to conduct preliminary assessments in relation to the circumstances prevailing at the farms in question in order to determine how much betaine should be added to diets.

Abbreviations

3-MST	3-mercaptopyruvate sulfurtransferase
ADG	average daily gain
αKB	α-ketobutyrate
ALP	alkaline phosphatase
ALT	alanine aminotransferase
apoE	apolipoprotein E
AS	ascites
AST	aspartate aminotransferase
ATP	adenosine triphosphate
BBR	berberine
BHMT	betaine-homocysteine S-methyltransferase
BW	body weight
Ca	calcium
CAT	cysteine aminotransferase
CBS	cystathionine-β-synthase
CO	carbon monoxide
CSE	cystathionine-γ-lyase
CumFI	cumulative feed intake
Cys	cysteine
Cyss R	cystine reductase
DL-Met	D- and L-isomers of Met
DNA	deoxyribonucleic acid
FCR	feed conversion ratio
FLKS	fatty liver and kidney syndrome
FLS	fatty liver syndrome
GGT	gamma-glutamyl transferase
GSH	glutathione
GSH-Cyss TH	glutathione-cystine transhydrogenase
GSH-Px	glutathione peroxidase
H_2S	hydrogen sulfide
Hcy	homocysteine
HMG-CoA	β-hydroxy-β-methylglutaryl-coenzyme A
HTL	homocysteine thiolactone
IL-17A	interleukin-17A
IL-17F	interleukin-17F
IL-1β	interleukin-1beta
IL-6	interleukin-6
L-cysteine	L-isomer of cysteine
LDL	low-density lipoprotein

Lys	lysine
MDA	malondialdehyde
Met	methionine
MHA-FA	DL-methionine hydroxy analogue-free acid
mRNA	messenger ribonucleic acid
MS	methionine synthetase
NH_4^+	ammonium ion
NO	nitrogen-monoxide/nitric oxide
P	phosphorus
RNA	ribonucleic acid
ROS	reactive oxygen species
SAH/AdoHyc	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SAM/AdoMet	S-adenosyl-L-methionine
SOD	superoxide dismutase
TAC	total antioxidant capacity
tHcy	total homocysteine
TNF-α	tumor necrosis factor alpha
tRNA	transfer ribonucleic acid
TSP	transsulfuration pathway

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