

Effects of Supplemental Glutamine and Lysine on Growth Performance of Broiler Chickens

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Abstract

The optimum levels of Lysine and Glutamine needed for growth performance and maintenance of the chicken broilers were evaluated in a randomized 3 × 4 factorial arrangement of dietary treatments. The battery cages measured 99 × 66 × 25 cm that can be sufficient for 5 birds. Day old Chicken broilers totaling 180 were assigned to dietary treatments comprising of 3 concentrations of Lysine (0.85, 1.14, and 1.42) each in combination with 4 concentrations of Glutamine (0, 1, 2, and 3). Each dietary treatment was replicated 3 times and each replication had 5 birds. The birds were given feed and water *ad libitum* with a 23-hour light regimen for a period of 4 weeks. Then, the experimental birds were evaluated for body weight gain, feed consumption, and feed conversion in order to determine their optimum requirement for dietary Lysine and Glutamine. Based on the findings of this study, the highest performance was observed in birds fed the diet supplemented with 1.42 lysine and 1% glutamine, but the highest improvement in feed conversion was observed in diet contain 1.14 and 1.42 with 1% and 3% glutamine, respectively. Birds fed 1.42 lysine and 1% glutamine had the highest total body weight gain and feed consumption. The lysine requirements in the diet for Chicken are between 1.14 and 1.42 with glutamine level of 1%.

Keywords

Broiler Chickens, Lysine, Glutamine, Amino Acid, Dietary Protein, Essentials AAs, Non-Essential Amino Acids

1. Introduction

The dietary protein requirement concept has been debatable since the discovery

of the amino acid (AA), the building block of proteins. In actual fact, there are some dietary essential AAs that provide better performance and growth of chickens. There are 10 essential AAs that must be included in the broiler diet, and among these 10 AAs. There are 3 essential AAs that take part in the synthesis of non-essential AAs that are required for bodily protein synthesis [1]. Over the last five decades, the addition of purified AAs or precursors of AAs in the broiler diet has become well-known. Purified AAs or precursors allow researchers to reduce crude protein in the diet by the addition of adequate levels of essential and non-essential AAs. The requirement of each essential AA has been documented as a function of other AA levels in the diet. If the ideal ratio between essential AAs is maintained along with high levels of total AAs, then it can increase growth and carcass yield, decrease fat accumulation, improve the efficiency of feed utilization and increase growth [1] [2]. In the category of essential AAs, lysine is closely correlated with the potential of bodily protein deposition and it is considered the 2nd most limiting AA in the soybean and corn-based diets for broilers [3]. In terms of the “ideal protein” concept, lysine is chosen as the reference AA while all other AA concentrations are dependent on the fixed concentration of lysine [3] [4] [5]. Many researchers have reported that the saccharopine-dependent pathway is used in the catabolism of lysine, and in mammals and poultry, it is reflected as the principal pathway for the degradation of lysine [6] [7] [8] [9] [10]. In the saccharopine-dependent pathway, lysine is transformed to saccharopine with the help of the lysine α -ketoglutarate reductase (LKR) enzyme and forms saccharopine with α -ketoglutarate (α -KG) from the condensation of lysine and this step is NADPH dependent. Subsequently, the oxidation of saccharopine occurs in a NAD⁺-dependent reaction that is catalyzed by dehydrogenase and produces glutamate and α -amino adipate- γ -semialdehyde [11] [12]. The non-essential amino acids, glutamine and glutamate, occur naturally in common feedstuffs and are closely related to each other. Glutamate and glutamine can be converted into one another in various organs such as the kidney, liver and intestine; in the broiler chicken, both amino acids are related to gastrointestinal tract development. There is a considerable amount of research concerning the influence of glutamine on bird performance, villi length, and the immune system but there is no data available that indicates the effect of high glutamine concentrations with the combination of different levels of lysine in the diet on the performance of birds [13] [14] [15]. Glutamine comes under the category of nonessential amino acid which is quantitatively the most plentiful free amino acid in blood plasma compared to other free amino acids [13] [14] [16] [17]. Moreover, it is important for different physiological functions and maintenance of cell functions. It acts as the substrate for several aminotransferases involved in the synthesis of purines, glucosamine, pyrimidine's and asparagine. Glutamine is also involved in protein, peptide, and nucleic acid synthesis. It is available as a source of oxidative energy and in the biosynthesis of glucose, amino sugars and glutathione [13] [14] [18]. In the body, there are different types of functions that occur by Glutamine: 1) during amino acid metabolism, the exces-

sively produced nitrogen is carried by glutamine; 2) act as the substrate for the production of energy in the rapidly dividing cell; 3) act as nucleotide's precursor; and 4) use for the synthesis of protein [19] [20]. In different types of conditions, such as post-intense exercise, illness or stress, there could be a decreased amount of glutamine, and after these conditions, a higher consumption of dietary glutamine could be helpful [21]. The major place for glutamine synthesis is in skeletal muscle and in muscles. The most plentiful free amino acid is glutamine [13]. The glutamine concentration in plasma is depleted due to muscle fatigue conditions and prolonged exercise [22] [23]. This is the reason, during exercise, the muscles are recovered with the supplementation of dietary glutamine. However, more work is still needed to clarify whether the supplementation of oral glutamine has an important role in the repair of muscle after the damage induced by extensive exercise [24]. Glutamine also prevents and controls the subsequent sepsis and translocation of bacteria. Glutamine might have antioxidant activity for macrophages and lymphocytes, regulate the production of cytokines, and precursors for nucleotide synthesis, and act as respiratory fuel [13]. The amino acid from glutamic acid, glutamine, is measured as a major fuel for the metabolism of enterocytes in the gut [25]. During the early development of birds, lymphoid cells are produced in the bursa and thymus, primary organs of the immune system. On the other hand, the ceecal tonsils and spleen are secondary immune system organs that provide a defense to the respiratory and digestive tract, respectively. Glutamine can elevate the lymph node numbers on the mucosal membranes of the gastrointestinal and respiratory tract. Moreover, glutamine provides energy to the cell and enhances cell division [26] [27]. The glutamine supplementation at 0.5% improved broiler weight gain and performance. Dietary glutamine has been observed as the most significant fuel for the small intestinal mucosa which is associated with glutamate formation. Many studies have revealed that the accumulation of glutamine in the broiler's diet could enhance the relative weights of the jejunum and duodenum [28] [29]. The broiler diet inoculated with *Eimeria maxima* could decrease the score of intestinal lesions, promote intestinal development, and enhance the integrity of intestinal maintenance [30]. It is understood that during the early life stages of chickens, longer villi could enhance food utilization and efficiency in the small intestine of broilers while improving broiler performance. Different studies have revealed that supplementation of glutamine in the diet could enhance the length of villi in several small intestine sections [31].

In animals, lysine is an essential amino acid. Lysine is found primarily as a component of body protein, and is approximately 6% of total body protein by weight. It acts both as a structural and a functional component of protein. One functional role of protein-bound lysine is its capacity to undergo acetylation of histones, which relaxes the DNA-histone interaction, allowing for DNA replication, gene expression, and nuclear division. An additional example of the functional role of lysine residues is their ability to be cross-linked in structural proteins like collagen, contributing to the distinct physical properties of this protein

[32] [33]. Intracellular and circulating free lysine also plays functional roles in whole-body homeostasis. Lysine is strictly ketogenic since it is converted to acetyl-CoA, which is subsequently oxidized in the TCA cycle. An additional role of free lysine is to serve as a precursor for functional metabolites generated from pathways of lysine oxidation [34]. The limiting amino acid may be known as the essential amino acid in the shortest dietary supply compared to its requirement. Lysine is frequently the 1st limiting amino acid for the growth of human and swine cereal grain-based diets [35] [36]. Additionally, it serves as the second most restrictive amino acid in the diets of chickens and fish [37] [38]. Therefore, protein deficiencies that result in this cereal grain consuming population are often the result of an insufficient intake of dietary lysine, rather than a shortage of amino acids as a whole. When dietary lysine deficiency occurs growth, feed efficiency, and overall health of an animal are compromised [39]. Thus, meeting the lysine requirement is imperative in a livestock production system to avoid the monetary losses associated with suboptimal animal performance and the cost of feeding amino acids as an energy source [37] [39]. During the metabolism of lysine, it is degraded into several significant metabolites, and not amazingly. Lysine is metabolized with the help of various pathways. A coenzyme A is acetyl CoA that is formed by the degradation of lysine, which is a critical catalyst for different reactions inside the body and also acts as a vital nutrient in the metabolism of carbohydrate. During lysine metabolism, it also assists in transamination (the transfer of amino group) by the formation of peptide bonds, or the linkage, between enzyme transaminase such as SGOT and SGPT, and for the occurrence of this activity, the coenzyme pyridoxal phosphate is needed. Lysine has the property to do this due to the presence of 2 amino groups: one joins with coenzyme pyridoxal phosphate and the other joins with transaminase enzyme. These reactions are performed by the enzymes that are present in tissues of the skin, brain, thymus gland, adrenal gland, heart, kidney and liver (in order of increased activity) [40]. Lysine is degraded in chicken liver by an L-amino acid oxidase and lysine-ketoglutarate reductase, leading to the formation of pipercolic acid and saccharopine, a tricarboxylic amino acid, respectively. Carbon-14 studies indicate that the sacchariopine pathway may be the major one for the *in vivo* degradation of L-lysine in chicks [41]. Both metabolites are subsequently converted to α -amino adipate and eventually to CO₂. D-Lysine is catabolized via pipecolate, which is further metabolized. In this the chicken differs from the rat, and probably other mammals, which excrete pipecolate in the urine without further catabolism [42]. The rapid elimination of catabolic products of both lysine isomers speaks against a transformation of D- and L-lysine; however, if the capacity to catabolize D-amino acids readily were common to birds, it would be useful for the utilization of D-amino acids found in lower animals and insects that are ingested by birds. There seems to be a similar difference between birds and mammals in the degradation of D-hydroxylysine [42] [43].

The animals that face the deficiency of protein are more susceptible to disease due to impaired function of the immune system. The dietary protein deficiency

causes the reduction in the amino acid availability inside the plasma, mostly for cysteine, tryptophan, arginine and glutamine [44]. There are well established that cysteine, arginine and glutamine can most important role in the improvement of the function of the immune [45]. For example, glutamine is a major fuel for lymphocytes and essential for their proliferation and function [45] [46].

The purpose of this research is to determine lysine and glutamine requirements for optimum growth performance of chicken broilers.

Specific aims

- 1) To find the most optimal levels of lysine and glutamine for the chicken broiler's body weight gain, feed consumption, and feed conversion.
- 2) To investigate the interaction between lysine and glutamine supplementation on broiler growth performance.

2. Materials and Methods

2.1. Experimental Design

The study had a total of 12 treatments consisting of 3 replicates per treatment and 5 birds per replicate totaling 180 chicken broilers. The study was randomized 3 × 4 factorial arrangement of dietary treatments comprising 3 Lysine concentrations (0.85, 1.14, and 1.42) and 4 Glutamine supplement concentrations (0%, 1%, 2%, and 3%). Variables that were measured include “body weight gain”, “feed consumed”, and “feed conversion ratio (FCR)”.

2.2. Animal Management

At the day first, 1-day-old chicken broilers were weighed and wing banded. The birds were assigned to “Petersime battery brooders (Petersime Incubator Co., Gettysburg, OH)”. The battery cages measured 99 × 66 × 25 cm that can be sufficient for 5 birds. During the experiment, brooders provided artificial heat. Birds were fed *ad libitum* water and feed during the whole period of experiment. Feed was provided in mash form.

2.3. Dietary Treatments

Experimental diets contained Lysine levels ranging from 0.85 - 1.42 with digestible lysine in increments of 25%, and Glutamine levels ranging from 0 - 3 with digestible glutamine in increments of 1%. The diets were replicated 3 times and arranged in 12 treatments × 3 replications × 5 birds (Table 1). The rations contained 3100 Kcal of metabolizable energy (ME) in each kg of the diet and 23% CP at 0 - 4 week of age (WOA). Water and feed was provided *ad libitum* during experimental period. Mortality was documented when it occurred.

2.4. Parameters Measured

2.4.1. Body Weight Gain

Experimental birds were weighed prior to assignment to dietary treatments. Then, the birds were weighed weekly until 4 WOA. Weekly body weight gains

Table 1. Layout of dietary treatment fed to chicken broilers from hatch to 4 weeks of age.

Lysine	Glutamine	# Of birds	Replications	Birds/Treatments
0.85 ^a	0 ^a	5	3	15
1.14 ^a	0 ^a	5	3	15
1.42 ^a	0 ^a	5	3	15
0.85	1	5	3	15
0.85	2	5	3	15
0.85	3	5	3	15
1.14	1	5	3	15
1.14	2	5	3	15
1.14	3	5	3	15
1.42	1	5	3	15
1.42	2	5	3	15
1.42	3	5	3	15

a = Control diet.

were determined by calculating the difference in body weights from one week to the next week. Body weight gains and body weight were expressed in grams units.

2.4.2. Feed Consumption

Consumption of feed was determined after each week until 4 WOA. Feed consumption was calculated as the feed supplied for the entire week minus feed wastage and the feed left in the feed troughs. Feed consumption was also expressed in grams units.

2.4.3. Feed Conversion Ratio

Feed conversion was determined by dividing the amount of feed consumed within a week by the number of bird days of the experimental birds, the quotient was then multiplied by 7 (which represents 7 days in a week). Bird days are defined as the total number of birds alive and on the experiment at the end of the week multiplied by the days, these birds were on the experiment. The number of birds that died during the week was multiplied by the number of days they were alive for the week.

2.5. Statistical Analysis

Data were analyzed using TWO WAY ANOVA model of SAS. Variables that were measured: body weight gain, feed consumption, and feed conversion ratio. When there is a significant F-value, means will be separated using the Least Square means option. The results of the trials will be presented in **Table 2** and **Table 3** forms.

Table 2. Composition of dietary total % of chicken broilers fed diets varying in lysine and glutamine concentrations.

Lysine %	75	100	125	75	75	75	100	100	100	125	125	125
Glutamine %	0	0	0	1	2	3	1	2	3	1	2	3
Ingredients	%											
Corn	50.61	49.22	50.3	48.51	46.41	44.61	47.28	45.38	43.38	48.55	46.36	44.46
Soybean meal	14.1	30.92	31.2	14.5	14.8	14.8	31.2	31.5	31.1	31.5	31.9	32.2
Corn gluten meal	17.6	6	6	17.6	17.6	17.8	6	6	6.7	6	6	6
Wheat middlings	10.36	4.4	2.67	10.36	10.36	10.36	4.4	4.4	4.4	2.67	2.67	2.67
Alfalfa meal	1	1	1	1	1	1	1	1	1	1	1	1
Poultry blend fat	2.3	4.62	4.62	3.1	3.9	4.5	5.4	6	6.7	5.2	6	6.6
Dicalcium phosphate	1.74	1.73	1.73	1.74	1.74	1.74	1.73	1.73	1.73	1.73	1.73	1.73
Limestone flour	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45
D, L-Methionine	0.03	0.11	0.12	0.03	0.03	0.03	0.11	0.11	0.11	0.12	0.12	0.12
L-Arginine	0.2	0	0	0.2	0.2	0.2	0	0	0	0	0	0
L-Lysine	0.06	0	0.36	0.05	0.05	0.05	0	0	0	0.35	0.34	0.34
L-Glutamine	0	0	0	0.91	1.91	2.91	0.88	1.88	2.88	0.88	1.88	2.88
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin-mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

Table 3. Calculated analysis for diets fed to chicken broilers at hatch—4 weeks of age.

Lysine %	75	100	125	75	75	75	100	100	100	125	125	125
Glutamine %	0	0	0	1	2	3	1	2	3	1	2	3
Feed ingredient	%											
Crude protein	23.03	23.05	23.01	23.05	23.02	23.00	23.03	23.02	23.09	23.01	23.03	23.02
ME, Kcal/kg	3101.02	3103.41	3111.82	3105.70	3107.93	3104.02	3108.89	3101.51	3107.89	3107.84	3109.5	3102.11
Calcium	1.04	1.07	1.07	1.04	1.04	1.04	1.07	1.08	1.07	1.07	1.07	1.07
Total phosphorus	0.72	0.71	0.70	0.72	0.72	0.71	0.71	0.71	0.70	0.70	0.70	0.69
Av. Phos	0.45	0.45	0.44	0.45	0.44	0.44	0.45	0.44	0.44	0.44	0.44	0.44
Methionine	0.50	0.50	0.51	0.50	0.49	0.49	0.50	0.50	0.50	0.51	0.51	0.51
Cysteine	0.42	0.39	0.39	0.42	0.41	0.41	0.39	0.39	0.39	0.39	0.38	0.38
Meth + Cys	0.92	0.89	0.90	0.92	0.91	0.91	0.89	0.89	0.89	0.90	0.90	0.89
Isoleucine	0.94	0.97	0.97	0.94	0.94	0.94	0.97	0.97	0.98	0.97	0.97	0.98
Lysine	0.85	1.14	1.42	0.85	0.85	0.85	1.14	1.14	1.14	1.42	1.42	1.42
L-Glutamine	0	0	0	1	2	3	1	2	3	1	2	3
Arginine	1.32	1.42	1.42	1.33	1.33	1.33	1.43	1.43	1.42	1.42	1.43	1.43
Valine	1.08	1.08	1.08	1.08	1.08	1.08	1.08	1.08	1.08	1.08	1.08	1.08
Threonine	0.82	0.86	0.86	0.82	0.82	0.81	0.86	0.86	0.86	0.86	0.87	0.87
Leucine	2.92	2.30	2.31	2.91	2.90	2.90	2.30	2.29	2.32	2.30	2.29	2.29
Tryptophan	0.22	0.29	0.29	0.22	0.22	0.22	0.29	0.29	0.29	0.92	0.29	0.29

ME, Kcal/kg = Metabolizable Energy/Av. Phos = Available Phosphorus/Meth + Cys = Methionine + Cysteine.

3. Results and Discussion

3.1. Body Weight Gain

The average body weight gains of Chicken broilers that were fed on the diet supplemented with different levels of Lysine and Glutamine were recorded after each week that are shown in **Table 4**.

After the first week (0 - 7 days), the diet supplemented with 1.42 lysine and 1% of glutamine showed significantly ($P < 0.05$) higher average body weight gain from other diets supplemented with 0.85 lysine with 0%, 1%, 2%, and 3% of glutamine, and diets supplemented with 1.14 lysine with 0% glutamine. On the other hand, it was not significantly higher than the diet supplemented with 1.42 lysine with 0%, 2% and 3% glutamine, as well as the diet supplemented with 1.14 lysine with 1%, 2%, and 3% glutamine. During the first week, the most significant ($P < 0.05$) lower body weight gain was attained by the bird fed on the diet that contained 0.85 lysine with 0%, 1%, 2% and 3% glutamine, and these lower body weight gains were not significantly different from the average body weight gain from diets supplemented with 1.14 lysine with 0% of glutamine. The birds fed on the diet containing 1.14 lysine with different levels of glutamine attained

Table 4. Mean body weight gains of broiler chicken fed diets varying in lysine and glutamine concentrations.

Lysine	Glutamine	Weeks of age				Total
		1	2	3	4	
g/birds/week						
0.85	0	72.00 ^c	111.73 ^d	168.26 ^e	213.93 ^f	565.92
0.85	1	62.00 ^c	91.33 ^d	132.40 ^e	186.13 ^f	471.86
0.85	2	64.42 ^c	90.85 ^d	116.85 ^e	188.14 ^f	460.26
0.85	3	70.84 ^c	106.61 ^d	147.84 ^e	228.15 ^f	553.44
1.14	0	81.53 ^{bc}	154.26 ^e	245.33 ^c	330.93 ^e	812.05
1.14	1	117.46 ^a	184.00 ^{abc}	386.80 ^a	459.06 ^{bc}	1147.32
1.14	2	100.66 ^{ab}	186.80 ^{ab}	342.46 ^{ab}	425.80 ^{cd}	1055.72
1.14	3	107.00 ^a	163.42 ^{bc}	274.42 ^{cd}	362.42 ^{de}	907.26
1.42	0	107.46 ^a	191.86 ^{ab}	273.20 ^{cd}	374.53 ^{de}	947.05
1.42	1	120.64 ^a	197.85 ^a	343.85 ^{ab}	536.57 ^a	1198.91
1.42	2	115.78 ^a	184.28 ^{abc}	315.78 ^{bc}	532.64 ^{ab}	1148.48
1.42	3	114.13 ^a	173.46 ^{abc}	344.33 ^{ab}	534.20 ^a	1166.12
	SEM	4.51	6.74	11.91	15.94	
	Lys	<0.0001	<0.0001	<0.0001	<0.0001	
	Gln	0.0032	0.3569	<0.0001	<0.0001	
	Lys * Gln	0.0002	0.0002	<0.0001	<0.0001	

^{a-f}Means within a column with different superscripts differ significantly ($P < 0.05$).

average body weight gain lower than the diet contain 1.42 lysine with different level of glutamine but higher body weight gain than the diets containing 0.85 lysine with different levels of glutamine. The different levels of lysine were significantly ($P < 0.05$) different from each other, as well as different levels of glutamine also showed significant ($P < 0.05$) differences between each other. The average body weight gain was significant ($P < 0.05$) affected due to the interaction between lysine and glutamine. Overall, after the first week of the experimental period diet supplemented with 1.42 lysine and 1% glutamine showed much higher average weight gain. Yi *et al.* (2005) described turkeys supplemented with glutamine (1%) in diet during the 1st week after hatch as having enhanced feed efficiency and weight gain in comparison to turkeys given the control diet based on corn-soybean meal (SBM). Moreover, Yi (2001) noted an increase in the height of the villus in poults supplemented with glutamine.

After the second week (8 - 14 days), the results of body weight gain were quite similar to the results of the first week. The diet supplemented with 1.42 lysine and 1% of glutamine showed significantly ($P < 0.05$) higher average body weight gain from other diets supplemented with 0.85 lysine with 0%, 1%, 2%, and 3% of glutamine, and diets supplemented with 1.14 lysine with 0% and 3%, but it was not significantly higher than the diet supplemented with 1.42 lysine with 0%, 2% and 3% glutamine, as well as the diet supplemented with 1.14 lysine with 1% and 2% glutamine. During the second week, the most significant ($P < 0.05$) lower body weight gain was attained by the bird fed on the diet that contained 0.85 lysine with 0%, 1%, 2% and 3% glutamine, and these lower body weight gains were significantly ($P < 0.05$) different from the average body weight gain from all other experimental diets. The different levels of lysine were significantly ($P < 0.05$) different from each other, but different levels of glutamine were not significantly different from each other. There was significant ($P < 0.05$) interaction that was present between lysine and glutamine during the second week.

After the third week (15 - 21 days), the diet supplemented with 1.14 lysine and 1% of glutamine showed significantly ($P < 0.05$) higher average weight gain from all other diets supplemented with 0.85 lysine with 0%, 1%, 2% and 3% of glutamine, 1.14 lysine with 0% and 3% glutamine, and 1.42 lysine with 0% and 2% glutamine, but not significantly different from 1.14 lysine with 2% glutamine and 1.42 lysine with 1 and 3% glutamine. The average body weight gain of a diet supplemented with 0.85 lysine with 0%, 1%, 2%, and 3%, significantly ($P < 0.05$) lower than the highest weight gain, but not significantly different from each other. The different levels of lysine were significantly ($P < 0.05$) different from each other, as well as, the different level of glutamine was also significant ($P < 0.05$) different from each other. There was significant ($P < 0.05$) interaction that was present between lysine and glutamine during the third week.

After the fourth week (22 - 28 days), the diet supplemented with 1.42 lysine and 1% of glutamine showed significantly ($P < 0.05$) higher average body weight gain from other diets supplemented with 0.85 and 1.14 lysine with 0%, 1%, 2%, and 3% of glutamine, but it was not significantly higher than the diet supple-

mented with 1.42 lysine with 2% and 3% glutamine. During the third week, the most significant ($P < 0.05$) lower body weight gain was attained by the bird fed on the diet that contained 0.85 lysine with 0%, 1%, 2%, and 3% of glutamine, and these lower body weight gains were not significantly different from each other. The different levels of lysine were significantly ($P < 0.05$) different from each other, as well as, the different level of glutamine was also significantly ($P < 0.05$) different from each other. There was significant ($P < 0.05$) interaction that was present between lysine and glutamine during the fourth week.

Overall, in four-week data (0 - 28 days), the diet supplemented with 1.42 lysine with 1% glutamine showed the highest body weight gain, which means an increase in the lysine percentage simultaneously increase the body weight gain. The higher the lysine content in the diet higher will be the body weight gain. These results are supported by Sibbald and Wolynetz (1987) [47], who demonstrated that 10-day-old male chicks required high levels of lysine for higher body weights. For higher breast meat yield, higher levels of lysine are required in the diet, more than the required level of lysine in the diet [48]. Likewise, Nasr and Kheiri (2012) found that male broilers fed high levels of lysine from 1 to 14-day-old had higher breast meat yields by the age of 49-day-old [49]. On the other hand, no effect was observed with higher levels of dietary lysine between 15 to 49-day-old broilers. Chicken and poultry diets that are supplemented with required amounts of lysine produce positive effects on the overall performance of birds including improved feed efficiency, increased muscle weights, and increased growth rates [50]. In the case of glutamine, the 1% was the most suitable level for the higher weight gain. Devi Priya *et al.*, (2010) and later on Jazideh *et al.*, (2014) observed that the glutamine supplementation at 0.5% improved broiler weight gain and performance [28] [29].

Bartell and Batal (2007) reported that in the broiler diet the addition of 1% glutamine can improve growth performance, blood parameters, and the immune system of birds. They used high levels of glutamine up to 4% and observed depression in weight gain with the higher level of supplemental glutamine [16]. Later, Soltan (2009) also reported that 1% glutamine can improve growth performance, blood parameters, and the immune system of birds. They used high levels of glutamine up to 2% and observed depression in weight gain with the higher level of supplemental glutamine. In both these two researches the authors concluded that there could be a toxic effect when glutamine is supplied at higher concentrations such as 2% and 4% [31]. These results support the results of the present studies.

There are different reports that explain the interaction of lysine metabolism with the other supplements in the diet. Wang & Nesheim (1972) reported that a higher level of arginine produced an effect on the pathway of lysine degradation by the reduction of lysine- α -ketoglutarate reductase that is essential for the completion of the process [41]. So, the higher level of arginine is not suitable with a higher level of lysine. Later on, there was another study conducted by Scott & Austic, (1978), who reported that a high level of potassium in a chicken

diet increases the lysine metabolism. The potassium can increase the production of lysine- α -ketoglutarate reductase that ultimately increases the growth rate [51]. Glutamine is synthesized from the lysine because glutamine accepts the nitrogen that comes from the lysine during the lysine catabolism [52]. So, the higher level of the supplemented glutamine can affect the catabolism of the lysine that was observed during the present study. The body weight gain results show fluctuation during the entire study period. The result of the present study indicated that there is an interaction present between glutamine and lysine. The high level of glutamine must have an effect on the lysine degradation, which causes the reduction in body weight gain.

Overall, the results of body weight gain undoubtedly indicated that the different levels of lysine were significantly ($P < 0.05$) different from each other, as well as, the different levels of glutamine were also significantly ($P < 0.05$) different from each other except the second week. There was significant ($P < 0.05$) interaction which was present between lysine and glutamine.

3.2. Feed Consumption

The average feed consumption of Chicken broilers that were fed on the diet supplemented with different levels of Lysine and Glutamine are shown in **Table 5**.

Table 5. Mean feed consumption of broiler chicken fed diets varying in lysine and glutamine concentrations.

Lysine	Glutamine	Weeks of age				Total
		1	2	3	4	
g/birds/week						
0.85	0	86.22 ^{ef}	179.27 ^f	268.55 ^{fg}	329.77 ^{fg}	863.81
0.85	1	80.22 ^{fg}	143.50 ^g	241.88 ^{gh}	310.88 ^g	776.48
0.85	2	81.57 ^f	149.72 ^g	228.05 ^h	316.21 ^{fg}	775.55
0.85	3	73.69 ^g	181.80 ^f	284.25 ^f	364.48 ^f	904.22
1.14	0	89.44 ^{de}	215.77 ^e	375.11 ^e	477.33 ^e	1157.65
1.14	1	100.55 ^{abc}	276.66 ^b	525.55 ^{ab}	650.22 ^c	1552.89
1.14	2	95.55 ^{cd}	306.53 ^a	549.17 ^a	647.33 ^c	1598.85
1.14	3	99.38 ^{bc}	236.00 ^{de}	401.14 ^{de}	535.42 ^d	1271.94
1.42	0	88.77 ^e	251.33 ^{cd}	417.55 ^d	507.55 ^{de}	1265.2
1.42	1	98.92 ^{bc}	304.14 ^a	507.85 ^b	786.42 ^a	1697.33
1.42	2	106.42 ^a	267.85 ^{bc}	472.02 ^c	650.11 ^c	1496.4
1.42	3	104.40 ^{ab}	269.06 ^{bc}	476.00 ^c	707.26 ^b	1556.72
	SEM	1.41	4.60	6.41	10.16	
	Lys	<0.0001	<0.0001	<0.0001	<0.0001	
	Gln	<0.0001	<0.0001	<0.0001	<0.0001	
	Lys*Gln	<0.0001	<0.0001	<0.0001	<0.0001	

^{a-g}Means within a column with different superscripts differ significantly ($P < 0.05$).

After first week (0 - 7 days), the diet supplemented with 1.42 lysine with 2% of glutamine showed significantly ($P < 0.05$) higher average feed consumption from other diets supplemented with 0.85 lysine with 0%, 1%, 2%, and 3% of glutamine, 1.14 lysine with 0%, 2%, and 3% of glutamine, and 1.42 lysine with 0% and 1% glutamine, but it was not significantly higher than the diet contains 1.14 lysine with 1% glutamine and diet containing 1.42 lysine with 3% glutamine. The diet supplement with 0.85 lysine with 3% glutamine shows significant ($P < 0.05$) lower average feed consumption from all other experimental diets. Overall, the diet supplemented with 0.85 with 0%, 1%, 2% and 3% of glutamine have lower average feed consumption. From data of average feed consumption, it was revealed that diets supplemented lysine with 0.85, 1.14 and 1.42 lysine with 0% glutamine show significantly ($P < 0.05$) lower average feed consumption. Generally, the conclusion from the first week's data is that the lysine content in the diet is directly proportional to the feed consumption, which means more the lysine in diet than more feed consumption. The average feed consumption in different levels of lysine was significantly ($P < 0.05$) different from each other, as well as, the average feed consumption in different level of glutamine were also significantly ($P < 0.05$) different from each other. The feed consumption was significantly ($P < 0.05$) affected due to the interaction of lysine and glutamine. These results, are supported by the result of Rezaei, *et al.*, (2004), who reported that during the starter period, of broiler diet, the feed consumption was higher by the birds, which fed on the diet with a higher level of lysine [53].

After the second week (8 - 14 days), the diet supplemented with 1.14 lysine with 2% glutamine, and 1.42 lysine with 1% glutamine showed significantly ($P < 0.05$) higher average feed consumption from all other experimental diets. The higher feed consumption was followed by the birds fed on the diet supplemented with 1.14 lysine with 1% glutamine and 1.42 lysine with 2% and 3% glutamine. The diet supplement with 0.85 lysine with 1 and 2% glutamine show significant ($P < 0.05$) lower average feed consumption from all other experimental diets. Overall, the diet supplemented with 0.85 with 0%, 1%, 2% and 3% of glutamine have lower average feed consumption. The average feed consumption in different levels of lysine was significantly ($P < 0.05$) different from each other, as well as, the average feed consumption in different levels of glutamine were also significantly ($P < 0.05$) different from each other. After the second week, the feed consumption was significantly ($P < 0.05$) affected due to the interaction of lysine and glutamine.

After the third week (15 - 21 days), the results were quite similar to the second week results. The diet supplemented with 1.14 lysine with 2% glutamine showed significantly ($P < 0.05$) higher average feed consumption from other diets supplemented with all other experimental diets but was not significantly different from the diet containing 1.14 lysine with 1% glutamine. The higher feed consumption was followed by the birds fed on the diet supplemented with 1.42 lysine with 1% glutamine, afterward followed by 1.42 lysine with 2% and 3% glutamine. The diet supplement with 0.85 lysine with 2% glutamine show signifi-

cant ($P < 0.05$) lower average feed consumption from all other experimental diets. Overall, the diet supplemented with 0.85 with 0%, 1%, 2% and 3% of glutamine has lower average feed consumption. The average feed consumption in different levels of lysine was significantly ($P < 0.05$) different from each other, as well as, the average feed consumption in different level of glutamine was also significantly ($P < 0.05$) different from each other. The feed consumption was significantly ($P < 0.05$) affected due to the interaction of lysine and glutamine after the third week.

After the fourth week (22 - 28 days), the diets supplemented with 1.42 lysine with 1% glutamine show a completely different pattern of mean feed consumption from the previous first and third weeks because the diet shows significantly ($P < 0.05$) higher mean body weight gain than the all-other diets. The diets supplemented with 1.14 lysine with 0%, 1% and 2% glutamine show significantly ($P < 0.05$) higher mean feed consumption than the diets supplemented with 0.85 lysine with 0%, 1%, 2% and 3% glutamine. The diet supplement with 0.85 lysine with 1% glutamine show significant ($P < 0.05$) lower average feed consumption from all other experimental diet. Overall, the diet supplemented with 0.85 with 0%, 1%, 2% and 3% of glutamine has lower average feed consumption. The average feed consumption in different levels of lysine were significantly ($P < 0.05$) different from each other, as well as, the average feed consumption in different level of glutamine were also significantly ($P < 0.05$) different from each other. The feed consumption was significantly ($P < 0.05$) affected due the interaction of lysine and glutamine after fourth week.

In general, in the four-week data (0 - 28 days), the diet supplemented with 1.42 lysine with 1% glutamine showed the significantly ($P < 0.05$) higher feed consumption, which means as increase in the lysine percentage simultaneously increase the feed consumption. The higher the lysine content in the diet higher with different levels of glutamine will be in the feed consumption. There are many different literature reported that various levels of lysine in diet produced variations in feed consumption, due to different factors that cause these variations and these factors include amino acid profile, reduced levels of crude protein, industrial amino acid or different levels of protein in diets of broilers [40]. Albino *et al.*, (1999), also described that feed consumption can be altered by the amino acid imbalance or deficiency in diets of birds [54]. On the other hand, Smriga *et al.* (2004) found that if a diet contains suboptimal amino acid level, then birds try to meet the requirement and more intake of diet was observed [39]. Soltan (2009) reported regarding to supplementation of 0.5% and 1% of glutamine in the broiler diets was non-significantly effect on the feed intake, but in higher glutamine level in broiler diet decreased the feed consumption [31]. Higher level of glutamine (4%) in the broiler diet, decrease the feed consumption [16].

Birds fed diet containing different levels of glutamine showed similarity in their consumption of glutamine (Table 6). Regardless the lysine concentration consumed by the birds, the diet contains 3% of glutamine showed significantly

Table 6. Mean glutamine consumption per bird of broiler chicken fed diets varying in lysine and glutamine concentrations.

Lysine	Glutamine	Weeks of age				Total
		1	2	3	4	
		g/bird/week				
0.85	0	0.00 ^g	0.00 ^g	0.00 ⁱ	0.00 ^h	0
0.85	1	0.80 ^f	1.43 ^f	2.41 ^h	3.10 ^g	7.74
0.85	2	1.63 ^d	2.99 ^e	4.56 ^g	6.32 ^f	15.50
0.85	3	2.21 ^b	5.45 ^d	8.52 ^e	10.93 ^d	27.11
1.14	0	0.00 ^g	0.00 ^g	0.00 ⁱ	0.00 ^h	0
1.14	1	1.00 ^e	2.76 ^e	5.25 ^f	6.50 ^f	15.51
1.14	2	1.91 ^c	6.13 ^c	10.98 ^c	12.94 ^c	31.96
1.14	3	2.98 ^a	7.08 ^b	12.03 ^b	16.06 ^b	38.15
1.42	0	0.00 ^g	0.00 ^g	0.00 ⁱ	0.00 ^h	0
1.42	1	0.98 ^e	3.04 ^e	5.07 ^{fg}	7.86 ^e	16.95
1.42	2	2.21 ^b	5.37 ^d	9.30 ^d	12.94 ^c	29.82
1.42	3	3.13 ^a	8.07 ^a	14.28 ^a	21.21 ^a	46.69
	SEM	0.03	0.08	0.11	0.18	
	Lys	<0.0001	<0.0001	<0.0001	<0.0001	
	Gln	<0.0001	<0.0001	<0.0001	<0.0001	
	Lys * Gln	<0.0001	<0.0001	<0.0001	<0.0001	

^{a-h}Means within a column with different superscripts differ significantly ($P < 0.05$).

higher ($P < 0.05$) consumption of glutamine compared with, 1% and 2%. The diet contains 1% of glutamine show significantly lower ($P < 0.05$) consumption of glutamine per bird. The diet contains 2% of glutamine show in between value of diet contain 3% and 1%, but these showed significantly different ($P < 0.05$) from all other diets. Higher the concentration of lysine in the diet higher the bird consumed glutamine. The average glutamine consumption per bird in different level of glutamine were also significantly different ($P < 0.05$) from each other. The glutamine consumption per bird were also significantly ($P < 0.05$) affected due the interaction of lysine and glutamine.

There is higher variation was in the data of lysine consumption per bird, shown in **Table 7**. The significant higher ($P < 0.05$) lysine was consumed per bird fed the diet containing 1.42 of lysine. The lower consumption of lysine per bird was observed in the diet containing 0.85 of lysine regardless the glutamine concentration and these are significantly different ($P < 0.05$) from the other diets contain 1.14 and 1.42 of lysine. The diet contains 1.14 of lysine show in between value of diet contain 0.85 and 1.42, but these showed significantly ($P < 0.05$)

Table 7. Mean lysine consumption per bird of broiler chicken fed diets varying in lysine and glutamine concentrations.

Lysine	Glutamine	Weeks of age				Total
		1	2	3	4	
g/bird/week						
0.85	0	0.64 ^e	1.34 ^f	2.01 ^d	2.47 ^e	6.46
0.85	1	0.60 ^e	1.07 ^f	1.81 ^d	2.33 ^e	5.81
0.85	2	0.61 ^e	1.12 ^f	1.71 ^d	2.37 ^e	5.81
0.85	3	0.55 ^e	1.36 ^f	2.13 ^d	2.73 ^e	6.77
1.14	0	0.89 ^d	2.15 ^e	3.75 ^c	4.77 ^d	11.65
1.14	1	1.00 ^{cd}	2.76 ^d	5.25 ^b	6.50 ^e	15.51
1.14	2	0.95 ^d	3.06 ^{cd}	5.49 ^b	6.47 ^e	15.97
1.14	3	0.99 ^d	2.36 ^e	4.01 ^c	5.35 ^d	12.71
1.42	0	1.10 ^c	3.14 ^c	5.21 ^b	6.34 ^e	15.79
1.42	1	1.23 ^b	3.80 ^a	6.34 ^a	9.83 ^a	21.20
1.42	2	1.39 ^a	3.49 ^{ab}	6.14 ^a	8.44 ^b	19.46
1.42	3	1.30 ^{ab}	3.36 ^{bc}	5.95 ^a	8.84 ^b	19.45
	SEM	0.02	0.06	0.09	0.13	
	Lys	<0.0001	<0.0001	<0.0001	<0.0001	
	Gln	<0.0001	<0.0001	<0.0001	<0.0001	
	Lys * Gln	<0.0001	<0.0001	<0.0001	<0.0001	

^{a-d}Means within a column with different superscripts differ significantly ($P < 0.05$).

different from all other diets. Higher the concentration of lysine in diet showed higher consumption lysine per bird. Overall, in data of combined glutamine and lysine consumption per bird, the diet containing lysine 1.42 and 1.14 with glutamine 1% have the higher consumption of lysine per bird. The different level of lysine was also significantly different ($P < 0.05$) from each other. The lysine consumption per bird were also significantly ($P < 0.05$) affected due the interaction of lysine and glutamine.

4. Feed Conversion

The average feed conversion of broilers Chicken that were fed the diet supplemented with different levels of Lysine and Glutamine are shown in **Table 8**.

After first week (0 - 7 days), the diet supplemented with 1.42 lysine with 0% and 1% glutamine showed significantly higher ($P < 0.05$) feed conversion when compared to the diets supplemented with 0.85 lysine and either 0%, 1%, or 2% of glutamine, 1.14 lysine with 0% glutamine. The similar result was found with diets supplemented with 1.14 and 1.42 lysine and 1% of glutamine that were

Table 8. Mean feed conversion of broiler chicken fed diets varying in lysine and glutamine concentrations.

Lysine	Glutamine	Weeks of age				Average
		1	2	3	4	
g feed/g body weight gain/week						
0.85	0	1.21 ^{abc}	1.63 ^{abc}	1.62 ^{bc}	1.55 ^{abc}	1.50
0.85	1	1.35 ^a	1.66 ^{abc}	2.00 ^{ab}	1.83 ^a	1.71
0.85	2	1.32 ^{ab}	1.76 ^{ab}	2.01 ^{ab}	1.71 ^{ab}	1.70
0.85	3	1.07 ^{abcde}	1.87 ^a	2.09 ^a	1.65 ^{abc}	1.67
1.14	0	1.16 ^{abcd}	1.44 ^{bc}	1.57 ^c	1.47 ^{bc}	1.41
1.14	1	0.87 ^{de}	1.52 ^{abc}	1.37 ^c	1.43 ^{bc}	1.29
1.14	2	0.99 ^{cde}	1.66 ^{abc}	1.62 ^{bc}	1.54 ^{abc}	1.45
1.14	3	0.96 ^{cde}	1.48 ^{abc}	1.48 ^c	1.51 ^{abc}	1.35
1.42	0	0.82 ^e	1.32 ^c	1.53 ^c	1.37 ^c	1.26
1.42	1	0.82 ^e	1.55 ^{abc}	1.50 ^c	1.47 ^{bc}	1.33
1.42	2	1.01 ^{bcde}	1.47 ^{abc}	1.39 ^c	1.33 ^c	1.30
1.42	3	0.93 ^{cde}	1.56 ^{abc}	1.40 ^c	1.34 ^c	1.30
	SEM	0.06	0.08	0.08	0.07	
	Lys	<0.0001	<0.0001	<0.0001	<0.0001	
	Gln	0.1307	0.0471	0.4979	0.2636	
	Lys * Gln	0.0033	0.4701	0.0024	0.2525	

^{a-f}Means within a column with different superscripts differ significantly ($P < 0.05$).

significantly higher ($P < 0.05$) feed conversion. The diet supplemented with 0.85 lysine and 1% glutamine show significantly low ($P < 0.05$) feed conversion. Overall, the diet supplemented with 1.42 with either 0% or 1% glutamine had higher average feed conversion when compared with all other treatments. These results was supported by Acar *et al.*, (2001) who reported that improved feed conversion was observed in the bird, supplemented with higher level of lysine in their diets [55]. The average feed conversion at different levels of lysine were significantly different ($P < 0.05$) from each other, however the average feed conversion in different level of glutamine were not significantly different from each other. There were significant interactions in feed conversion ($P < 0.05$) between lysine and glutamine dietary treatments.

After the second week (8 - 14 days), in the diets that contains 0.85 lysine with 0%, 1%, 2%, and 3% of glutamine were not significantly different from each other, however these showed significantly lower ($P < 0.05$) feed conversion from all other diets, whereas, the diet supplemented with 1.42 lysine and 0% of glutamine had significantly higher ($P < 0.05$) feed conversion from all other diet ex-

cept diets containing 0.85 lysine with 2% and 3% glutamine. Overall, during second week, the diet containing 1.42 lysine with either 0% or 1% glutamine showed better results. The average feed conversion at different levels of lysine were significantly different ($P < 0.05$) from each other, also the average feed conversion in different level of glutamine were significantly different from each other ($P < 0.05$). However, there were no significant interactions between lysine and glutamine present during second week.

After the third week (15 - 21 days), the diet supplemented with 1.14 and 1.42 lysine with either 0%, 1%, 2% or 3% glutamine showed significantly higher ($P < 0.05$) feed conversion than birds supplemented 0.85 lysine with 0%, 1%, 2%, and 3% glutamine. The diet supplemented with 0.85 lysine and 3% glutamine showed significantly lower ($P < 0.05$) feed conversion than other dietary treatments with the exception of birds fed diet containing 0.85 lysine with 0%, 1%, and 2% glutamine. Different levels of lysine showed significantly different ($P < 0.05$) however, different levels of glutamine were not significantly different from each other. The variation in the data of feed conversion showed a significant interaction ($P < 0.05$) between lysine and glutamine. Overall, the diet containing 1.14 lysine with 1% glutamine showed better results after the end of the third week. These results were supported by Oliveira *et al.*, (2013) who reported that the broiler during 8 - 21 days showed improved feed conversion and body weight gain when the lysine level was higher in the diet of the chicks. Soltan, (2009), reported the same results that feed conversion was much improved when the broiler diet contains 1% of glutamine [31].

After the fourth week (22 - 28 days), the diet supplement with 1.42 lysine with 0%, 2% and 3% glutamine showed significantly improved ($P < 0.05$) feed conversion however, results from all diets supplemented with 0.85 lysine with 1% and 2% glutamine showed constant result after each week. On the other hand, diets containing 1.14 and 1.42 lysine with 0%, 1%, 2%, and 3% glutamine and 0.85 lysine with 0% and 3% glutamine were not significantly different from each other. Different levels of lysine showed significant differences ($P < 0.05$) in average feed conversion, however, different levels of glutamine were not significantly different from each other. The data of feed conversion showed no significant interaction between lysine and glutamine.

Overall, after the whole period of study (0 - 28 days), the diet supplemented with 1.42 and 1.14 showed improvement in feed conversion with 0% and 1% of glutamine, respectively. Earlier, Yi *et al.*, (2001) got the same result of improvement in feed conversion when 1% glutamine supplement in turkeys' diet. There is various literature that reported that higher lysine in the broiler diet significantly ($P < 0.05$) improves the feed conversion [56] [57] [58].

5. Conclusion

On the basis of the result found in the present study, a dietary higher level of lysine showed a positive effect on the parameter of the performance, while an op-

timium level of glutamine showed a positive effect on the performance parameters when there was a higher concentration of lysine. Overall, the highest performance by the mean of body weight gain and feed consumption was found in the birds fed the diet supplemented with 1.42 lysine with 1% glutamine, but the improved feed conversion was found with a diet containing 1.14 and 1.42 with 1% and 0% glutamine, respectively. Generally, 1% glutamine regardless of the supplemented lysine had the highest overall performance by means of body weight gain, feed consumption, and feed conversion. Overall, poor performance was related to a diet containing higher glutamine levels. The lysine requirements in the diet for Chicken are between 1.14 to 1.42 with a glutamine level of 1%. In the birds, the highest performance by the mean of body weight gain and feed consumption was found in the birds fed the diet supplemented with 1.42 lysine with 1% glutamine, but the improved feed conversion was found with a diet containing 1.14 and 1.42 with 1% and 3% glutamine, respectively. Birds fed 1.42 lysine and 1% glutamine had the highest total body weight gain and feed consumption, as well as glutamine consumption. Experimental results suggest that a higher level of lysine improves bird performance, whereas, glutamine can improve the performance of birds in the presence of a sufficient level of supplemented lysine. The lysine requirements in the diet for Chicken are between 1.14 and 1.42 with glutamine level of 1%.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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