Litter quality of broiler fed with to different levels of sulfur amino acid

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Abstract The objective of this study was to determine the effect of sulfur amino acid (methionine+cystine) supplementation included in the diet of broiler chickens raised under Brazilian commercial conditions on the concentration of ammonia gas, moisture, pH, temperature, and nitrogen excretion in litter on the 14th, 28th, and 42nd day of breeding. A total of 900 male chicks of the Cobb500 line were used, distributed in a completely randomized design with five treatments, with six replicates of 30 birds. A basal diet (without methionine) was formulated and was supplemented with DL-methionine (0.072, 0.168, 0.239, 0.311% and 0.058, 0.134, 0.192, 0.250% for days 1 to 21 and days 22 to 42 of breeding respectively) replacing the corn starch in order to achieve the desirable digestible methionine + cysteine levels (0.545 (basal diet), 0.616, 0.711, 0.782 and 0.853%) and (0.514 (basal diet); 0.571; 0.647; 0.704 and 0.761% and digestible methionine + cysteine for the phase 1 to 21 and 22 to 42 days of breeding, respectively. There was no significant effect on the temperature and concentration of ammonia gas in any of the phases evaluated. Met+cys supplementation influenced moisture and pH of litter in all the evaluated phases. For nitrogen, a significant effect was observed at 14 days, not exhibiting effects during the other phases, suggesting that nitrogen excretion increases with increasing levels of met+cys in the diet for up to 14 days.

Keywords: crystalline amino acid, poultry, litter, methionine+cystine

Introduction

A major problem in the poultry industry concerns amino acid nutrition, of which poultry require a perfect balance. Imbalances influence the quality of the breeding environment, and could be responsible for excess nutrient deposition in litter, which in turn may impact the environment.

Methionine is the first limiting amino acid in corn- and soybean-based poultry diets (Kim et al 2006; Wen et al 2014; Zhang et al 2015). The nitrogen and sulfur atoms that constitute part of the methionine structure may, upon its breakdown, yield odoriferous volatile compounds in henhouses, such as mercaptan, hydrogen sulfide and dimethyl sulfide, phenol, cresol, propionic acid, and butanoic acid, through the microbial decomposition of the litter (Pillai 2011; Murphy et al 2014).

In Brazil, broiler chicken litter is commonly used in the fertilization of pastures and of crops such as eucalyptus, coffee, oranges, and vegetables. Its application provides nutrients such as P, K, and N, as well as other micro- and macro-nutrients; an average unit of litter contains 4% N (Assity-Duffey et al 2015). Excess N excretion by poultry arises mainly from dietary amino acids, and may increase the volatilization of ammonia from animal production systems, which can affect air quality (Carter e Kim 2013).

Factors such as litter moisture, pH, and temperature influence microbial aerobic degradation reactions, which convert uric acid to ammonia and carbon dioxide, which are found in henhouses (Li 2006). The litter moisture level is critical in hen house management, as it influences the severity and incidence rate of injuries in carcasses, and controls the volatilization of ammonia gas, since increased moisture enhances its release (Qiu e Guo, 2010).

The accumulation of ammonia and fecal matter raises the litter pH, which typically ranges between 7.0 and 8.5 (Rehbeger 2002). pH values above 7.0, when combined with high moisture, typically stimulate bacterial growth in the litter and increase ammonia production (Traldi et al 2007). Ammonia levels should be kept below 10 ppm in all breeding stages (Cobb-Vantress 2008).

Variables such as pH, moisture, temperature, nitrogen excretion, and concentration of ammonia gas in poultry litter, and their indirect influence on performance and direct influence on the quality of the breeding environment, render their evaluation under different nutritional conditions important.

The present study aimed to determine the effect of sulfur-containing amino acid supplementation (methionine+cysteine) in the diet of broiler chickens (bred under Brazilian commercial conditions) on the concentration of ammonia gas, moisture, pH, temperature, and nitrogen excretion in litter after 14, 28, and 42 days of breeding.

Materials and Methods

The experiment was conducted between December 2014 and January 2015 at the Industrial Aviary of the Department of Animal Science, School of Veterinary and Animal Science, Federal University of Goiás in Goiânia, Goiás, Brazil (Latitude 16º40'43"S, longitude 49º15'14"W, altitude 749 m). Nine-hundred male Cobb500 broiler chicks were distributed in a completely randomized design of five treatments, each with six replicates of 30 birds.

This study was conducted in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol procedures with experimental animal use was approved by the Ethics Committee for Animal Experimentation of the Federal University of Goiás (Goiás, Brazil), under number 010/2015.

The birds were housed in 30 individual cages measuring 1.80×1.60 m (2.88 m²), mounted in the central area of the negative pressure industrial henhouse measuring 12×125 m (1,500 m²), containing seven exhaust fans, a misting system, and an air inlet with evaporative plate. The configuration of the hen house also included masonry side guard rails measuring 0.40 m in height, and a 2.80 meter-high wire mesh, with a ceiling height of 3.20 m, in an east-west orientation. The birds were raised according to Brazilian trading conditions.

Each cage contained a water dispenser with 10 nipple-type outlets, plus a chick- or adult-appropriate tube bird feeder (for ages of 1–7 and 8-42 days, respectively). The ratios of feeder and water dispenser per number of birds were 1:30 and 1:3, respectively. The birds were vaccinated in the hatchery against Marek's disease, and against Gumboro disease through drinking water at 14 days of age. Throughout the experimental period, the birds were provided feed and water "ad libitum", and raised following the lighting, temperature, moisture, and management recommendations of the Cobb-Vantress Management Guide (2008).

The control feed was not supplemented with the addition of DL-Methionine (99%). The methionine+cysteine

(%) content was acquired solely from the ingredients used in the feed and was thus the lowest. The experimental feeds were supplemented with increasing DL-Methionine levels, (0.00, 0.072, 0.168, 0.239, 0.311% and 0.00, 0.058, 0.134, 0.192, 0.250% in the initial and growth phases respectively) which replaced the inert material (corn starch) in the basal feed, resulting in experimental feeds containing (0.545% - basal diet; 0.616%, 0.711%; 0.782%, 0.853%) and (0.514% - basal diet, 0.571%, 0.647%, 0.704%, 0.761%) of digestible methionine+cystine in the initial and growth phases (Table 1), respectively.

The diets were based on corn and soybean meal and formulated according to the nutritional recommendations of the supplier, based on (Rostagno et al 2011).

The first batch of rice husk litter with a height of 8–10 cm was used. The litter was collected after 14, 28, and 42 days of breeding and analyzed for moisture, pH, volatilized ammonia, and total nitrogen.

The temperature evaluation was performed in loco using a Non-Contact Infrared Thermometer with Laser Targeting (Cen-tech ITEM 69465). The procedure to determine the pH and moisture, volatilized ammonia, and total nitrogen contents involved collecting litter at six different points in each box separately, immediately after temperature evaluation, avoiding the areas adjacent to and below the feeding tubes and water dispensers.

Possible alterations in moisture, pH, and ammonia content measurements were circumvented by immediate analysis after collection in the Food Analysis Laboratory of the DPA/EVZ, UFG-Goiânia-GO. The pH was measured using a digital pH meter, PM608 ANALION®, according to the methodology described by (Oliveira et al 2003).

The amount of volatilized ammonia in the litter was determined using the method described by (Hernandes et al 2001), using the formula: $A = V \times 17 \times 0.05$, wherein A = amount of volatilized ammonia (mg/100 g), V= volume of H2SO4 used for titration (mL); 17= molecular weight of ammonia, and 0.05 = H2SO4 normality. A 100-g sample was used for ammonia determination.

Litter samples were ground after pre-heating, and subsequently subjected to moisture and nitrogen determination. The moisture content was determined according to the recommendations by the Ministry of Agriculture (Brasil 1992).

The litter nitrogen content was determined according to the Dumas (1831) method using an automated apparatus (Leco) at the Food Analysis Laboratory of the São Paulo State University "Júlio de Mesquita Filho" UNESP Jaboticabal Campus, SP.

An analysis of variance (ANOVA) was performed to assess the significance of treatment effects. Upon significant difference between treatments, a polynomial regression analysis ($P < 0.05$) was applied using R computer software.

Table 1 Percentage composition and nutritional values of the experimental diets for broilers.

Ingredients (%)	Initial phase (1 to 21 days)	Growth phase (22 to 42 days)
Corn (%)	62.99	64.56
Soybean meal 45.5% (%)	28.38	24.56
Meat and bone meal 44% (%)	3.20	2.60
Poultry by-Product meal (%)	2.00	2.67
Limestone 37% (%)	0.62	0.65
Salt (%)	0.40	0.24
Poultry Fat (%)	1.33	3.81
DL-Methionine 99% (%)	0.00	0.00
Biolys 70% (%)	0.40	0.34
L-Threonine 98% (%)	0.10	0.07
Choline 75% (%)	0.06	0.03
Others (%)	0.13	0.13
Starch (%)	0.29	0.24
Mineral Supplement[1] (%)	0.05	0.05
Vitamin Supplement[2] (%)	0.05	0.05
Total (%)	**100.0**	**100.0**
Calculated composition		
Metabolizable Energy (kcal/kg)	3109	3300
Crude Protein (%)	21,803	20,21
Calcium (%)	0,952	0,903
Available Phosphorus (%)	0,451	0,421
Digestible Methionine+Cysteine (%)	0,545	0,514
Digestible Lysine (%)	1,190	1,058
Digestible Threonine (%)	0,749	0,066
Sodium (%)	0,201	0,190

[1]Mineral supplement per kg of feed (Mineral mix): Mn, 60 g; Fe, 80 g; Zn, 50 g; Cu, 10 g; Co, 2 g; I, 1 g; vehicle up to 500 g. [2]Vitamin supplement per kg of feed (Protein mix): Vit. A - 15,000,000 IU; Vit. D3 - 1,500,000 IU; Vit. E - 15,000 IU; Vit. B1 - 2.0 g; Vit. B2 - 4.0 g; Vit B6 - 3.0 g; Vit. B12 - 0.015 g; Nicotinic acid - 25 g; Pantothenic Acid - 10 g; Vit. K3 - 3.0 g; Folic acid - 1.0 g; Zinc bacitracin - 10 g; Selenium - 250 mg; vehicle up to 1,000 g.

Table 2 Moisture, temperature, pH, nitrogen, and ammonia concentration in a litter of 14-day-old broilers subjected to different levels of digestible met+cys.

Met+cys levels (%)	Variables				
	Moisture	Litter temperature	pH	Nitrogen	Ammonia
Basal	9.3167	24.4667	7.2950	1.9050	1.3917
0.616	12.4100	24.9333	7.2217	2.0050	1.4050
0.711	11.9783	24.5000	7.0550	2.4267	1.9850
0.782	12.1817	24.7667	7.1600	2.2033	1.8150
0.853	10.7733	24.9500	6.9950	2.4383	1.6167
CV%	19.50	2.59	3.34	14.57	29.62
Regression	**	ns	*	*	ns

Variables	Regression equation		Effect
Moisture	$\hat{Y} = -38.4347 + 142.3352x - 99.3523x^2$	$R^2 = 0.82$	Quadratic
pH	$\hat{Y} = 7.7447 - 0.8545x$	$R^2 = 0.74$	Linear
Nitrogen	$\hat{Y} = 1.0363 + 1.6529x$	$R^2 = 0.70$	Linear

CV%: Coefficient of Variation. *, ** Linear and Quadratic effect with ($P < 0.05$), respectively.

Results and Discussion

The litter temperature, pH, moisture, concentration of ammonia, and total nitrogen excretion content on the 14th day of breeding for poultry fed with different levels of digestible methionine+cystine are shown in Table 2.

There was an influence ($P < 0.05$) of digestible met+cys levels on nitrogen excretion, moisture, and pH of the litter (Table 1; Figures 1A, B, and C, respectively).
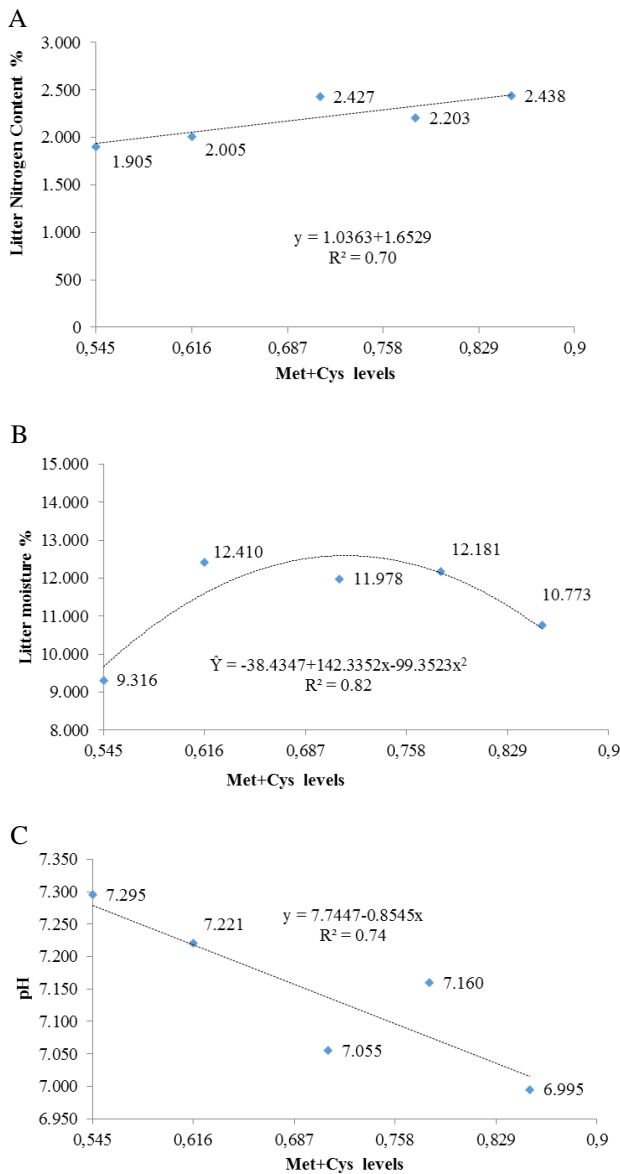
Figure 1 Results of the nitrogen concentration (A), moisture (B), and pH (C) in litter of 14-day-old broilers bred with different levels of digestible methionine+cystine.

In Figure 1A, there is a linear correlation between digestible met+cys content and nitrogen excretion ($\hat{Y}$ = 1.0363 + 1.6529x; R² = 0.70), showing that an increase in digestible met+cys content increases nitrogen excretion in the litter.

The results suggest that the presence of non-starch polysaccharides (NSPs), which are plant cell wall components, present in corn and also in greater amounts in soybeans, contributed to imbalances in dietary amino acid, enhancing N excretion in the litter. The lack of one dietary amino acid may have enhanced the catabolism of other amino acids, and consequently the higher nitrogen excretion at this phase.

Non-ruminant animals acquire nitrogen from their diet in the form of proteins and other foodstuff (Bergen e Wu 2009). Protein digestion in poultry yields amino acids and peptides, which are, in part, absorbed by the organism and used in new protein synthesis, and in part, eliminated by the kidneys (Corzo et al 2011). Thus, poultry excretion primarily serves to eliminate nitrogenous substances (Donsbough et al 2010); uric acid is the primary metabolite excreted in urine (as a whitish paste).

Excess amino acids (i.e. in levels above requirements) are deaminated, and nitrogen (N) is excreted as uric acid (Namroud et al 2008). Amino acid shortage can enhance the catabolism of other amino acids (Bertechini, 2006). Excess or insufficient supplementation of dietary methionine increases nitrogen excretion and environmental emissions (Kim et al 2006). Nitrogen excretion can be decreased by adapting the amount of protein in feed to the requirements of the different phases of broiler breeding (Oviedo-Rondón, 2008; Dozier et al 2008).

NSPs adversely compromise the bioavailability of important nutrients to poultry such as N and P, which are consequently eliminated in excess in the litter and which become harmful to the environment (Alvarenga et al 2011).

Regression analysis showed a quadratic effect of digestible met+cys levels on litter moisture ($\hat{Y}$ = −38.4347 + 142.3352x − 99.3523x2; R² = 0.82). Moisture increased with the digestible met+cys content; according to the obtained equation, the largest moisture content was observed in diets supplemented with 0.716% digestible methionine+cysteine, reaching a plateau from then onwards (Figure 1B).

Litter moisture is a major concern in broiler production due to its negative impact on poultry health, well-being, and productive performance (Owada et al 2007; Qiu e Guo, 2010; Garcia et al 2012; Sharma et al 2016). Multifactorial causes influence litter moisture in an industrial henhouse, stemming from interactions between bird nutrition, handling, and intestinal health (Oviedo-Rondón, 2008). Sodium, chlorine, potassium, and dietary fiber levels; the digestibility of dietary protein; water consumption; inadequate use of phytase in feed; type of water dispenser; and ventilation, among others, are noteworthy factors that contribute to litter moisture (Oviedo-Rondón, 2008; Qiu e Guo, 2010).

An increase in dietary protein enhances the consumption of water and consequently its content in excreta (Ziaein et al 2007; Collett, 2012). This phenomenon becomes more relevant when the dietary protein content is higher or lower than the desired level, i.e., when the amino acid profile is imbalanced (Collet, 2006; Namroud et al 2009). In such cases, excess amino acids—which are not used in synthetic pathways—should be catabolized, with nitrogen eliminated as uric acid through excreta and urine (Kim et al 2006; Van der Hoeven-Hangoor et al 2014), which consequently increases water loss.

In birds, the elimination of nitrogen as uric acid employs specific enzymes, with a concomitant loss of water;

this represents an important adaptation of birds to the terrestrial environment (Devilin, 2011; Capitelli e Crosta, 2013). Elevated nitrogen excretion correlates with higher moisture content in excreta (Namroud et al 2008; Van der Hoeven-Hangoor et al 2014).

Thus, the present study indicates that the low litter moisture in treatments with lower dietary digestible met+cys levels can be ascribed to the lower excretion volume of uric acid.

The hydrogen potential (pH) (Figure 1C) correlated in a negative linear manner with dietary digestible methionine+cysteine content (P < 0.05), i.e., the former decreased as the latter increased. The highest digestible met+cys content (0.853%) yielded the lowest pH value (6.99).

Increased methionine+cysteine levels exerted no effect (P < 0.05) on litter temperature and concentration of ammonia of 14-day-old broilers. The conversion of nitrogen to ammonia in excreta is related to litter temperature, moisture, and pH, and the ventilation rate of the breeding environment (Miles, 2008). In poultry manure, the dominant nitrogen form is the ammonium ion (NH_4^+), which is converted into ammonia (NH_3^+) with increasing pH and humidity (Oviedo-Rondón, 2008).

The concentration of ammonia gas tends to increase with an increase in excreta moisture content; several microbial species depend on water and are activated by the increased litter moisture. Some studies have found that the water content in litter affects microbial activity (Wadud et al 2012), ammonia emissions, and odors (Miles et al 2011; Sharma et al 2015).

Equipment that improve hen house internal conditions in broiler breeding, such as misting systems, are employed virtually only in the final breeding phase; the fact that water consumption in the first breeding phase is minimal, generated a consensus that the use of water in poultry facilities is lower in the first weeks of birds' lives, thereby reducing the excreta volume (uric acid + water) produced.

Thus, the present study indicates that litter moisture content is below the ideal value for enhanced microbial proliferation, yielding an insignificant ammonia concentration.

An analysis of Table 3 and Figures 2A and B found statistical differences for litter moisture and pH among the variables evaluated in 28-day-old-birds.

Table 3 Moisture, temperature, pH, nitrogen, and ammonia concentration in litter at a broiler age of 28 days, subjected to different levels of digestible met+cys.

Met+cys levels (%)	Variables			
	Moisture	Litter temperature	pH	Ammonia
Basal	12.1583	27.8833	8.3200	5.1033
0.571	17.0833	27.5000	8.3040	5.0883
0.647	17.6517	28.0500	8.2033	4.8900
0.704	17.2833	27.8500	8.0983	4.5917
0.761	18.1633	27.4000	8.1417	4.2683
CV%	21.73	4.16	1.93	30.54
Regression	*	ns	*	ns

Variables	Regression Equation	Effect
Moisture	$\hat{Y} = 4.0411+19.4353x$ ($R^2 = 0.60$)	Linear
pH	$\hat{Y} = 8.7875-0.8994x$ ($R^2 = 0.84$)	Linear

CV%: Coefficient of Variation. *, ** Linear and Quadratic effect with (P < 0.05), respectively.

Litter moisture (Figure 2A) correlated linearly (P < 0.05) with increased levels of digestible dietary methionine+cysteine.

Poultry whose diets were supplemented with the highest levels of digestible met+cys consumed more water and eliminated larger volumes of uric acid and water in excreta, thereby increasing litter moisture.

There is evidence that the consumption of water affects the nitrogen metabolism of non-ruminant animals. (Bressani e Braham, 1964) assumed that a high consumption of water (either ad libitum or forced) in non-ruminant animals led to a decrease in nitrogen retention.

For pH values, a negative linear correlation was observed (P < 0.05), i.e., its value in the litter decreased as dietary digestible met+cys increased. The pH value remained in the alkaline region in all treatments after 28 days of breeding, which compromised the internal conditions of the henhouse. One of the most important factors that can affect NH_3 release is litter pH (Choi e Moore, 2008). Small quantities of ammonia are released under acidic pH; however, pH values above 8.0 favor the decomposition of uric acid ($C_5H_4N_4O_3$) (Hristov et al 2011; Behera et al 2013). H^+ ions and pH values below 7.0 decrease the NH_3/NH_4^+ ratio, leading to a reduction of nitrogen losses from ammonia volatilization, as the ammonium ion is non-volatile (Hristov et al 2011; Behera et al 2013). pH values below 7.0, in addition to reducing the litter bacterial load, reduce ammonia volatilization, thereby improving the

environmental conditions of the aviary (Oviedo-Rondón, 2008; Carvalho et al 2011).
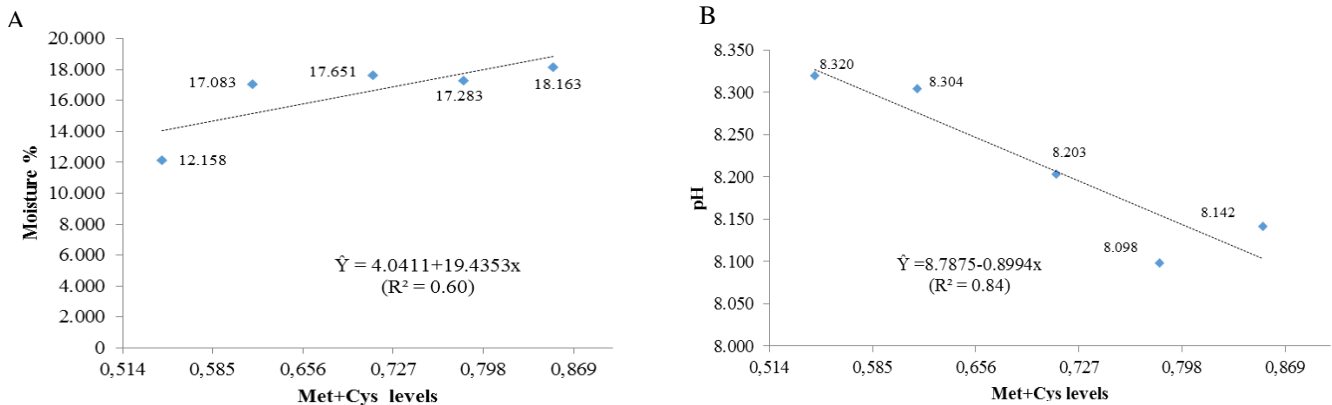
Figure 2 Litter moisture (A) and pH (B) after 28 days of broiler age, subjected to different levels of digestible methionine+cystine.

The present study showed that an alkaline pH value—favorable to microbial growth in the litter—did not compromise ammonia concentration, and it is thus likely that this increase in pH stemmed from the increased accumulation of excreta and the gradual increase of litter moisture as poultry breeding progressed, and not from the dietary digestible met+cys content.

No significant difference was found (P > 0.05) in ammonia levels between the different treatments at the 28th day of breeding (Table 2).

The litter nitrogen content, moisture, pH, and the ammonia volatilization potential at the 42nd day of breeding are described in Table 4.

Table 4 Litter moisture, temperature, pH, nitrogen, and ammonia concentration of 42-day-old-broilers subjected to different levels of digestible met+cys.

Met+cys levels (%)	Variables				
	Moisture	Litter temperature	pH	Nitrogen	Ammonia
Basal	20.6667	29.1400	8.6783	3.9500	14.8917
0.571	20.6600	28.3667	8.5567	3.8367	19.0567
0.647	19.6967	29.0833	8.6967	3.8567	14.0133
0.704	23.3550	27.9667	8.1367	3.7233	11.8017
0.761	27.6050	28.7000	8.6080	3.8533	14.9917
CV%	18.98	2.44	1.04	6.04	33.00
Regression	*	Ns	**	ns	ns
Variables	**Regression Equation**			**Effect**	
Moisture	$\hat{Y}=111.4610-312.2721x+65.4141x^2$ (R²=0.95)			Quadratic	
pH	$\hat{Y}=12.0161-10.1599x+7.2250x^2$ (R² = 0.13)			Quadratic	

CV%: Coefficient of Variation. *, ** Linear and Quadratic effect with (P < 0.05), respectively.

The methionine+cysteine content on the litter's hydrogenionic potential (pH) and moisture after 42 days exhibited a quadratic effect (P<0.05), $\hat{Y}= 12.0161 − 10.1599x + 7.2250x^2$ (R²= 0.13), $\hat{Y}= 111.4610 − 312.2721x + 265.4141x^2$, (R²= 0.95), with a positive trend up to 0.703% and 0.588% of methionine+cysteine, respectively (Table 4 and Figures 3A and B).
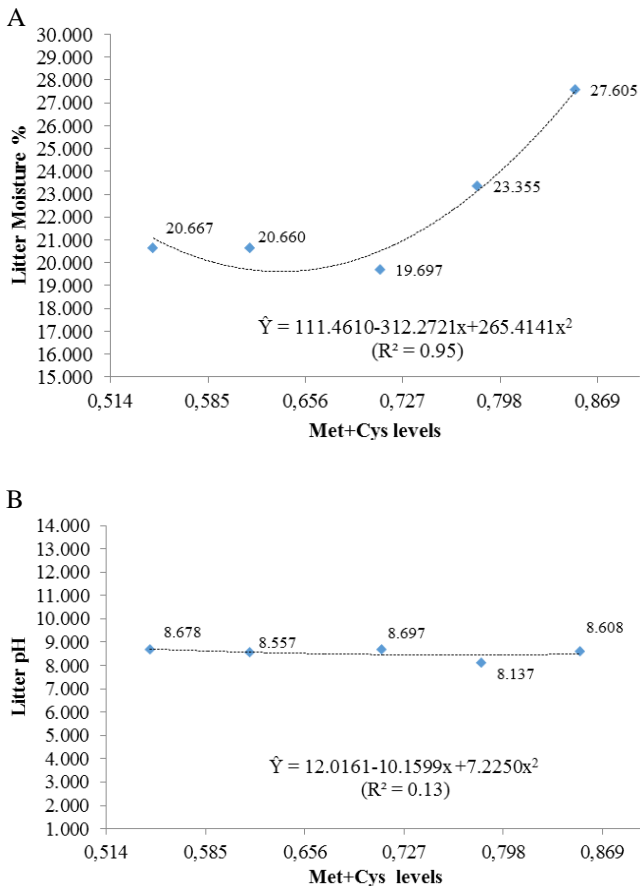
A litter with moisture content below 20% increases dust concentration within the facility, which irritates the respiratory system of birds and predisposes them to the development of infection. On the other hand, excessive litter moisture (above 35%) may cause health and wellbeing issues

in birds, increased incidence of breast lesions, skin burns, pododermatitis, convictions, and loss of quality in carcasses (Nagaraj et al 2007; Traldi et al 2007; Cobb-Vantress, 2008; Shepherd e Fairchild, 2010).

There was no influence of digestible met+cys content (P<0.05) on nitrogen excretion, ammonia concentration, and litter temperature on the 42nd day of breeding.

In the present study, an increase in litter temperature was observed throughout the evaluation period. This increase in litter temperature is related to the duration that birds spend lying down as they age.

The concentration of ammonia in the litter was not significantly affected (P < 0.05) in any of the evaluated periods; however, ammonia volatilization gradually increased with bird age. This increased concentration of ammonium in the litter is probably related to excreta produced with the increasing bird age, moisture, and litter pH alkalinity.

A

$\hat{Y} = 111.4610 - 312.2721x + 265.4141x^2$
$(R^2 = 0.95)$

B

$\hat{Y} = 12.0161 - 10.1599x + 7.2250x^2$
$(R^2 = 0.13)$

Figure 3 Litter moisture (A) and pH (B) after 42 days of broiler age, subjected to different levels of digestible methionine+cysteine.

Conclusions

Litter temperature and concentration of ammonia gas were not significantly affected in any of the evaluated phases. The different treatments exerted effects on litter moisture and pH in each evaluated phase. The nitrogen content was significantly affected after 14 days, but not in the remaining stages, suggesting that nitrogen excretion increases with increasing levels of dietary met+cys up to 14 days.

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