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Bioactive amines in ingredients and feeds of broilers and storage effects on their levels

Aminas bioativas em ingredientes e rações de frangos de corte e efeitos da estocagem nas concentrações destas

Aminas bioactivas en ingredientes y alimentos para pollos de engorde y efectos del almacenamiento en sus concentraciones

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Abstract
To quantify amines in ingredients and in different types of feed and to verify spermine inclusion influence in polyamine levels at storage of experimental diets of broiler, ingredients to formulate diets and commercial starting and growth feeds and were purchased. Spermidine, spermine, putrescine, cadaverine, agmatine, tyramine and histamine were detected by ion pair high performance liquid chromatography in ingredients, commercial diets, control and 0.06 and 012% spermine included formulations. Bioactive amines levels were analysed weekly to initial and growth diets, respectively until 21st and 49th days of storage at 26 ± 3°C and 72% relative humidity. All amines were detected in commercial feed. Spermidine and spermine predominate in soybean, wheat and corn, and tyramine in meat and bone meal. To initial diets averages of cadaverine reduced significantly in day 21, except for 0.06% spermine inclusion; agmatine decreased ($p<0.05$) in times, except for 0.12% spermine inclusion; spermidine were smaller ($p<0.05$) at 14 and 21 days and increased significantly with the level of inclusion. In growth diets cadaverine oscillated ($p<0.05$) between 0.35 at 28 days in control and 1.15 mg.100g-1 in 0.06% spermine inclusion at 49 days. Agmatine were 0.36 to 0.91 mg.100g-1 in control; spermidine vary significantly at 7 days in control and in 0.12% spermine inclusion at 28 days; spermine vary from 1.33 at 42 days to control to 72.55 mg.100g-1 at 7 days in 0.12% spermine inclusion. Polyamine levels were low in ingredients, commercial feed and initial and growth experimental formulations even when stored for 21 or 49 days.
Keywords: Feed; Avian; Food quality.

Resumo

Para quantificar aminas em ingredientes e rações e verificar a influência da inclusão de espermina no armazenamento de dietas experimentais de frangos de corte foram adquiridos insumos e rações comerciais inicial e de crescimento. Espermidina, espermina, putrescina, cadaverina, agmatina, tiramina e histamina foram detectadas por cromatografia líquida de alta eficiência de pares iônicos em ingredientes, dietas comerciais, controle e formulações com 0,06 e 012% de espermina. As bioaminas foram analisadas semanalmente nas dietas inicial e de crescimento, respectivamente, até o 21º e 49º dias de armazenamento a 26±3°C e 72% de umidade relativa. Todas foram detectadas nas rações comerciais. Espermidina e espermina predominaramm em soja, trigo e milho e tiramina, na farinha de carne e ossos. Em dietas iniciais, cadaverina reduziu significativamente no dia 21, exceto com 0,06% de espermina; agmatina diminuiu ($p<0,05$) nos tempos, exceto na inclusão de 0,12% de espermina; espermidina foi inferior ($p<0,05$) aos 14 e 21 dias e aumentou ($p<0,05$) com o nível de inclusão. Nas dietas de crescimento cadaverina (mg.100g-1) oscilou ($p<0,05$) entre 0,35 aos 28 dias no controle e 1,15 com 0,06% de espermina aos 49 dias; agmatina, entre 0,36 a 0,91 mg.100g-1 no controle; espermidina variou ($p<0,05$) aos 7 dias no controle e com 0,12% de espermina aos 28 dias e espermina (mg.100g-1), de 1,33 aos 42 dias para o controle a 72,55 aos 7 dias com 0,12% de espermina. As concentrações de poliamina foram baixas em ingredientes, rações comerciais e formulações inicial e de crescimento, mesmo quando armazenadas por 21 ou 49 dias.

Palavras-chave: Ração; Aves; Qualidade alimentar.

Resumen

Para cuantificar bioaminas en ingredientes y piensos y verificar la influencia de la inclusión de espermina en el almacenamiento de dietas para pollos, se adquirieron insumos y piensos de iniciación y crecimiento. Se detectaron espermidina, espermina, putrescina, cadaverina, agmatina, tiramina e histamina por cromatografía líquida de alta resolución de pares iónicos en ingredientes, piensos, formulaciones control y con 0,06 y 012% de espermina. Se analizaron semanalmente las dietas iniciadora y de crecimiento, respectivamente, hasta los días 21 y 49 de almacenamiento a 26±3°C y 72% de humedad. Todas fueron detectadas en piensos. Espermidina y espermina predominaron en soja, trigo y maíz y tiramina, en harina de carne y huesos. En dietas iniciadoras, cadaverina se redujo significativamente el día 21, excepto con 0,06% de espermina; agmatina disminuyó ($p<0,05$) con el tiempo, excepto con 0,12% de espermina; la espermidina fue menor ($p<0,05$) a los 14 y 21 días y aumentó ($p<0,05$) con el nivel de inclusión. En dietas de crecimiento, cadaverina (mg.100g-1) varió ($p<0.05$) entre 0.35 a los 28 días en control y 1.15 con 0.06% de espermina a los 49 días; agmatina, entre 0,36 a 0,91 mg.100g-1 en control; espermidina varió ($p<0.05$) a los 7 días en control y con 0.12% de espermina a los 28 días y espermina (mg.100g-1), de 1.33 a los 42 días en control a 72.55 a los 7 días con 0.12 % de espermina. Concentraciones bajas de poliaminas ocurriran en ingredientes, piensos y formulaciones de iniciación y crecimiento, incluso cuando se almacenaron durante 21 o 49 días.

Palabras clave: Alimento; Ave; Calidad alimentaria.

1. Introduction

Biogenic amines are low molecular weight nitrogenous compounds formed by microbial action during the storage of natural, processed and fermented foods of animal or plant origin (Assis et al., 2015; Brito et al., 2017; Driehuis et al., 2018). The formation of amines in foods requires free amino acids availability, which are formed during proteolysis via the action of endogenous or microbial enzymes. *Enterobacteriaceae*, *Clostridium* spp., *Lactobacillus* spp., *Streptococcus* spp., *Micrococcus* spp. and *Pseudomonas* spp. and other microorganisms that decarboxylate amino acids and can cause amines formation (Ahmad et al., 2020; Brito et al., 2017; Liu et al., 2020; Silva & Glória, 2002).

High concentrations of biogenic amines may exert toxic effects on broilers and other species; according to Gilbert et al. (2018) and Hashemi et al. (2014), increasing dietary levels of these amines results in a higher deposition of these amines in poultry tissues. Several biogenic amines, such as putrescine and cadaverine-formed by microbial action in products of animal origin-may be present in feed, especially when it is made from poor-quality raw materials or conditioned under improper storage conditions (Alvarez & Moreno-Arribas, 2014). According to Gilbert et al. (2018), high levels of phenylalanine, putrescine, cadaverine and histamine can cause chickens low performance and intestinal lesions that delay avian development.

Moreover, nitrites in food, or nitrites present in the saliva during food consumption, act on certain polyamines, such as spermine, and may form nitrosamines. Several of these have carcinogenic, mutagenic, teratogenic and embryopathic activity. The presence of high levels of polyamines in tissues can further cause putrefying odors and unpleasant flavors that affect meat quality (Ruiz-Capillas & Herrero, 2019).

Although biogenic amines already have been identified as toxic substances that cause disease in both humans (Zhang et al., 2019) and animals (Gilbert et al., 2018). Studies have suggested that the presence of polyamines in chicken diets is important because it can act as growth promoter (Hashemi et al., 2014) and do not cause health problems to broiler chicken (Bermudez & Firman, 1998). Among the polyamines, spermine and spermidine stabilize membranes, stimulate and regulate growth, participating nucleic acids and proteins synthesis (Lima & Glória, 1999; Lima et al., 2006). These substances also have mitogenic, metabolic and immunological effects, promoting growth and differentiation of the immature gastrointestinal tract (Bogusławska-Tryk et al., 2020; Liu et al., 2020).

Polyamines are inherent to cells; spermidine is predominant in plant tissues and spermine in animal tissues. Since most of the ingredients that make up the diet of poultry are of plant origin, the predominance of spermidine in feed is common (Lima et al., 2006; Liu et al., 2020). Meat and bone meal is the most susceptible ingredient to lead biogenic amines formation in broiler diets because microbiological deterioration of meat is relatively ease and microorganisms involved are capable to form amines (Silva & Glória, 2002; Brito et al., 2017; Caires et al., 2010; Liu et al., 2020).

Few studies have been evaluated the quality of commercial chicken diets, particularly those focused on bioactive amines levels. Due to the scarcity of studies and in order to clarify this gap, the aim of this work were to quantify amines in ingredients used in broiler diets production and in different types of feed and to verify the length of storage influence on polyamine levels in diets with spermine inclusion.

2. Methodology

2.1 Materials

Six brands of commercial feeds of both starting and growth types were purchased from the retail market of Belo Horizonte, Minas Gerais, Brazil. The formulations of the feeds were similar, and the ingredients were corn, wheat bran, soybean meal, and meat and bone meal. The experimental diets were formulated according to the descriptions in Table 1. It were made in the feed factory of the Prof. Hélio Barbosa Experimental Farm located at lgarapé, Minas Gerais, Brazil.

Table 1. Basic composition of initial and growth broiler chickens experimental diets

Ingredients	% of Dry Matter Basis	
	Initial	Growth
Calcitic limestone	0.511	0.714
DL-methionine	0.235	0.160
Meat and bone meal	5.089	4.211
Soybean meal	28.010	25.816
L-lysine	0.188	0.025
Corn	65.119	68.213
Mineral supplement[1]	0.100	0.100
Sodium Chloride	0.348	0.361
Vitamin and coccidiostatic supplement[2]	0.400	0.400
TOTAL	100.000	100.000

[1] Selenium-45mg; iodide - 175mg; Iron-12.525mg; Copper-2.500mg; Manganese-19.500mg; Zinc-13.750mg. [2] Chemical composition per kilogram of the product: Vitamin A 2.000.000UI; Vitamin D3 375.000UI; Vitamin E 3.750mg; Vitamin K3 500mg; Vitamin B1 250mg; Vitamin B2 750mg; Vitamin B6 500mg; Vitamin B12 3.750mcg; Niacin 6.250mg; Pantotenic Acid 2.500mg; Biotin 10mg; Folic Acid 125mg; Colin 75.000mg; Avilamicin 15.000mg; Narasin 12.250mg; Butyl Hydroxy Toluene 500mg; Vitamin C 12.500mg Source: From authors

The diets were transported to the Department of Technology and Inspection of the Veterinary School of the Federal University of Minas Gerais (UFMG), where it were stored for the duration of the study. Initial and growth diets contained 60 (0.06%) and 120 mg (0.12%) amines/100 g of feed. Spermine analytical (≥99.0% - Sigma Aldrich™) was incorporated into the

feed through a mixer in 50 kg aliquots to ensure homogeneity of the sample. The starting and growth diets were stored for 21 and 49 days, respectively, at 26 ± 3°C and 72% relative humidity.

2.2 Determination of bioactive amines

The feed samples were crushed in a multiprocessor (Arno™), homogenized, and used for the determination of bioactive amine levels. All samples were determined in duplicate. The extraction of the amines was performed by adding 7 mL of 5% trichloroacetic acid (TCA) to 5g of the comminuted and homogenized sample. The samples were shaken for 5 min. on a vortex mixer and centrifuged at 10,000x g at 4°C for 20 min. This step was repeated two more times with 7 and 6 mL of the TCA solution, respectively. The supernatants were combined, the volume was completed to 20 mL, and the final extract was filtered through a membrane of 0.45 µm and injected into the liquid chromatograph (Silva & Glória, 2002).

The separation of the amines was performed using µBondapak C_{18} reverse-phase columns (3.9 m × 300 mm, 10 µm, Waters™), µBondapak C_{18} pre-columns (Waters™) and an elution gradient system at room temperature (23.0 ± 1.0°C) in a liquid chromatograph. The mobile phases used included the following: A. 0.2 M acetate buffer containing 10 mM octanesulfonic acid sodium salt, pH adjusted to 5.0 with acetic acid; and B. 10 mM octanesulfonic acid sodium salt dissolved in 100 mL of water:acetonitrile:methanol (1:9:1, v/v), using the following schedules (tempo-min / %B): 0.01/12%; 20/12%; 22/13%; 35/13%; 43/26%; 66/26%; and 71/12% at a flow rate of 0.7 mL/min. The mobile phases were filtered through membranes (47 mm diameter and 0.45 µm pore size) of the HAWP type (Millipore Corporation™) for aqueous solvent and of the HVWP type (Millipore Corporation™) for organic solvent.

The liquid chromatography system consisted of a Shimadzu™ LC-10AD model device with a low pressure mixing chamber; piston automatic washing set; spectrofluorimetric detector RF-55 1 model (Shimadzu™) with 340 nm excitation and 445 nm emission and a CBM-10AD control unit connected to a microcomputer.

The post-column derivatization system was assembled using a mixing chamber (dead volume equal to zero) installed between the column outlet and the detector. A 2 m long and 0.25 mm diameter Teflon tube was attached (protected from light) between the mixing chamber and the detector. An LC-10AD (Shimadzu™) pump pumped the derivatizing solution into the mixing chamber at a flow rate of 0.4 mL/min. The derivatizing solution was prepared by dissolving 25 g of boric acid and 22 g of potassium hydroxide in 500 mL of liquid chromatography-grade water, pH adjusted to 10.5 with potassium hydroxide. A total of 1.5 mL of Brij 35 (Merck™), 1.5 mL β-mercaptoethanol (Merck™) and 0.2 g of o-phthalaldehyde (Sigma™) dissolved in 3.0 mL of methanol were added to this solution, as described by Vale and Glória (1997). The derivatizing solution was prepared daily, filtered with a HAWP membrane of 47 mm diameter and 0.45 µm pore size (Millipore Corporation™) and kept in the dark.

The identification of the bioactive amines was based on the retention times, and confirmation was achieved via the addition of a standard solution containing the suspected amine to the sample. The amine content was calculated directly by interpolation in the calibration curve.

The standard solution was prepared as follows: stock solutions I were prepared by weighing 17.6 mg of agmatine sulfate; 17.1 mg of cadaverine dihydrochloride; 16.6 mg of histamine dihydrochloride; 13.0 mg of 2-phenylethylamine dihydrochloride; 18.3 mg of putrescine dihydrochloride; 22.0 mg of 5-hydroxytryptamine (serotonin) complexed with creatinine sulfate monohydrate; 17.5 mg of spermidine trihydrochloride; 17.2 mg of spermine tetrahydrochloride; 10 mg of tryptamine and 10 mg of tyramine. These substances were separately dissolved in 10 mL of 0.1 N hydrochloric acid, prepared with liquid chromatography-grade water at a final concentration of 1 mg/mL of each amine. All standards and spermine added to the experimental feeds were purchased from Sigma™ (Saint Louis, MO, USA).

Stock solutions II were prepared from stock solutions I by transferring aliquots of 1 mL into a vial, forming a pool containing 100 µg/mL of each amine. The stock solutions III were prepared by withdrawing 1 mL of stock solutions II and diluting to 10 mL with 0.1 N hydrochloric acid, yielding a concentration of 10 µg/mL. The working solutions were prepared from stock solution III by withdrawing aliquots of 50, 400 and 800 µg/mL. All solutions were stored at 4°C. The pH stability of these solutions was monitored and new solutions were prepared when necessary.

2.3 Statistical analysis

All data collected were submitted to analysis of variance, using Statistical Analysis System (SAS, 1985). The comparison of means obtained at different timepoints of the experiment was performed according to the Student Newman-Keuls test (Banzato and Kronka, 2006).

3. Results

In Table 2 results regarding profile and levels of the amines for ingredients used in feed preparation were presented. Among ten amines investigated, spermidine, spermine, putrescine, cadaverine and agmatine were present in all analyzed products whereas phenylethylamine, serotonin and tryptamine were not detected in any samples. The highest levels of bioactie amines were 2.13 mg.100g^{-1} of spermine in corn, 13.05 mg.100g^{-1} of spermidine in soy bran, 3.89 mg.100g^{-1} of spermine in wheat bran and 17.15 mg.100g^{-1} of tyramine in meat and bone meal (Table 2).

Table 2. Amine levels in broiler feed ingredients acquired in Belo Horizonte, Minas Gerais, Brazil.

Ingredients	n	Amine levels (mg.100g^{-1})						
		Spd	Spm	Put	Cad	Agm	Tim	Him
Corn	1	0.35	2.13	0.47	0.08	0.08	0.05	Ud
SB	1	13.05	3.20	0.42	1.35	1.23	Ud	Ud
WB	1	3.89	1.62	0.37	0.02	0.13	Ud	Ud
MBM	1	0.82	0.89	1.26	0.45	0.83	17.15	0.32

Spd = spermidine, Spm = spermine, Put = putrescine, Cad = cadaverine, Agm = agmatine, Tim = tyramine, Him = histamine. SB - Soy Bran, WB - Wheat Bran, MBM - Meat and Bone Meal. Detection limit = 0.02 mg.100g^{-1}. Ud = undetected, corresponds to a value lower than the detection limit of the method. Source: From authors

The mean values of the amine levels found in the starting and growth diets on the market for broilers are presented in Table 3. Seven amines were detected: spermine, spermidine, putrescine, cadaverine, tyramine, histamine and agmatine. Total amine levels (mg.100g^{-1}) varied from 6.34 to 16.53 and 7.49 to 18.13 in the starting and growth diets, respectively (Table 3).

Table 3. Average amine levels in different commercial broilers diets acquired in Belo Horizonte, Minas Gerais, Brazil.

Producer	Mean amine levels (mg.100g^{-1})																
	Initial diets									Growth diets							
	n	Spd	Spm	Put	Cad	Agm	Tim	Him	Total	Spd	Spm	Put	Cad	Agm	Tim	Him	Total
1	4	4.12	2.45	1.87	3.36	0.48	1.69	2.49	16.53	3.75	2.31	0.49	0.64	0.30	0.16	0.22	7.87
2	4	5.03	3.18	0.48	0.71	0.43	0.08	0.15	10.06	3.99	2.63	0.59	0.55	0.41	0.19	0.40	8.80
3	4	2.67	2.26	0.45	0.43	0.28	0.12	0.13	6.34	3.50	2.27	0.56	0.63	0.22	0.09	0.23	7.49
4	4	3.86	1.85	0.29	0.40	0.44	0.31	0.16	7.29	3.32	1.62	1.28	1.50	0.44	7.05	2.82	18.13
5	4	4.61	2.69	0.54	0.39	0.37	0.08	0.03	8.71	3.95	2.18	0.95	0.54	0.31	0.53	0.39	8.84
6	4	3.78	3.78	1.45	1.07	0.39	0.25	0.19	10.90	3.60	1.96	1.08	0.63	0.17	0.28	0.18	7.89

Spd = spermidine, Spm = spermine, Put = putrescine, Cad = cadaverine, Agm = agmatine, Tim = tyramine, Him = histamine. Detection limit = 0.02 mg.100g^{-1}. Source: From authors.

Amine levels in starting diets supplemented with 0.06% and 0.12% of spermine in three weeks were demonstrated in table 4. During the storage, putrescine averages were similar among weeks and inclusion level of spermine, ranging from 0.36 to 0.68 mg.100g^{-1}. Cadaverine averages oscillated between 0.62 to 1.74 mg.100g^{-1} and reduced significantly in the third week, except for 0.06% spermine supplementation. Agmatine averages were detected between 0.49 to 1.58 mg.100g^{-1} and decreased ($p<0.05$) in times, except for 0.12% spermine supplementation. Spermidine averages ranged from 4.72 to 10.18 mg.100g^{-1} and were smaller ($p<0.05$) in weeks two and three. Spermine averages from 2.20 to 80.64 mg.100g^{-1} were found and increased significantly with the level of inclusion of this substance (Table 4).

Table 4. Amine levels in starting feed (control) for broilers and supplemented with inclusion of 0.06% and 0.12% spermine (Spm) during storage at 26 ± 3°C.

Spermine (% of inclusion)	Storage days	Amine levels (mg.100g^{-1})					
		Put	Cad	Agm	Spd	Spm	Total
0,00 (Control)	7	0.65^{a}	1.74^{a}	1.58^{a}	10.18^{a}	2.34^{c}	16.49
	14	0.38^{a}	0.89^{b}	0.49^{b}	5.06^{b}	2.20^{c}	9.02
	21	0.42^{a}	0.98^{b}	0.56^{b}	5.32^{b}	2.43^{c}	9.71
0,06	7	0.78^{a}	2.01^{a}	0.77^{b}	9.56^{a}	47.67^{b}	60.79
	14	0.38^{a}	0.62^{b}	0.64^{b}	4.72^{b}	30.36^{b}	36.72
	21	0.50^{a}	1.10$^{a.b}$	0.65^{b}	5.20^{b}	44.10^{b}	51.55
0,12	7	0.63^{a}	0.87^{b}	0.81$^{a.b}$	8.20^{a}	80.29^{a}	90.80
	14	0.86^{a}	1.11$^{a.b}$	1.11^{a}	6.46^{b}	80.64^{a}	90.18
	21	0.36^{a}	0.92^{b}	0.92^{a}	5.89^{b}	75.59^{a}	83.68

Put = putrescine, Cad = cadaverine, Agm = agmatine, Spd = spermidine, Spm = spermine. Detection limit = 0.02 mg.100g^{-1}. ^{a,b,c} Averages with different letters in the same column differ among each other according to the Student's t-test ($p<0.05$). Source: From authors

In Table 5 were presented amine levels in growth diets with 0.06% and 0.12% spermine inclusion during storage of diet or seven weeks. Putrescine levels were similar among weeks and inclusion level of spermine, ranging from 0.18 to 0.59 mg.100g^{-1} during the storage. Cadaverine averages oscillated ($p<0.05$) between 0.35 in week four in control diet and 1.15 mg.100g^{-1} in 0.06% spermine supplementation diet at seventh week. Agmatine averages were detected between 0.36 to 0.91 mg.100g^{-1} in control diet. Spermidine averages ranged significantly from 2.86 to 6.43 mg.100g^{-1} in the week one of control diet and week four in 0.12% spermine inclusion diet, respectively. Spermidine concentrations reduced with time in control and 006 spermidine inclusion diet. Were found spermine averages from 1.33 in the sixth week to control diet to 72.55 mg.100g^{-1} in the first week to 0.12% spermine inclusion diet (Table 5).

Table 5. Variation in amine levels in growth (control) diet for broilers and with inclusion of 0.06% and 0.12% spermine (Spm) during storage at 26 ± 3°C.

Spermine (% of inclusion)	Storage days	Amine levels (mg.100g^{-1})					
		Put	Cad	Agm	Spd	Spm	Total
0.00 (control)	7	0.52^{a}	1.05^{a}	0.91^{a}	6.43^{a}	2.03^{c}	2.60
	14	0.59^{a}	1.14^{a}	0.79^{a}	5.59^{a}	3.64^{c}	11.75
	21	0.47^{a}	0.73^{b}	0.36^{b}	4.37$^{a.b}$	2.77^{c}	8.70
	28	0.36^{a}	0.35^{c}	0.80^{a}	3.39^{b}	2.63^{c}	7.53
	35	0.39^{a}	0.92^{a}	0.58$^{a.b}$	4.62$^{a.b}$	2.00^{c}	8.51
	42	0.21^{a}	0.72^{b}	0.47^{b}	3.34^{b}	1.33^{c}	6.07
	49	0.18^{a}	0.45^{c}	0.48^{b}	3.22^{b}	1.87^{c}	6.20
0.06	7	0.41^{a}	0.43^{c}	0.63$^{a.b}$	6.39^{a}	35.27^{b}	43.13
	14	0.51^{a}	0.37^{c}	0.65$^{a.b}$	4.92$^{a.b}$	34.42^{b}	40.87
	21	0.54^{a}	0.81$^{a.b}$	0.54$^{a.b}$	3.81^{b}	39.30^{b}	45.00
	28	0.56^{a}	0.76^{b}	0.53$^{a.b}$	4.59$^{a.b}$	30.97^{b}	37.41
	35	0.43^{a}	0.80$^{a.b}$	0.51^{b}	3.75^{b}	32.16^{b}	37.65
	42	0.56^{a}	1.13^{a}	0.47^{b}	2.94^{b}	35.42^{b}	40.52
	49	0.39^{a}	1.15^{a}	0.62$^{a.b}$	3.97^{b}	37.83^{b}	43.96
0.12	7	0.41^{a}	0.36^{c}	0.56$^{a.b}$	3.58^{b}	72.55^{a}	77.46
	14	0.35^{a}	0.65^{c}	0.45^{b}	4.60$^{a.b}$	69.79^{a}	75.84
	21	0.50^{a}	0.91$^{b.c}$	0.60$^{a.b}$	4.20$^{a.b}$	67.82^{a}	74.03
	28	0.53^{a}	1.14^{a}	0.54$^{a.b}$	2.86^{b}	67.76^{a}	72.83
	35	0.46^{a}	0.95^{a}	0.46^{b}	3.73^{b}	70.10^{a}	75.70
	42	0.30^{a}	1.08^{a}	0.42^{b}	4.48$^{a.b}$	68.57^{a}	74.85
	49	0.29^{a}	0.59$^{b.c}$	0.87^{a}	5.41^{a}	72.07^{a}	79.23

* Put = putrescine, Cad = cadaverine, Agm = agmatine, Spd = spermidine, Spm = spermine. Detection limit = 0.02 mg.100g^{-1}.
^{a,b,c} Different letters in the same column differ among each other according to Student's t-test ($p < 0.05$). Source: From authors

4. Discussion

The results observed in this work for corn indicate the presence of seven amines, predominantly spermine (Table 2), which is compatible with the values reported by Pinho et al. (2008) that verifiy 2.42 mg.100g^{-1} of spermine in green maize. Bandeira et al. (2012) observed 2.79 mg.100g^{-1} of spermine in corn kernels. Contrary to the results of Table 2, Bandeira et al. (2012) did not detected histamine in these product. Seed cultivars, agricultural practices, environmental characteristics and processing steps can be associated with the variations, as described by Glória et al. (2005) and Bandeira et al. (2012), justifying these differences.

The results can suggest that in soybean and wheat bran, spermidine is the predominant amine, followed by spermine (Table 2). Bardócz et al. (1993) and Silva (2000) also reported higher concentrations of spermidine in raw soy or in soy protein products, respectively, with levels varying from 3.32 to 6.21 mg.100g^{-1} for spermidine and 2.97 to 3.43 mg.100g^{-1} for spermine in raw soy and 4.20 for spermidine and 1.26 mg.100 g^{-1} for spermine in soy protein products.

For meat and bone meal, tyramine was the predominant amine (Table 2) and this amine was related to the putrefaction of meats by Bellaver (2002). This remains one of the main challenges related to the use of animal meal in feed processing and Tamim and Doerr (2000) affirmed that the presence of this amine at a concentration above 55 mg.100g^{-1} indicates a toxicity risk to animals. However, since this ingredient is used in small percentages, maximum 3 to 5% in the broiler's diet (Caires et al., 2010), the final levels (Table 2) would be considered non-toxic for this product.

Amines have been identified as substances that can cause toxicosis when ingested in high quantities by animals. Putrescine at a concentration above 200 mg.100g^{-1}may be considered growth-promoting, but it is toxic at level of 1 g.100g^{-1}. Spermine has been considered toxic and detrimental to performance when the concentratiom is 100 mg.100g^{-1} in the diet (Bellaver, 2002).

Generally, the predominant amine was spermidine, followed by spermine. After these two amines, and in alternating positions, where putrescine, cadaverine, tyramine, histamine and agmatine. However, one starting diet showed a predominance of cadaverine over spermine (Table 4). In one growth diet, there was a predominance of spermine, putrescine and cadaverine at the same proportions; in another brand, tyramine levels were approximately two or more times greater than means of other amines detected (Table 5). In general, the maximum values observed for the different amines were approximately 0.03%, levels well below those considered to be toxic or that would result in decreased bird performance according to Sousadias and Smith (1985), Smith (1990) and Smith et al. (1996).

Although the polyamine levels observed in this work were low or even decreased during storage (Table 4 and 5), it is important carefully evaluation of raw materials and broilers diets quality. Stability observed for other biogenic amines indicates that risks remain during storage and that other synergistic effects between the various amines can be present, according to Ahmad et al. (2020), Jaguey-Hernández et al. (2021) and Alvarez et al. (2014).

Spermine levels (Tables 4, 5) varied in different groups of samples, which was expected as a function of this amine different supplementation levels. Spermine and putrescine averages did not change significantly during the storage period, indicating stability and a decreasing was observed in cadaverine, agmatine and spermidine levels over time (Tables 4, 5). The results (Table 4) were similar to those observed for the growth diet (Table 5) indicating that during storage of 49 days, no change was observed in the levels of spermine in the final product.

The concentrations of biogenic amines in these diets remain low (Tables 4, 5). It would not exert toxic effects on broilers and other species, as described by Gilbert et al. (2018) and Hashemi et al. (2014) because spermine concentratiom is lower than 100 mg.100g^{-1} in the diets (Bellaver, 2002).

These levels of biogenic amines (Tables 4, 5) indicated that feed were made with good quality raw materials and storage under proper conditions, as related by Alvarez and Moreno-Arribas (2014) and if the diets were offered to broiler poor performance would not ocurr (Bermudez & Firman, 1998; Gilbert et al., 2018) as well low meat quality (Assis et al., 2015; Ruiz-Capillas & Herrero, 2019). Besides, spermine and spermidine could stimulate and regulate gastrointestinal growth (Lima, Glória, 1999; Lima et al., 2006; Bogusławska-Tryk et al., 2020, Liu et al., 2020) and can act as growth promoter (Hashemi et al., 2014).

5. Conclusion

The biogenic amine levels were low in ingredients used in broiler diets production, in commercial formulations and in experimental initial and growth feed even when stored respectively for 21 and 49 days and supplemented with 0.06 and 0.012% of spermine. Despite being present in various foods for humans and animals, the number of publications about bioamines is limited and few authors are encouraged to research it. However, other studies need to be made with other foods supplied to different categories and animal species, using new levels of inclusion and even additional temperatures and storage times.

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