



## Supra-nutritional Levels of Selected B Vitamins in Animal or Vegetable Diets for Broiler Chicken

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### ■ Keywords

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### ABSTRACT

The objective of this study was to evaluate the effects of supra-nutritional level of selected B vitamins in different types of diet on broiler performance. Two experiments were conducted using male and female one-day-old chicks (n=288 each; initial body weights in experiment I and II was, respectively,  $47.57 \pm 0.43$ , and  $47.98 \pm 0.31$ ) reared in batteries up to 18 days. In experiment I, the chicks were fed a corn and soybean meal-based diet and, in experiment II, a diet containing oxidized animal by-product meals and soybean oil was used. Both experiments followed a completely randomized design in a  $3 \times 2$  factorial arrangement, consisting of the factors: i) supplementation levels of selected B vitamins (control, 3- or 6-times control of the vitamins riboflavin, pantothenic acid, niacin, folic acid and vitamin B<sub>12</sub>); ii) dietary nutritional density (low or high), totaling 6 treatments and 8 replicates of 6 birds each (3 males and 3 females). As result of this study, in Exp. I, chicks showed higher weight gain (741.1 g vs. 697.3 g) and feed intake (920.2 vs. 878.5 g) when fed low-nutritional density diet with supra-nutritional vitamin level 6-times higher than the control. However, this effect was not found in the performance of chickens fed high-nutritional density diet. Despite the poor quality of the ingredients used in Exp. II, no statistical effect was shown of the use of vitamin super-dose in rations with different dietary nutrient density. Feed conversion ratio (FCR) was significantly improved for chickens fed high-nutritional density diet (1.191 vs. 1.246 in experiment I, 1.244 vs. 1.275 in experiment II,  $p < 0.01$ ). We conclude that birds fed a vegetable diet formulated with low-dietary density improved body weight (BW) and feed intake (FI) when receiving supra-nutritional levels of vitamins 6-times higher than the control.

### INTRODUCTION

Most of the poultry diets in Brazil are based on corn and soybean meal, which supplies the greater part of energy and protein in the feed. The use of by-products of the meat processing industry is a safe way to use low-cost products that contribute to the nutritional quality and cost of the diets (Caires *et al.*, 2010). Among these ingredients are residues such as inedible viscera, feathers, bones, blood and fat, which have no commercial purpose for human consumption. Despite of the use of antioxidants in the processing of this ingredients, animal by-products are susceptible to autoxidation. Because its rich nutritional composition, especially the high percentage of fat, makes them very susceptible to chemical and bacterial spoilage (Amaral *et al.*, 2018).

Together, different components of food, nutrients, and dietary patterns have a high capacity to change the growth of different microbial species and/or the modulation of community dynamics of the gut microbiota (Ishiguro *et al.*, 2018). For example, *Bacteroides*



spp. are associated with diets containing animal protein sources, while *Prevotella* spp. are related to vegetable diets (Wu *et al.*, 2011). Diets rich in vegetable ingredients including polyphenols and fibers are shown to be beneficial for gut health by providing substrates such as vitamins, short chain fatty acids, etc. to the host during microbial fermentation (Zoetendal & De Vos, 2014). Moreover, the consumption of diets containing carbohydrates from vegetable sources and fibers provides a greater variety of bacteria than diets containing animal protein (Martínez *et al.*, 2015). In humans, it has been shown that microbial diversity was reduced in association with worse metabolic and inflammatory status (Rosario *et al.*, 2016).

Dietary nutrient density is also a factor that has an impact on the health and growth of animals (Coelho & McNaughton, 1995). A physiological stress condition can be promoted using diets with high nutritional density due to increased rates of growth and metabolism. The levels of nutrients provided in the feed may not be adequate for the functioning of the immune system and animals' maximum resistance to disease under these circumstances. Thus, the requirements for certain nutrients may increase in order to achieve the maximum performance of poultry raised in intensive commercial systems.

Broilers raised in commercial systems are exposed to adverse environmental conditions that can increase stress and diseases. Coelho *et al.* (2001) reported that the use of super-dose 16-times higher than the basal level recommended by the NRC (1994) of the vitamins riboflavin, pantothenic acid, niacin, folic acid, and vitamin B<sub>12</sub> resulted in improved performance when chickens were submitted to stress conditions similar to those found in the field. These vitamins are closely linked to the metabolism of carbohydrates, amino acids, synthesis of methyl groups, and nucleic acids. The use of super-doses of water-soluble vitamins may be used to meet the metabolic needs, as well as the needs of intestinal microorganisms, being a simple alternative to be implemented in the field. Therefore, our purpose was to evaluate the effect of the use of supra-nutritional levels of selected B vitamins, riboflavin, pantothenic acid, niacin, folic acid, and vitamin B<sub>12</sub>, on broiler performance using different dietary patterns.

## **MATERIAL AND METHODS**

All procedures used in these experiments were approved by the institutional animal care and use

committee of the College of Agriculture "Luiz de Queiroz", University of Sao Paulo (process n. 2017.5.1809.11.9). Two experiments were conducted using male and female one-day-old chicks Ross AP 95 (n=288 each) housed in metallic batteries up to 18 days. Chicks were weighed by cage for equal distribution. Both the experiments consisted in a completely randomized design using a 3 × 2 factorial arrangement with supplementation levels of selected B vitamins (control, 3- or 6-times control of vitamins riboflavin, pantothenic acid, niacin, folic acid and vitamin B<sub>12</sub>), and dietary nutritional density (low or high) as a factors, totaling 6 treatments and 8 replicates of 6 birds each (3 males and 3 females). The basal feed differed between experiments: Exp. I – corn and soybean meal-based diet; Exp. II – diet containing oxidized animal by-products (meat and bone meal, feather meal, and poultry by-products meal) and oxidized soybean oil (Table 1).

The treatments for both experiments were as follows: Lo-Cont (low-dietary density with control level of selected B vitamins); Lo-3× (low-dietary density with 3-times control level of selected B vitamin); Lo-6× (low-dietary density with 3-times control level of selected B vitamin); Hi-Cont (high-dietary density with control level of selected B vitamins); Hi-3× (high-dietary density with 3-times control level of selected B vitamin); and Hi-6× (high-dietary density with 3-times control level of selected B vitamin). The birds had *ad libitum* access to water and feed in mash the entire experimental period.

The feed and vitamin supplement were formulated to meet the nutrient specifications of the Brazilian Tables (Rostagno *et al.*, 2017), and are presented in Tables 1 and 2. At the feed mill, the control diets (Lo-Cont and Hi-Cont) were produced in a single batch and subdivided for the addition of the super-dose of the vitamin supplement. These super-doses were included in the diets over the top. The total weight of these ingredients represents 0.17% and 0.43% for the treatments with 3- or 6-times control of selected B vitamin, respectively. Thus, it ensured that there was no difference between the experimental diets, except for vitamin supplementation. The amount of vitamins present in the control diets were accounted for when the over the top supplementation was calculated.

At 6, 12 and 18 days of age, the chickens and the feed were weighed by cage to calculate the body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR).



**Table 1** – Ingredients and nutrient composition of a corn and soybean-meal-based diet (experiment I) and a diet containing oxidized animal by-products and soybean oil (experiment II).

Ingredients (%)	Experiment I		Experiment II	
	Low	High	Low	High
Corn (8.05% CP)	52.34	47.84	59.20	54.72
Soybean meal (46.26% CP)	40.56	43.52	29.82	32.76
Meat and bone meal (44.75% CP)	-	-	3.00	3.00
Poultry by-product meal (79.27% CP)	-	-	2.00	2.00
Feather meal (65.20 % CP)	-	-	2.00	2.00
Soybean oil <sup>1</sup>	3.395	4.871	1.555	3.024
Dicalcium phosphate	1.633	1.684	1.094	1.145
Limestone	0.866	0.884	0.000	0.007
Salt	0.502	0.511	0.422	0.430
DL-Methionine	0.311	0.372	0.323	0.339
L-Lysine HCl	0.126	0.102	0.290	0.267
Vitamin premix <sup>2</sup>	0.100	0.100	0.100	0.100
Mineral premix <sup>3</sup>	0.050	0.050	0.050	0.050
Choline chloride (60%)	0.080	0.080	0.080	0.080
L-Threonine	0.041	0.040	0.074	0.073
Total	100.00	100.00	100.00	100.00
Calculated composition (analyzed composition are in parentheses)				
Metabolizable energy (kcal/kg)	3,050	3,100	3,050	3,100
Crude protein (%)	23.31(22.62)	24.30 (23.40)	23.31 (23.40)	24.30 (22.91)
Digestible lysine (%)	1.256	1.306	1.256	1.306
Digestible met + cys (%)	0.929	0.966	0.929	0.966
Digestible methionine (%)	0.515	0.535	0.515	0.535
Digestible threonine (%)	0.829	0.862	0.218	0.862
Calcium (%)	0.878 (0.788)	0.907 (0.852)	0.878 (0.809)	0.907 (0.852)
Available Phosphorus (%) <sup>4</sup>	0.419 (0.788)	0.432 (0.808)	0.419 (0.891)	0.432 (0.914)

<sup>1</sup>Soybean oil oxidized was used in experiment II; <sup>2</sup>DSM Nutricional Products, Composition per kg of feed: Vit. A – 12,216 UI; Vit. D<sub>3</sub> – 3,054 UI; Vit. E – 45.8 UI; Vit. K<sub>3</sub> – 2.44 mg; Vit. B<sub>1</sub> – 3.28 mg; Vit. B<sub>2</sub> – 8.17 mg; Vit. B<sub>6</sub> – 4.58 mg; Vit. B<sub>12</sub> – 20 µg; Nicotinic acid – 42 mg; Pantothenic acid – 16.42 mg; Biotin – 0.115 mg; Folic acid – 1.15 mg; Selênio – 0.3 mg. <sup>3</sup>DSM Nutricional Products, Composition per kg of feed: Manganese – 80 mg; Iron – 50 mg; Zinc – 50 mg; Copper – 10 mg; Cobalt – 1.0 mg; Iodo – 1.0 mg. <sup>4</sup>Total phosphorus values analysis is presented in parentheses.

**Table 2** – Vitamin supplementation levels per kg of feed.

Vitamin	Unit	Control <sup>1</sup>	3-times vitamin	6-times vitamin
Retinoids (A)	UI	12,216	12,216	12,216
Calciferol (D <sub>3</sub> )	UI	3,054	3,054	3,054
Tocopherol (E)	UI	45.8	45.8	45.8
Phytonadione (K <sub>3</sub> )	mg	2.44	2.44	2.44
Tiamin (B <sub>1</sub> )	mg	3.28	3.28	3.28
Puridoxine (B <sub>6</sub> )	mg	4.58	4.58	4.58
Biotin	mg	0.115	0.115	0.115
Riboflavin (B <sub>2</sub> )	mg	8.17	24.51	49.02
Pantothenic acid	mg	16.42	49.26	98.52
Niacin	mg	49.6	148.8	297.6
Folic acid	mg	1.145	3.435	6.870
Cobalamin (B <sub>12</sub> )	mg	0.019	0.059	0.119

<sup>1</sup>Vitamin supplementation levels for starter phase of chickens (Rostagno *et al.*, 2017). Supra-nutritional levels are indicated in bold.

## MEASURING INGREDIENTS QUALITY

In order to ensure that the animal by-product meals and soybean oil used in experiment II had lower quality standards, these ingredients were submitted to the laboratory analyzes (Table 3). For comparative purpose, the analyzes of the soybean oil used in experiment I

was also included. The analyzes of peroxide value, acidity (method 27 for animal by-products meal, and method 28 for vegetable oils), and rancidity were performed according to the methodologies described by the Compêndio Brasileiro de Alimentação Animal (SINDIRAÇÕES, 2017). Thiobarbituric acid reactive substances (TBARS) determination was quantified in



**Table 3** – Peroxide value, acidity, TBARS and rancidity of the animal by-product meals and the soybean oil fresh (experiment I) and oxidized (experiment II) used in the feed.

Ingredients	Peroxide value	Acidity	TBARS	Rancidity
	mEq/kg		mg MDA/ kg	
Meat and bone meal	9.88	8.30 mg NaOH/g	3.0818	Negative
Feather meal	0.00	3.84 mg NaOH/g	0.6878	Negative
Poultry by-product meal	86.98	4.74 mg NaOH/g	7.1383	Positive
Soybean oil (experiment I)	7.55	0.17% Oleic acid	6.1041	Negative
Soybean oil (experiment II)	10.03	1.72% Oleic acid	57.4202	Negative

triplicate for each ingredient according to AOAC (1990) recommendations with some modifications. It was used as standard 1,1,3,3-tetraethoxypropane (TEP), which on acid hydrolysis produces malonaldehyde ratio of 1 mol: 1 mol, to obtain a standard curve consisting of five points of different concentrations (0.6; 1.0; 2.5; 5.0; 10.0  $\mu\text{mol} / \text{L}$  of TEP). For each sample of animal by-product meals, the aldehydes were extracted by ultrahomogenization at 3500 rpm using Ultra Turrax (Ika T18 basic, Wilmington, North Carolina, USA) of 15 mL of trichloroacetic acid solution (7.5%) with addition of 0.015 g of propyl gallate, and 0.015 g of ethylenediaminetetraacetic acid, with approximately 7 g of sample. After filtration of the homogenate, 2.5 mL of the filtrate were transferred to a test tube and added to 2.5 mL of thiobarbituric acid reagent 46 mM. These samples were immersed in water bath at 95 °C for 35 min and then cooled in an ice bath for about 5 min. The absorbance was measured at 532 nm using spectrophotometer (Shimadzu, UV-Vis mini 1240, Chiyoda-ku, Tokyo, Japão). For these quantification, standard solutions of malonaldehyde (MDA) in 7.5% TCA were prepared from TEP and calibration curves. The results were calculated from the TEP curve and expressed in mg MDA per kg of sample.

For the determination of the TBARS values of the soybean oil samples, the analysis was adapted according to the procedure described by Papastergiadis *et al.* (2012) for the extraction of aldehydes. The aqueous layer was collected, and the procedure was repeated twice. The collected extract reacted with the TBA reagent, as described above.

### Vitamin analyzes of feed samples

The levels of riboflavin, pantothenic acid, niacin, folic acid and vitamin B<sub>12</sub> present in the feed were determined at the laboratory Eurofins CLF (Friedrichsdorf, Germany). The assay comprised extraction of riboflavin and its coenzyme forms in an autoclave using diluted sulfuric acid. Phosphor ester bonds were cleaved enzymatically. After dilution and centrifugation of the extract, the riboflavin content

was determined by reversed phase HPLC using fluorimetric detection (Rubaj *et al.*, 2008). Nicotinic acid and nicotinamide were extracted from feed using 0.001N-sulfuric acid. After dilution of the extract with mobile phase and centrifugation an aliquot of the clear solution was chromatographed using a reversed phase HPLC system (detection at 260 nm) (Van Niekerk *et al.*, 1984). Free pantothenic acid was extracted with water and analyzed by reversed-phase HPLC. Folic acid and vitamin B<sub>12</sub> were also determined by HPLC methodology (Heudi *et al.*, 2005).

### Statistical analysis

The data of productive performance was analyzed by ANOVA with procedures appropriate for a completely randomized design in a factorial arrangement 3 × 2 using the GLM procedure of the statistical program SAS 9.4, and the means were compared using Tukey's test. All data were checked for homogeneity of variances and normality of residues.

## RESULTS AND DISCUSSION

The analyzed concentration of the vitamins riboflavin, pantothenic acid, niacin, folic acid, and vitamin B<sub>12</sub> in the experimental diets are reported in Table 4, along with the expected values. The laboratory analyzes are one of the biggest issues in research with vitamins, because even with the attention at the feed mill, sample collection, etc., the analyzed values in the complete feed may not coincide with the expected ones. In this study, feed processing did not affect severely the retention of the vitamins used in the diets, since there was no heat treatment in the feed such as pelleting, expansion or extrusion. However, values for folic acid were higher than expected in the diet with supra-nutritional levels of vitamins, whereas the other vitamins analyzed presented values relatively close to the expected. In agreement to our results, Stahly *et al.* (2007) evaluated the same 5 test vitamins in a swine experiment and found a high concentration of folic acid in the feed compared to the calculated value.



**Table 4** – Riboflavin, pantothenic acid, niacin (sum of nicotinamide and nicotinic acid), folic acid, and vitamin B<sub>12</sub> content of diets in experiment I and II (expected values are in parentheses).

Experiment	Treatment <sup>1</sup>	Riboflavin	Pantothenic acid	Niacin	Folic acid	Vitamin B <sub>12</sub>
		mg/kg	mg/kg	mg/kg	mg/kg	µg/kg
I	Lo-Cont	10.70 (8.2)	29.95 (16.4)	61.60 (49.6)	1.70 (1.1)	16.20 (19.0)
	Lo-3x	23.75 (24.5)	63.85 (49.2)	179.0 (148.8)	13.10 (3.4)	50.30 (59.0)
	Lo-6x	41.40 (49.0)	100.3 (98.52)	315.0 (297.6)	25.20 (6.9)	99.40 (119.0)
	Hi-Cont	10.05 (8.2)	24.01 (16.4)	56.10 (49.6)	1.50 (1.1)	14.55 (19.0)
	Hi-3x	25.30 (24.5)	46.18 (49.2)	165.0 (148.8)	11.00 (3.4)	38.95 (59.0)
	Hi-6x	43.03 (49.0)	97.52 (98.52)	312.0 (297.6)	23.40 (6.9)	86.45 (119.0)
II	Lo-Cont	8.65 (8.2)	20.56 (16.4)	54.20 (49.6)	1.30 (1.1)	13.10 (19.0)
	Lo-3x	16.40 (24.5)	37.54 (49.2)	101.0 (148.8)	5.90 (3.4)	33.45 (59.0)
	Lo-6x	42.30 (49.0)	97.98 (98.52)	311.0 (297.6)	27.00 (6.9)	87.50 (119.0)
	Hi-Cont	-	-	-	-	-
	Hi-3x	22.25 (24.5)	48.02 (49.2)	126.0 (148.8)	9.60 (3.4)	51.45 (59.0)
	Hi-6x	-	-	-	-	-

<sup>1</sup>Exp. I – corn and soybean meal-based diet; Exp. II – diet containing oxidized animal by-products and soybean oil. The treatments for both experiments were as follows: Lo-Cont (low-dietary density with control level of selected B vitamins); Lo-3x (low-dietary density with 3-times control level of selected B vitamin); Lo-6x (low-dietary density with 3-times control level of selected B vitamin); Hi-Cont (high-dietary density with control level of selected B vitamins); Hi-3x (high-dietary density with 3-times control level of selected B vitamin); and Hi-6x (high-dietary density with 3-times control level of selected B vitamin).

In both experiments, the same supplement of the selected B vitamins was added in the feed over the top and the same equipment was used to mix these feeds; even though the values of the vitamins recovered in the feed Lo-3x were analytically lower in experiment II than in experiment I.

In experiment I, BW ( $p=0.032$ ) and BWG ( $p=0.040$ ) were affected by vitamins supplementation level in the 1-6d period (Table 5). Body weight and body weight gain were higher when chicks were fed the 6-times vitamin

level. Feed conversion ratio also tended ( $p=0.082$ ) to be improved in this treatment. There was a significant ( $p=0.051$ ) vitamin supplementation × dietary nutrient level interaction for FI. Feed intake of chicks increased when the 6-times vitamin level was used in the diet formulated with low-dietary nutrient density (Table 6). However, FI was not affected by supra-nutritional levels of vitamins when the high-density diet was fed.

When the period of 1 to 12 days was evaluated (Table 5), there was also a significant ( $p=0.036$ )

**Table 5** – Effect of vitamin supplementation and dietary nutritional density on 6, 12, and 18-day broiler performance in experiment I.

Treatment <sup>1</sup>	0 d		1 to 6 d			1 to 12 d				1 to 18 d				
	BWI (g)	BW (g)	WG (g)	FI (g)	FCR	BW (g)	WG (g)	FI (g)	FCR	BW (g)	WG (g)	FI (g)	FCR	
Lo-Cont	47.89	148.1	100.2	101.9	1.018	393.3	345.4	391.8	1.134	745.2	697.3	878.5	1.261	
Lo-3x	47.44	138.1	90.70	92.18	1.018	373.7	326.2	368.9	1.132	727.4	679.9	839.8	1.235	
Lo-6x	47.40	155.8	108.4	109.4	1.011	416.5	369.1	415.4	1.126	788.5	741.1	920.2	1.242	
Hi-Cont	47.79	149.2	101.4	102.1	1.007	408.0	360.3	389.2	1.080	764.8	717.0	864.1	1.206	
Hi-3x	47.12	145.5	98.40	104.1	1.061	404.9	357.8	387.1	1.083	790.3	743.4	875.1	1.178	
Hi-6x	47.75	150.4	102.6	102.7	1.006	414.3	366.6	393.5	1.074	786.1	738.4	879.6	1.191	
Main factors <sup>2</sup>														
Vitamin Suppl.	Control	47.84	148.6ab	100.8ab	102.0	1.012	400.7ab	352.8ab	390.5	1.107	755.0	707.2	871.3	1.234
	3x	47.28	141.8b	94.5b	98.14	1.040	389.3b	342.0b	378.0	1.107	758.8	711.6	857.5	1.207
	6x	47.58	153.1a	105.5a	106.1	1.008	415.4a	367.9a	404.5	1.100	787.3	739.7	899.9	1.217
Dietary Density	Low	47.58	147.3	99.72	101.2	1.016	394.5b	346.9b	392.1	1.131a	753.7	706.1	879.5	1.246a
	High	47.55	148.3	100.8	103.0	1.025	409.1a	361.6a	389.9	1.079b	780.4	732.9	872.9	1.191b
<i>p-value</i>														
Vitamin suppl.		0.032	0.040	0.114	0.082	0.005	0.005	0.005	0.633	0.038	0.037	0.009	0.075	
Dietary density		0.761	0.752	0.551	0.464	0.020	0.020	0.73	<0.0001	0.018	0.018	0.544	<0.0001	
Interaction		0.313	0.285	0.051	0.154	0.089	0.083	0.036	0.955	0.053	0.050	0.022	0.973	
CV <sup>3</sup> (%)		7.041	10.48	9.128	3.696	4.624	5.269	4.806	2.067	4.334	4.621	3.820	2.410	

a-b Means within a column without a common superscript are significantly different ( $p<0.05$ ). <sup>1</sup> Treatments: Lo-Cont (low-dietary density with control level of selected B vitamins); Lo-3x (low-dietary density with 3-times control level of selected B vitamin); Lo-6x (low-dietary density with 3-times control level of selected B vitamin); Hi-Cont (high-dietary density with control level of selected B vitamins); Hi-3x (high-dietary density with 3-times control level of selected B vitamin); and Hi-6x (high-dietary density with 3-times control level of selected B vitamin). <sup>2</sup>Main factors are: Vitamin suppl. – vitamin supplementation level, and dietary density – dietary nutritional density. <sup>3</sup> Coefficient of variation.



interaction for FI, similar to that observed in the initial period (Table 6). Body weight ( $p=0.005$ ) and body weight gain ( $p=0.005$ ) improved when chickens were fed a diet supplemented with 6-times vitamin level. In addition, broilers fed with high-dietary nutrient density had better performance compared with those fed the low-density ( $p<0.001$ ).

**Table 6** – Interactions between vitamin supplementation level and dietary nutrient density in experiment I.

		Feed intake (g) – 1 to 6 d		
		Vitamin supplementation level		
Dietary nutritional level		Control	3-times	6-times
Low		101.9ab	92.18b	109.4a
High		102.1	104.1	102.7
		Feed intake (g) – 1 to 12 d		
Dietary nutritional level		Control	3-times	6-times
Low		391.8b	368.9b	415.4ab
High		389.2	387.1	393.5
		Body weight (g) – 1 to 18 d		
Dietary nutritional level		Control	3-times	6-times
Low		745.2ab	727.4Bb	788.5a
High		764.8	790.3A	786.1
		Weight gain (g) – 1 to 18 d		
Dietary nutritional level		Control	3-times	6-times
Low		697.3ab	679.9Bb	741.1a
High		717.0	743.4a	738.4
		Feed intake (g) – 1 to 18 d		
Dietary nutritional level		Control	3-times	6-times
Low		878.5ab	839.8b	920.2a
High		864.1	875.1	879.6

Means followed by different lowercase letters (upper case) in the rows (columns) differ from each other by the Tukey test.

For the entire experimental period (1-18d), there were significant interactions between vitamin supplementation and dietary nutrient density for BW ( $p=0.053$ ), BWG ( $p=0.050$ ) and FI ( $p=0.022$ ). In this respect, super-dose of 6-times control of selected B vitamins affected more the BW, BWG, and FI of chickens fed low-dietary nutritional density than with the high-density diets (Table 6). Feed conversion ratio was improved ( $p<0.001$ ) for chickens fed a diet formulated with high-dietary nutritional density, irrespective of vitamin supplementation level.

The impact of the dietary levels of the same five vitamins tested were evaluated by Stahly *et al.* (2007) on performance of pigs with a high or moderate genetic capacity for lean tissue accretion. In that study, as the concentration of vitamins increased in the diet, body weight gain and feed conversion ratio improved. The results were more pronounced for animals with high lean tissue growth and this finding may be explained by the changes in metabolic pathways and not the

highest energy intake or body energy incorporation rate.

In our experiment, we expected an increase in performance responses of chickens fed the supra-nutritional levels of the selected B vitamins compared with the control level with advancing age. Weight gain and feed intake were stimulated by the super-dose of 6-times vitamin levels in both dietary nutrient densities. Nevertheless, less pronounced response was noted for chickens receiving high-density diets. As we know, birds fed high energy density diets have improved body weight and feed efficiency (Lott *et al.*, 1992). Because of that, vitamin super-dose did not influence the performance of chickens fed high-dietary nutrient density in our research.

The treatment 3-times vitamin levels with low-nutrient density diet resulted in lower performance of the chickens, beginning in the first period evaluated (1-6d). This effect was not observed for the high-density diet receiving the same vitamin dose. Because specific treatment effect of vitamin supplementation was not expected in this period, there is no reasonable explanation for this loss in performance other than an uncontrolled experimental error. This loss in performance was more pronounced in the latter periods.

In experiment II, no interactions between the main effects of vitamin supplementation and dietary nutrient level were observed for any of the traits studied (Table 7). However, feed conversion ratio was improved in broilers fed high-dietary nutritional density in the 1-18d period ( $p=0.008$ ).

The animal by-product meals and soybean oil used in experiment II had poor quality relative to the recommended standards according to Compêndio Brasileiro de Alimentação Animal (SINDIRAÇÕES, 2017). The analyses of lipid oxidation to characterize the quality of animal by-product meals and soybean oils (experiment I and II) should be studied together (Table 3). According to the industry, crude degummed soybean oil should have values of up to 2.0% acidity and 10.0 mEq/kg for peroxide value. The oil samples from both experiments were within the limits for acidity and peroxide value, but, the acidity for the oil in experiment II was 10-times as high and the TBARS value confirms that the oil used in experiment II had been oxidized. Although the birds fed a high pro-oxidant diet with 3% oxidized oil showed the higher inflammation scores at 21 d compared with the standard group receiving non oxidized oil (Lu *et al.*, 2014), suggesting a stress condition; in our study,



**Table 7** – Effect of vitamin supplementation and dietary nutritional density on 6, 12, and 18-day broiler performance in experiment II.

Treatment <sup>1</sup>	0 d		1 to 6 d			1 to 12 d				1 to 18 d				
	BWI (g)	BW (g)	WG (g)	FI (g)	FCR	BW (g)	WG (g)	FI (g)	FCR	BW (g)	WG (g)	FI (g)	FCR	
Lo-Cont	47.81	161.1	113.3	124.9	1.103	418.5	360.2	453.7	1.262	803.1	744.9	957.3	1.286	
Lo-3x	47.95	157.1	109.1	124.5	1.143	413.8	365.8	435.6	1.193	792.6	744.7	942.8	1.267	
Lo-6x	47.70	166.3	118.7	128.0	1.079	435.6	387.9	470.4	1.213	822.5	774.8	986.2	1.273	
Hi-Cont	48.05	161.3	113.2	127.7	1.134	430.4	377.4	457.0	1.213	816.7	763.7	962.8	1.261	
Hi-3x	47.98	161.1	113.1	125.4	1.109	425.2	377.2	450.0	1.193	827.2	779.2	969.2	1.244	
Hi-6x	48.08	163.0	114.9	124.3	1.085	428.7	380.6	448.4	1.181	825.2	777.1	951.3	1.226	
Main factors <sup>2</sup>														
Vitamin Suppl.	Control	47.93	161.2	113.3	126.3	1.118	424.4	368.8	455.3	1.237	809.9	754.3	960.0	1.273
	3x	47.96	159.1	111.8	124.9	1.126	419.5	371.5	442.8	1.193	809.9	761.9	956.0	1.256
	6x	47.89	164.7	116.8	126.1	1.082	432.1	384.2	459.4	1.197	823.8	776.0	968.8	1.249
Dietary Density	Low	47.82	161.5	113.7	125.8	1.108	422.6	371.3	453.2	1.222	806.1	754.8	962.1	1.275a
	High	48.04	161.8	113.7	125.8	1.109	428.1	378.4	451.8	1.195	823.0	773.3	961.1	1.244b
<i>P</i> -value														
Vitamin suppl.		0.223	0.199	0.856	0.973	0.322	0.215	0.215	0.118	0.598	0.387	0.780	0.206	
Dietary density		0.918	0.985	0.994	0.102	0.431	0.346	0.865	0.155	0.192	0.149	0.947	0.008	
Interaction		0.519	0.469	0.481	0.304	0.457	0.392	0.180	0.538	0.575	0.575	0.256	0.626	
CV <sup>3</sup> (%)		4.965	6.902	5.448	4.778	4.928	6.031	5.445	4.746	4.781	5.034	4.826	2.696	

a-b Means within a column without a common superscript are significantly different ( $p < 0.05$ ). <sup>1</sup> Treatments: Lo-Cont (low-dietary density with control level of selected B vitamins); Lo-3x (low-dietary density with 3-times control level of selected B vitamin); Lo-6x (low-dietary density with 3-times control level of selected B vitamin); Hi-Cont (high-dietary density with control level of selected B vitamins); Hi-3x (high-dietary density with 3-times control level of selected B vitamin); and Hi-6x (high-dietary density with 3-times control level of selected B vitamin). <sup>2</sup>Main factors are: Vitamin suppl. – vitamin supplementation level, and dietary density – dietary nutritional density. <sup>3</sup> Coefficient of variation.

the birds were healthy and well nourished, which may have limited the effects of using vitamin super-doses. Additionally, the final level of lipid oxidation in these diets was low, due to the low inclusion of animal by-products.

The quality standards by Compêndio Brasileiro de Alimentação Animal (SINDIRAÇÕES, 2017) for animal by-product meals are 6.0 mg NaOH/g acidity and 3.0 mEq/kg peroxide value. In our study, feather meal (10.0% ether extract) presented values within the standard for these parameters. On the other hand, meat and bone meal and poultry by-product meal (13.0 and 11.8% of ether extract, respectively) showed a high degree of oxidation. Rancidity was also detected in poultry by-products meal. Therefore it is possible to ascertain that these ingredients were in the process of auto-oxidation.

Studies on the animal by-product meals utilization in comparison to corn and soybean-meal-based diets for chickens showed an improvement in intestinal health. Birds fed a diet containing animal by-product meals had a low number of congestive or hemorrhagic points recorded in the intestine during the visual analysis of the duodenum at 21 days of age (Bellaver *et al.*, 2005) and a low lesion score for ileum histological analysis, such as lamina propria thickness, mixed inflammatory cell infiltration, necrosis and presence of oocysts, at 28 days of age (Belote *et al.*, 2017).

Unexplained experimental results in our study may be justified by the lack of knowledge about the interactions that occur between the intestinal microbiota and the nutrients provided in the rations. We did not collect intestinal contents for microbiota analysis. However, the microbiota analysis could show the variation between individual birds per dietary treatment are more pronounced in comparison to the variation caused by feed composition as reported by Van Der Hoeven-Hangoor *et al.* (2013). Moreover, Steinert *et al.* (2016) discussed different concepts for the characterization of a substance as a prebiotic for humans, and the use of vitamin riboflavin as a “new” prebiotic was proposed. Although it does not provide a direct substrate for microbial fermentation, riboflavin may beneficially modulate the composition of the gut microbiota by being metabolized and changing the gastrointestinal redox state. That is in accordance with the definition of Gibson & Roberfroid (1995) that prebiotics are ingredients that promote beneficial effects to the host by stimulating the growth and / or activity of one or a limited number of bacteria in the colon, promoting improvement in host intestinal health.

The dynamics of the microbiota is also affected by the facilities where the study is conducted. Birds raised in metallic batteries are more demanding in vitamins than those raised on floor pens, which exhibit coprophagy. Birds raised on the floor consume the



vitamins synthesized by the lower gut bacteria that are excreted to the environment because they are not absorbed in the cecum (Vispo & Karasov, 1997). On the other hand, these chickens are also exposed to other stress factors, interfering at the vitamin level for maximum growth. In addition, the variation in microbiota also occurred between individual birds with the same genetics and housed in the same environment (Van Der Hoeven-Hangoor *et al.*, 2013).

The use of supra-nutritional levels of vitamins riboflavin, pantothenic acid, niacin, folic acid, and vitamin B<sub>12</sub> resulted in improved productive performance for chickens raised in batteries and fed a corn and soybean-based diet formulated with low-dietary density. It is known that vegetable diets are more aggressive to the intestinal mucosa of birds in relation to diets containing animal by-product meals. Therefore, the results obtained in this study allow to conclude that when we use low nutritional density in corn-soybean meal-based diets, the use of supra-nutritional level of vitamin supplementation may be needed for the birds to reach their maximum production potential.

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