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Original Article

Levels of Polyamines in Feces of Laying Hens Fed with Agave Fructans (Agave Tequilana, Weber) in Association with the Quality and Production of Egg

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Tequilana weber, egg quality, fructans, supplementation, putrescine.

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ABSTRACT

In this study two experiments were carried out, the effect of a diet supplemented with fructans of Agave tequilana Weber, was analyzed in Hy-line hens in regards to their egg quality and production as well as on the levels of the polyamines, putrescine, spermidine and spermine in their feces. In the first study 300 Hy-line W-36 hens per group, which were aged 36 weeks, were randomly separated as follows for each of the three treatments. One group of 100 hens was fed with a diet supplemented with 0.1% fructans. Another group of 100 hens was supplemented with 0.2% fructans and a group of 100 hens without any fructans was added as a control group. Feed consumption was lower in the supplemented groups compared to the control group (p<0.05). Egg yolk quality was measured using Haugh units. The quality of the shell was studied using an Egg Force reader (g pressure/mm^2). Putrescine levels was measured in 10 animals in each group. In the second study, 1,155 laying hens, aged one day from the Hy-line W-35 genetic line were also randomly divided into three groups containing 385 hens in each one (A, B, C). Egg laying levels and weight was measured during 35 weeks. The egg laying percentage increased considerably in hens ingesting supplemented diets (p<0.05) and the egg weight was greater mainly in the supplementation with 0.1% fructans (p<0.05). Thus, it was shown that diet supplementation with fructans of agave improves egg quality and homeostasis and food consumption in the Hy-line hen.

INTRODUCTION

Fructans are made up of fructose units that can be obtained from *Agave tequilana*. Weber have the possibility of being added to a wide variety of dietary products, since they offer technological and nutritional benefits, such as acting as stress suppressors when decreasing intestinal traffic (Zhang *et al.*, 2007; Corzo *et al.*, 2015; Brady *et al.*, 2017). The so-called oligofructans (OLGFA) have several simple sugars linked together and they are produced by many types of plants. They are concentrated or stored in the tissues of the plant. In general, roots and rhizomes contain the highest concentrations (Corzo *et al.*, 2015).

Prebiotics are ingredients in food that are non-digestible and affect the host favorably by means of selective stimulation of the growth and/or activity of a limited number of bacteria in the colon (Gibson & Roberfroid, 1995), which converts Inulin-type fructans into prebiotics (Márquez-Aguirre *et al.*, 2013). These prebiotics are digested by the Bifidobacteria and it stimulates their growth. Such bacteria contribute, at the same time, to the homeostasis of intestinal cells, besides inhibiting the growth of pathogenic bacteria (Yoo & Kim, 2016). Park & Park (2012) showed that the supplementation of the diet with Helianthus tuberosus L. in laying hens with inulin-type OLGFA can improve further

egg production compared to the unsupplemented diet. However, there were no studies on the effects of fructans of *Agave tequilana* Weber on the diet of laying hens until this moment.

Egg production in Mexico is an activity comparable to that of developed countries with 4.7% of world production. In Mexico, poultry farming is a very important economic activity and it represented 1.0% of the total Gross Domestic Product (GDP) in 2016 (SAGARPA 2016). This economic activity contributed creating 1,000,236 direct and indirect jobs in the same year. Mexico is the main egg consumer worldwide. In 2016 consumption reached 22.8 kg of egg per year, per person (UNA 2014). Thus, the egg represents a whole food culture, given its high nutritional value, its accessibility to most of the population and versatility in the way it is served and eaten.

The polyamines putrescine (Pu), spermidine (Spd) and spermine (Spm) are ubi-molecule and show basic and low molecular weight, given their amino groups. They are obtained from intrinsic sources by intracellular biosynthesis, as well as from extrinsic sources in the case of dieting, intestinal flora and secretions related to digestion. They are considered necessary for homeostasis, cell division (Vargas *et al.*, 2012; Ruseva *et al.*, 2014), as well as cell differentiation, since they are involved in multiple metabolic pathways and molecular processes such as replication, transcription and translation. Therefore, they are considered indispensable molecules for survival and development (Majumdar *et al.*, 2016; Lenis *et al.*, 2017). The bioactive polyamines Spd and Spm, respectively, are degraded to Pu via a retroactive pathway, with the later being excreted. In plant model studies, these polyamines have been well recognized as indicators of stress for 40 years (Minocha *et al.*, 2014); Moreover, there are several studies on polyamine levels in the caecum in mammals (Sabater *et al.*, 2011; Delzenne *et al.*, 2000; Van der Meulen, *et al.*, 2004), but not in laying hen excretion.

Nevertheless, there is a controversy regarding the use of polyamines as molecular markers in different models and/or physiological states. Since it is argued that the levels of polyamines are the result of a total response of the body, when determined in fluids or excretions in cancer cases (Häkkinen *et al.*, 2013) and other conditions like pathological cell proliferation and differentiation and urinary polyamines in patients with Alzheimer's disease (Teti *et al.*, 2002; Paik *et al.*, 2006).

The purpose of this work was to research the effect of a diet supplemented with Fructans of *Agave tequilana*, Weber with regard to egg quality, production of laying hens and levels of polyamines, Pu, Spd and Spm in hen excretions.

MATERIALS AND METHODS

Experimental design

In the first study 300 Hy-line W-36 hens (Dallas, Iowa, U.S.A), aged 36 weeks were randomly separated for each of the following three treatments.

Study groups

One group of 100 birds was fed with a diet supplemented with 0.1% fructans (Bustar Alimentos, Zapopan, México). Another group, made of 100 hens, was supplemented with 0.2 % fructans (Bustar Alimentos, Zapopan, México) and another one, which was made of 100 hens and used as a control group, without fructans added. During the study, the birds were given feed in the morning and in the afternoon in accordance with the nutritional requirements reported by the National Research Council. The feed was served in hopper feeders, and water was provided in bell drinkers. For the OFA 0.1% and 0.2% groups, OFA was added to the feed in powder form. Total feed consumption was determined on a weekly basis and expressed as an average. Inoculations were carried out in accordance with the study zone and the technical reference of the avian. The experiment lasted 20 weeks and during this time values were obtained for the variables of bird development, feed consumption, egg quality in Haugh Units and shell resistance and a sample of 10 hens out of each experimental group was analyzed as well. In the second study, 1,155 laying hens, aged one day from the Hy-line W-35 genetic line were also randomly divided into three groups, containing 385 hens each one (A, B, C). The treatments were categorized, as follows: group A, with 0.1% of fructans (Bustar Alimentos, Zapopan, México) of agave added to the food, B 0.2% of fructans (Bustar Alimentos, Zapopan, México) of agave added to the food, and C control group without fructans of agave added. The amount of agave fructans (Bustar Alimentos, Zapopan, México) added was determined by preliminary experiments (Park & Park 2012). This experiment lasted 35 weeks, time when the test values were obtained for egg-laying percentage and egg weight. The birds were kept under accepted conditions and in accordance with the institutional bioethics regulations.

Agave Fructans

The agave pineapples were obtained from different crops in the southern state of Jalisco Mexico. The species corresponds to Weber tequilana agave, blue variety in a semi-dry climate, clayey soils and preferentially cultivated on the slopes, harvested between 5 and 7 years of age. Pineapples weighing between 28 and 33 kg were used. The minimum content of fructans was 95%, the rest of sugars depending on the origin are mainly fructans and dextrans. The molecular structure corresponds to branched chains with Beta 2-1 and Beta 2-6 bonds, with a maximum degree of polymerization (DP) of 32 and an average DP of 13 units. It is possible to provide complete spectroscopy. The agave extract complies with the official Mexican standard (PROY-NOM-002-SAGARPA-2015), is stable and withstands the high temperatures of the production process. The shelf life is established by official regulation in one year but it has been shown that during 2 years it does not denature, degrade or undergo changes in its composition. The main product of agave photosynthesis are fructans, which are defined as polypolymers of fructose linked by β-fructofuranosyl type bonds, soluble and with one terminal glucose per molecule. These are synthesized and stored mainly in the pineapple of the agave and its main function is that of carbohydrate as a reserve.

Feeding and handling

The bird lodging was in a floor with a corncob bed and a space conditioned for the egg-laying stage with nests of galvanized sheets. During the experiment, the birds were given food twice a day, one in the morning and the other in the afternoon in a controlled manner. All of these based on a feeding program according to their requirements and an appropriate and guaranteed consumption of nutrients according to the tables of the NRC (NRC 1992). The food was served in hopper feeders. The water was supplied in bell drinkers for poultry. Agave fructans (Bustar Alimentos, Zapopan, México) were added to the diet in the form of flour. A vaccination schedule (Avilab, Tepatitlán, México) was carried out according to the study area and the technical reference of the avian stock. The light period was adjusted to 17 hours. The birds were kept under these conditions for three weeks and then until they reached an average weight of 1.119 Kg and 21 weeks of age to start laying eggs. During this period of time, growth was evaluated based on weight and feed consumption.

Table 1 – Formula and chemical composition of the standard diet for the laying hens.

Ingredient	Kg
Sorghum	584
Soy meal	279
Calcium carbonate	88
Vegetable oil	28
Calcium phosphate	11
NaCl	4
Vitamin premix	2.5
Mineral premix	1
Choline chloride	0.5
Yellow pigment	1
Micotoxin sequestrant	0.5
Bacitracina	0.3
Antioxidant	0.2
Total	1,000
Nutriment calculated analysis	
Crude protein (%)	17.9
ME (Kcal/kg)	2,850
Lysine (%)	1
Total Calcium (%)	4
Phosphorus (disp) %	0.44

Production and egg quality

Egg weight was obtained daily and by that amount the percentage of egg-laying was established; Feed consumption was determined on a weekly basis and its values were expressed as an average. The egg quality was determined from the Haugh Units analysis (Haugh, 1937). Measurement of protein quality of the egg was based on the height of the albumin egg white. It was made with a QCH micrometer with a 11 mm stainless steel calibration block (inTech, Weston, Florida, U.S.A), which presented the results on the digital display and QCD power supply. With the egg production and the weight recorded daily and the dietary intake examined weekly, all the data were expressed as the average values of the entire experimental period. The egg quality assessment measured from 20 eggs was selected through the average weight per treatment each week. The Haugh Units, egg shell thickness, resistance to egg shell breakage and yolk color were measured immediately on eggs collected using the EMT-5200 multi-tester egg (Robotmation Co. Ltd. Tokyo, Japan).

Shell quality, in terms of resistance to breaking, was determined in g pressure/mm^2 (Stadelman 1995). The aforementioned was carried out through an "Egg Force" reader, showing the output data on the RS-232 screen (ORKA, West Bountiful, Utah, U.S.A.). In order to determine this, 10 eggs were randomly taken from each of the three test lots, and the analysis was made

in the laboratory of the association of poultry farmers of Los Altos de Jalisco.

Determination of polyamines

Concentrations of Pu, Spd and Spm were determined in excretions by Liquid Chromatography of High Resolution in Reverse Phase (RP-HPLC) with detection of fluorescence (Agilent 1200 Series, Hanover, Germany) according to Marcé *et al.* (1995) with small modifications, at the end of the test. 200 mg of the sample was taken and mixed with 1 ml of TCA-HCl (Sigma, San Louis, Missoury, USA), 40 µL of diaminoheptane (DAH) (Sigma, San Louis, Missoury, USA) was added as an internal standard at 1ngr/µL and centrifuged in Eppendorf tube at 14,000 rpm/20 min. 100 µL of the supernatant was taken and mixed in an amber vial with 40 µL of saturated sodium carbonate and 100 µL of dansyl chloride (5 mg/mL of acetone) was added (Sigma, San Louis, Missoury, USA). The vials were capped and allowed to stand overnight in the dark, dried at room temperature for 72 hrs and with nitrogen gas (PRAXAIR, Guadalajara, México) at 60 °C, respectively. They were re-suspended in 1 ml of water and filtered in Octadecil C18 cartridges (J. T. Baker, The Netherlands), which were previously activated with 1 ml of methanol (Sigma, San Louis, Missoury, USA). They were equilibrated with 1 ml of 20 mM sodium bicarbonate (Carlo Erba, Rome, Italy) at a pH 12. The dansylated sample was passed and the column was washed with 5 mL of water. The polyamines were eluted with 1 ml of acetonitrile (Sigma, San Louis, Missoury, USA), and 20 µL was injected into the Agilent 1200 Series liquid chromatograph (Agilent 1200 Series, Hanover, Germany).

To analyze the polyamine levels, the dansyl chloride dansylation method proposed by Marcé (1995) was utilized. The dansylated polyamines were separated by RP-HPLC, with Lichrosorb column 10-RP-18 (4.6x250 mm) (Merck, Darmstadt, Germany). As a mobile phase, an isocratic mixture of acetonitrile (Merck, Darmstadt, Germany): water (MilliQ, Paris, France) (90:10) at 1 mL/min, fluorescence detection, at 340 nm of excitation and 435 nm of emission was used (Agilent 1200 Series, Hanover, Germany). The concentration of polyamines was determined by the internal standard method (DHA as reference), and expressed in ng/g of Pu in feces.

Statistical analysis

The results for polyamines were analyzed using the Kruskal-Wallis test for mean difference and the Dunn method for the comparison of means, respectively. Values are expressed as average ± standard error (SEM). The analyses were developed with the statistical package SigmaPlot for Windows, version 11. Statistically significant difference was considered with the value of $p<0.05$. For productivity parameters the data were subjected to an analysis of variance and the Fisher's least significant difference test was applied with 95% confidence for the variables feed consumption, egg weight, percentage of laying, shell quality, HU and quantity of VFA., for this, the Minitab 16 Copyright 2014® program was used.

RESULTS

The gain in weight always showed higher results for the hens fed with the 0.2% diet supplemented with fructans of Agave ($p<0.05$). Even though there are fluctuations when comparing the control groups in contrast to the 0.1% diet supplemented with fructans of Agave, it was found that when measuring this parameter from the second week of age until the beginning of the laying period of 20 weeks, there were no significant differences compared to the control group at the end of this period.

Table 2 shows the weight gain or growth of the study birds from the second week of age to the start of the laying period. The results do not present a statistically significant difference for weeks 2, 15 and 18 in the three study groups. However, a better development or weight gain is observed in birds fed with the addition of 0.2% fructans compared to the other weeks analyzed.

Table 2 also shows Feed consumption on a weekly basis from the 5th week. Lower consumption is demonstrated by both groups added with Fructans at week 15 and 20 of the development being statistically significant in respect to the control group ($p<0.05$). However greater weight gain is recorded around the same period of time.

Table 3 shows the percentage of egg laying. In the first half of the study, 15 weeks, the 0.2% diet supplemented has a higher egg laying percentage in contrast to the control group without the diet supplemented ($p<0.05$). By the end of the second half of the same experiment, from week 15 to 30, a higher egg laying percentage was observed in the hens fed with the diet supplemented with 0.1% fructans of Agave tequilana ($p<0.05$).

Important differences in the percentage of laying are observed with the two supplements with respect to the control group ($p<0.05$) in all the weeks

Table 2 – Body weight gain of the laying hen (kg) and feed consumption (kg) in the development with the addition of 0.1% and 0.2% of Agave fructans.

Body weight gain	fructans			
Week	-	0.1 %	0.2 %	SE ±
2	0.11 [a]	0.11 [a]	0.11 [a]	0.008
5	0.26 [b]	0.27 [b]	0.30 [a]	0.016 *
7	0.40 [b]	0.37 [b]	0.41 [a]	0.023 *
9	0.53 [b]	0.52 [b]	0.58 [a]	0.027 *
11	0.68 [a]	0.63 [a]	0.70 [a]	0.042
13	0.70 [b]	0.74 [b]	0.81 [a]	0.041 *
15	0.87 [a]	0.89 [a]	0.93 [a]	0.035
18	1.09 [a]	1.00 [c]	1.08 [b]	0.046 *
Weekly consumption of feed (kg)				
5	0.026 [a]	0.024 [a]	0.025 [a]	0.002
10	0.058 [a]	0.052 [a]	0.055 [a]	0.011
15	0.072 [a]	0.064 [b]	0.066 [b]	0.002 *
20	0.087 [a]	0.079 [b]	0.080 [b]	0.002 *

* a, b letters show significant statistical difference $p<0.05$. SE = Standard Error.

considered in the test, except in week 15 (Table 3). The study groups supplemented with fructans, in general, present important differences at discharge and with significant statistical differences in all the weeks studied compared to the control group.

Table 3 also shows variance in egg weight, except for weeks 7 and 22. Higher values were seen in the groups supplemented with 0.1% and 0.2% fructans compared to the control during the test period, showing significant statistical difference in most weeks, with the group supplemented with 0.1% fructans the one that presented a trend of greater weight ($p<0.05$) during the weeks of study (Table 3).

The differences in egg quality between the three study groups (table 3) can be observed since the values for the Haugh Units show an important statistical

Table 3 – Percentage of egg laying; Egg weight (g); Values of Haugh Units during the laying period of hens fed with the addition of 0.1% and 0.2% of Agave fructans.

Percentage of egg laying of hens	fructans			
Week	Control Group	Group A 0.1 %	Group B 0.2 %	SE ±
1	3.57 [b]	1.86 [b]	5.91 [a]	1.27 *
5	64.67 [b]	63.91 [b]	74.92 [a]	6.25 *
10	75.38 [ab]	69.80 [b]	79.68 [a]	4.31 *
15	91.59 [a]	91.06 [a]	94.40 [a]	4.13
20	91.68 [b]	97.83 [a]	89.06 [b]	4.36 *
25	89.12 [b]	98.71 [a]	90.08 [b]	3.68 *
30	85.50 [ab]	88.86 [a]	80.26 [b]	4.86
Egg weight (g) of laying hens				
2	47.91 [b]	50.05 [a]	49.85 [a]	0.89 *
7	55.22 [a]	53.92 [a]	52.25 [a]	2.51
12	56.41 [b]	59.50 [a]	58.27 [a]	0.46 *
17	60.59 [b]	62.49 [a]	61.46 [b]	0.56 *
22	61.01 [a]	61.83 [a]	60.90 [a]	0.61
27	61.62 [b]	63.21 [a]	61.87 [b]	0.42 *
Values of Haugh Units during the laying period				SE ±/Quality
1°	65.6 [b]	71.2 [a]	75.7 [a]	1.77 *
2°	81.9 [a]	83.9 [a]	83.3 [a]	1.45/very good
3°	84.9 [ab]	87.6 [a]	83.0 [b]	1.17 */very good
4°	90.6 [b]	92.4 [a]	95.6 [a]	0.87 */excellent
Shell resistance values (g pressure/mm^2) of eggs				
1°	4392 [ab]	4479 [a]	4198 [b]	118.25 *
2°	4023 [a]	4005 [a]	4200 [a]	156.86
Quality	Excellent	Excellent	Excellent	-

* a, b Different letters show significant statistical difference $p<0.05$. SE = Standard Error.

difference (p<0.05) that is especially accentuated in the last analysis. The values for Haugh Units showed a difference of 5.6 and 10.1 mm in height of the albumin egg white of the group supplemented with 0.1% and 0.2% of fructans with respect to the control group. There was a statistically significant difference of 1.8 and 5 mm of the group added with 0.1% and 0.2% fructans supplemented with respect to the control group for the last analysis.

Shell resistance. Here the group added with 0.1% of fructans obtained better results in the first analysis with a statistically significant difference, compared to the other groups. Unlike the second analysis, where the highest values were for the group added with 0.2% fructans, compared to the control group and the one supplemented with 0.1%. However, the three groups obtained an excellent score in both analyses (Table 3).

In Figure 1. Putrescine levels in the feces of fructans supplemented hens were lower than in the control group. Putrescine in its non-acetylated form is the best indicator of stress for diets supplemented with fructans from Agave tequilana (Figure 1).

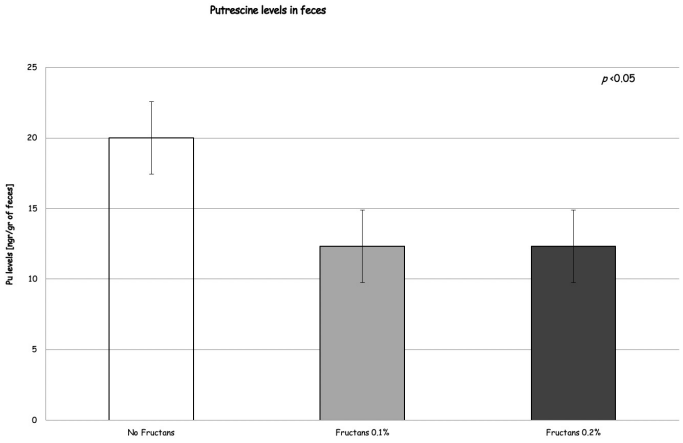

Figure 1 – Levels of putrescine in feces of laying hens fed a diet supplemented with fructans of *Agave tequilana*, Weber. The values correspond to the mean ± the standard error, obtained by the KruskarWallis test and the Dunn test for comparison of means.

In Figure 2, the retention time of each of the standards, Pu, Spd, Spm, as well as Diaminoheptane that corresponds to the internal standard in the analysis, used in the RP-HPLC test to determine polyamines can be observed. In addition, Figure 3 shows a typical chromatogram corresponding to a sample of feces of laying hens analyzed in the present work.

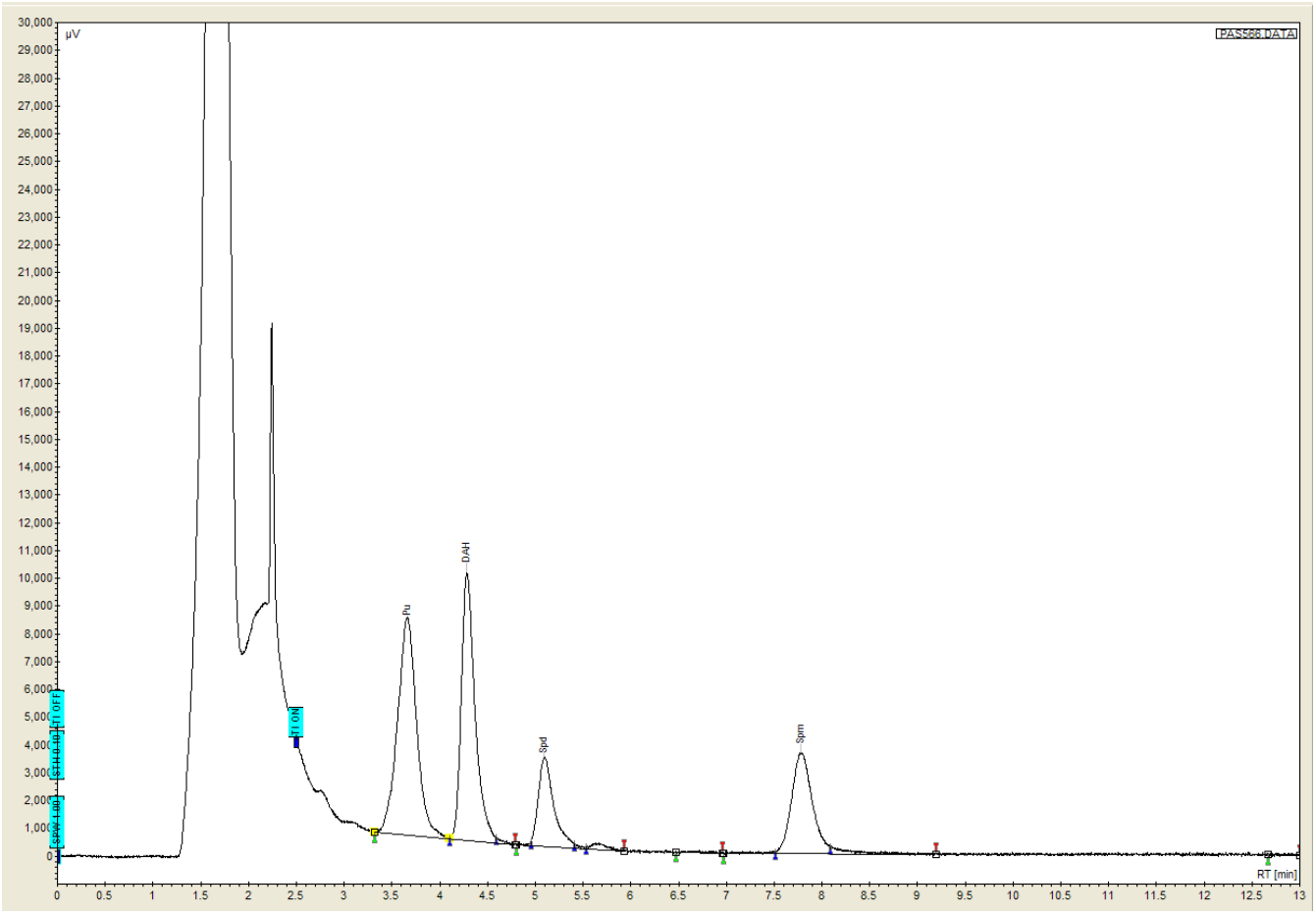

Figure 2 – Chromatogram corresponding to the polyamine standards: putrescine (Pu), spermidine (Spd) and spermine (Spm), as well as diaminoheptane (DAH) as internal standard and where the retention times of each molecule are included.

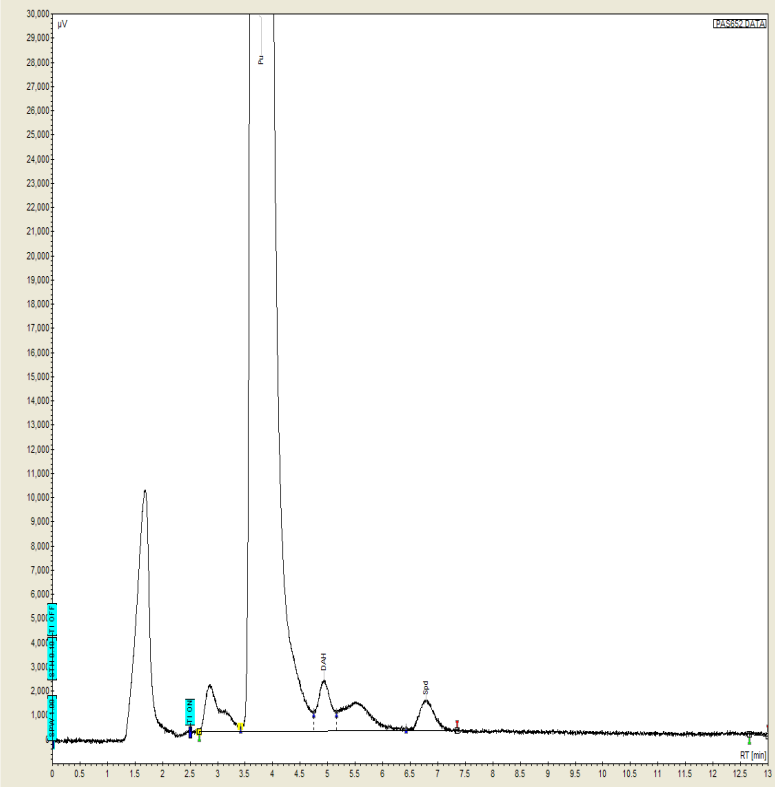

Figure 3 – Typical chromatogram of a feces sample of laying hens fed a diet supplemented with fructans of *Agave tequilana*, Weber and their respective retention times.

DISCUSSION

The results show a better development or weight gain of the birds that were added with 0.2% of fructans. The highest results were found in laying hens fed with a diet supplemented with 0.2% of fructans of Agave. According to Korczak *et al.* (2018) fructan fibers including inulin, fructooligosaccharides and oligofructose affect the feeling of fullness. Probably the dose of 0.2% of supplementation with fructans and the duration of it increases the appetite, therefore increasing the consumption as well as the weight gain in the present work. ~~Not~~ The same results were not found for the diet supplemented with 0.1%. For this last addition to the food, the values obtained were very close to the ones in the control group. The group of Korczak *et al.* (2018) also mentions that a higher dose of fiber such as oligofructose (16 g /day) and longer duration (12-16 weeks) is needed to detect differences in appetite and subsequent energy intake, while practical amounts of fructooligosaccharides, less than 10 g/day, generally do not affect satiety or food intake. Other aspects could also be considered such as the type of fructans, the genetic line or the birds used.

The published data on the deve-lopment of birds due to the inclusion of fructans in the ration are scarce and contradictory, being mainly on broilers. For instance, Ammerman *et al.* (1989) found that the live weight gain of the hens increased with the inclusion of fructans in the ration at a concentration of 3.75 g/kg. Chen & Chen (2003) observed that inulin or fructoligosaccharides, incorporated at a concentration of 10 g/kg improved the live weight increase and the rate of transformation in females, but not in males. Perhaps, it is necessary to work with larger quantities of fructans of Agave to achieve better weight gain.

Regarding the lower food con-sumption in week 10 in the present work, these results are in contrast to the results obtained by the Park & Park group (2012) where they used supplementation of the diet with *Helianthus tuberosus* L. in laying hens with inulin-type fructans, since they found higher feed intake with supplementation.

It is interesting to observe that regarding the egg laying porcentage at the beginning of the 15 days of the experiment, these ones are higher with the 0.2% diet supplemented with fructans of Agave. However, in the second half of the study, it is observed that the group supplemented with 0.1% fructans represents the highest production in such parameter. Both diets compared to the group that did not receive any supplements.

The 1% seaweed (1% CC y SG) increase in the egg weight and the yolk could be due to a major protein synthesis in the yolk and increasement in the availability of water and minerals (Novak *et al.*, 2004) as well as a higher food retention in the gastrointestinal tract.

The mechanism of action of fructans as prebiotics could be demonstrated given the higher dietary intake in the groups treated with the supplemented diets in this work, as well as the higher weight gain in these animals, which most likely achieved a greater absorption of nutrients in the large intestine, since non-digestible oligosaccharides, such as agave inulin, have these properties among others already known (de Vrese & Schrezenmeir, 2008).

Therefore, a better food ileal digesta (Piray *et al.*, 2007). The addition of agave fructans could be considered as a function that improves healthy bacteria which decrease reproductive health issues that decrease egg laying besides the availability of water, minerals and food in the gastrointestinal tract (Novak *et al.*, 2004; Piray *et al.*, 2007). It is likely that Agave fructans provide advantages for healthy microbiota in laying birds, since intake of prebiotics can

confer health benefits to poultry, such as modulating the colonic microbiota by increasing the number of specific probiotic bacteria, including Lactobacilli and Bifidobacteria (Tang *et al.*, 2017). Tests to this hypothesis are required to determine the populations of said bacteria and their development in the intestine of the studied birds.

These results of the egg laying percentage increase in egg weight (Table 3) can be explained by recognizing the effect of the mechanism of action of prebiotics that increases the rate of absorption of nutrients in the large intestine of animals and provides an excellent substrate for Bifidobacteria and Lactobacilli (Roberfroid 2000), which leads to improve the immune response, stimulating at the same time a better absorption of minerals such as calcium and magnesium (Scholz-Ahrens & Schrezenmeir 2002). It was not possible to measure immunological parameters or the absorption of minerals such as Calcium and Magnesium in the present work, although it is interesting to measure these parameters given their possible association, as well as in the Metabolic Syndrome (Crowley *et al.*, 2018; Rayssiguier *et al.*, 2010). It is estimated that egg production is lower in the control group due to these reasons. In previous studies, Park & Park (2012) found statistical differences when comparing the percentage of egg-laying of groups added with different levels of inclusion of inulin. Those groups with greater amount of inclusion being better. Chen *et al.*, (2005) reported that supplementing laying hens with Oligofructose and Inulin increased the percentage of egg laying and of egg weight in comparison to the control group (Table 3).

The data obtained in the present work on egg quality (Table 3) are in agreement with those obtained by Park & Park (2012), where supplementation was done with 250 mg of microencapsulated inulin oligosaccharide per kg of diet.

The results obtained by Park & Park's group (2012) are in agreement with the results obtained in the present study (Table 3) regarding the fact that supplementation provides greater resistance to the shell for these groups.

Although the polyamines are considered appropriate as indicators of physiological changes in diets, the acetylated forms of the polyamines (Fig. 2) Pu, Spd and Spm, in excretions (Muskiet *et al.*, 1995) Spd and Spm did not seem to be good indicators, given the fact that the recorded levels were very close to the lower detection limit (data not shown). The separation technique used in the present study allows the determination of the three polyamines Pu, Spd and Spm, considered classical in their different applications (Reynoso *et al.*, 2008), with the corresponding advantages, since the obtaining of the sample is non-invasive, among others, and for the model presented here (Fig. 3). It has been noted in other animal models that putrescine levels in the feces are associated with homestasis status (Apás *et al.*, 2010).

Although the use of polyamines as stress indicators is well established in the plant kingdom (Minocha *et al.*, 2014), this is not a fully accepted criterion in animals. However, there are studies in human models, showing that in pathologies such as cancer (Gugliucci *et al.*, 2004; Khuhawar & Qureshib 2001) putrescine is a marker for stress levels. However, physiological polyamines are proposed as primordial stress molecules (Rhee *et al.*, 2007) in the small intestine, the particularities of their metabolism does not allow them to be proposed as appropriate indicators of physiological changes since proline and not arginine is used efficiently for the synthesis of polyamines and its precursor ornithine (Flynn, 2009). In addition, since diets can shape the composition of the microbiome, the influence of diet on polyamine levels could also be explained by diet-induced change in the composition of the microbiota, the study model and composition should also be considered of the microbiota. On the other hand, the acetylated forms of Pu, Spd and Spm are not good candidates to consider given the exquisiteness with which the levels of polyamines are controlled under physiological conditions (Bekebrede, 2020).

Furthermore, significant physical differences were observed in the general state of the hens that were fed a diet supplemented with fructans of *Agave tequilana*. For instance, their plumage developed with better appearance, with these, the hens showed better behavior and less stress (Data not shown). The results show an association between the effect of diet with the content of Pu (Fig. 3), with the advantage of using a non-invasive sample type, with respect to other works with similar purposes (Gostner 2006; Gonzalez-Esquerra & Leeson 2006).

CONCLUSIONS

The data gathered in this study showed that supplementing the diet of egg laying hens with *Agave tequlilana*, Weber might be beneficial. Further studies would be needed to measure other egg quality parameters such as cholesterol levels and the development of the microbiota in hens.

CONFLICT OF INTEREST

The authors declare that no conflict of interests lies among them.

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