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Article

Supplementation with *Agave fourcroydes* powder on growth performance, carcass traits, organ weights, gut morphometry, and blood biochemistry in broiler rabbits

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Abstract:

The aim of this study was to evaluate the effect of dietary supplementation with *Agave fourcroydes* powder on growth performance, carcass traits, organ weights, gut morphometry, and blood biochemistry in broiler rabbits. A total of 40 male rabbits (New Zealand × Californian) weaned at 35 d were randomly selected for a control diet (CD) and CD + 1.5% of *A. fourcroydes* powder, with 10 replicates and two rabbits per replicate. After 60 d, *A. fourcroydes* powder increased body weight, feed intake, and weight gain ($P<0.05$), without affecting feed conversion ratio and viability ($P>0.05$). Furthermore, this natural product did not affect the edible portions and the indicators determined in the *Longissimus dorsi*, nor the organ relative weights and the intestinal morphometry ($P>0.05$); however, a decrease in cecal pH was observed and consequently an increase in

cecal beneficial bacteria (P<0.05) were found. Also, *A. fourcroydes* powder reduced (P<0.05) the serum concentration of glucose, harmful lipids, HDL and atherogenic index although without change for the ureic nitrogen, creatinine and VLDL (P>0.05). *Agave fourcroydes* powder as a zootechnical additive promoted better growth, in addition, it showed lipid-lowering and hypoglycemic effects, without modifying the edible portions and organs digestive.

Key words: *Agave fourcroydes*, Zootechnical additive, Rabbit; Natural growth promoter, Hypoglycemic effect, Lipid-lowering effect.

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Introduction

Modern rabbit production is characterized by high productive intensity, in which animals are subjected to different stress situations. These, in turn, cause in some cases imbalances on intestinal microbiota, with the development of pathogenic microorganisms, immunosuppression, inefficient feed conversion, high mortality, and decreased zootechnical response[(1)]. For the above reasons, over the decades, antibiotics are used as animal growth-promoting additives. However, as a consequence of food security problems, especially due to the indiscriminate use of preventive antibiotics, effective dietary alternatives have been identified, with acceptable results in the growth performance and edible portions of non-ruminant animals[(2)].

The scientific community and the industry of the livestock sector study and introduce new safe and innocuous additives to improve the health and productive indicators of animals, such as organic acids, prebiotics, probiotics, phytobiotics, enzymes, or their combination[(3,4)]. These natural products currently have various beneficial characteristics such as hypocholesterolemic, hypoglycemic, anti-inflammatory, antioxidants, immunity modulators, morphology, pH and intestinal microbiology[(4)], thus its constant use in small concentrations in diets, could contribute to maximize the genetic expression of animals, and in turn, the growth performance of farm animals[(5)].

The Agave genus, part of the Agavaceae family, is native to Mexico. The stem of the *Agave fourcroydes* is known to be high in oligosaccharides (fructans) and beneficial anti-inflammatory and bactericidal secondary metabolites such as saponins, flavonoids, anthocyanins, coumarins, reducing sugars and tannins[(6)]. In this sense, Iser *et al*[(7)] reported

that the use of *Agave fourcroydes* powder as a dietary supplement in rabbits promoted the body weight gain due to higher the feed intake and better gut health, which increased the villi height in the small intestine and IgG concentration, with a decrease in the crypts depth and unchanged in hematological parameters.

Despite the prebiotic benefits of *Agave* spp. according to our knowledge, no studies were found to demonstrate its effect on edible portions, chemical composition of *Longissimus dorsi* muscle, serum metabolic profile, cecal lactic acid bacteria and relative weight of immune and visceral organs in rabbits. For this study, it was hypothesized that dietary supplementation with *Agave fourcroydes* rich in fructans could promote the growth of cecal lactic acid bacteria and, therefore, modify the edible portions and decrease the harmful lipids of growing rabbits. Thus, the objective of this experiment was to evaluate the effect of dietary supplementation with *A. fourcroydes* powder on growth performance, carcass traits, organ weights, gut morphometry, and blood biochemistry in broiler rabbits.

Material and methods

Animal, treatment, and housing

This study was carried out in accordance with the Mexican guidelines for animal welfare and experimental protocol, which is approved by the Animal Care Committee (Document CINV.106/12). The experiment was carried out in the "Cofradia" experimental area of the University Center for Biological and Agricultural Sciences, University of Guadalajara, Mexico. The temperature was kept at 21 ºC (±2), and relative humidity was maintained between 63 % (±2).

A total of 40 male rabbits (New Zealand × Californian) weaned at 35 d with an initial BW of 768 ± 2 g were randomly selected to two dietary treatments, with 10 replicates and two rabbits per replicate. For the size of the experimental sample, the recommendations of García *et al*[8] were considered. The dietary treatments consisted of a control diet (CD) and CD+1.5% dried-stem powder of *A. fourcroydes*. For the level of *Agave fourcroydes* supplementation of the diet, the recommendations by Iser *et al*[7] were considered. Control diet was prepared according to the nutritional requirements of broiler rabbits[9]. It was used the same diet from a previous work[7], which met the nutritional requirements of rabbits from 35 to 95 d. The dried-stem meal of *Agave tequilana* was provided by the University Center for Biological and Agricultural Sciences.

The rabbits were placed in metal cages 76 x 76 x 45 cm long, wide, and high, respectively. Feed and water in tubular feeders and automatic nipple drinkers respectively were freely available during the entire experimental period.

Growth performance

During the experimental phase, the initial and final body weight (35 and 95 d old) of the rabbits were measured individually, always at the same time and before feeding them. For this, an OSBORNE® brand digital scale (Kansas, USA), model 37473®, was used with an accuracy of ± 0.1 g. Viability was computed by the number of rabbits during the experimental stage among those housed at the start of the experiment. The average feed intake (FI) was determined daily by the offer and reject method. The average daily gain (ADG) was determined considering the final and initial body weight and the number of experimental days. The feed conversion ratio (FCR) was calculated as the amount of feed eaten, for a gain of 1 kg of body weight.

Carcass traits

Ten (10) rabbits by treatment at 95-d old were sacrificed, by the method of bleeding from the jugular vein, in the experimental slaughterhouse of the University of Guadalajara, Jalisco, Mexico. Before slaughter, the animals for 12 h were fasted, only with water *ad libitum*[10]. For the characterization of the carcass and evaluation of its properties, the dissection of the carcasses was proceeded in fore legs, hind legs, loin, and abdominal wall and ribs[11]. The edible portions were weighed on an OSBORNE® digital scale (Kansas, USA), model 37473®, with an accuracy of ± 0.1 g and the relative weight was calculated according to the carcass weight. Also, the *Longissimus dorsi* muscle (LD, at the level of the 5th lumbar vertebra) was taken from each sacrificed animal and kept at -20 ^{o}C for future analysis.

pH, color tones, chemical composition, and sensorial quality of the *Longissimus dorsi* muscle

After 24 h of sacrifice, the chilled samples (10 rabbits per treatment) reached room temperature (23 °C) and the pH was determined by a Bantex digital potentiometer model 300 A calibrated with buffer solutions of pH 7 and 10. Also, the color tones of the *Longisimus dorsi* muscle, such as *L** (lightness), *a** (redness), and *b** (yellowness) values were measured using a Minolta CR-400/410 chromameter (Konica Minolta Sensing Inc., Osaka Japan). Moreover, in the samples the dry matter (DM), crude fat (CF), ashes and crude protein (CP) was prescribed, according to the methodology described by AOAC[12].

The sensory quality was evaluated by a panel of 16 trained tasters who consume rabbit meat daily, in excellent health and between the ages of 20 and 55 yr of age. Tasters were selected from the University Center of Biological and Agricultural Sciences of the University of Guadalajara, Jalisco, Mexico. The samples (50 g) were cooked without salt or spice at a temperature of 70 ºC for 1 h[13]. The criteria for the evaluation were: Aroma (normal and abnormal), juiciness (normal and abnormal), tenderness (normal, hard, very hard and very soft) and color (normal, pale, and intense).

Relative weight of the organs, morphometry, and gut pH

In the rabbit slaughter (at 95-d old), the viscera (liver and heart), spleen as an immune organ, and stomach were extracted and weighed. In addition, the small intestine, large intestine and cecum was weighed and measured using an OSBORNE® digital scale (Kansas, USA), model 37473®, with an accuracy of ± 0.1 g and a measuring tape, respectively. The relative weight of the organs was calculated according to the body weight at slaughter. At the time of sacrifice, several portions of stomach, small intestine, colon, and caecum were cut and homogenized in paste form in a porcelain mortar. Two grams of sample was weighed on a watch glass; 10 ml of distilled water was added and homogenized in a vortex for 2 min. The pH was determined by a Bantex digital potentiometer model 300 A (USA) calibrated with buffer solutions of pH 7 and 10.

Total count of viable mesophilic bacteria and cecal acid-lactic acid

The cecum sac of each animal was taken by treatment (10 animals per treatment). Then, each sample (1 g) was placed in a tube containing 9 mL of sterile peptone water (Cultimed Parnreac-Química-SAU), homogenized in distilled water at a ratio of 1/10 (w/v) and performed serial dilutions (1/10) until dilution 10^{12}. From each dilution, 1 mL was taken and seeded deep into plates with MRS agar (Difco Laboratories, Detroit, Mich.) and pH 6.2 at 37 ºC for 48 h in anaerobiosis (Gas Pak system, BBL, Cockeysville, USA). Subsequently, to determine the lactic acid bacteria, visual counting was carried out with a colony counter (XK97A, China).

Blood biochemistry

Of the rabbits sacrificed for each treatment (10 rabbits per treatment), 10 ml of blood was taken. To obtain the blood serum, the samples were left to stand for one hour in 20 ml vials, then centrifuged (Eppendorf centrifuge) at 10,000 rpm and 20 ºC for 25 min. In blood serum, glucose, creatinine, urea nitrogen, total lipids, triglycerides, total cholesterol, HDL, LDL and VLDL were determined by colorimetric methods, using a Humalyzer ultraviolet brand spectrophotometer and enzymatic kits. The atherogenic index was determined according to the formula of Dobiášová *et al*[14].

Statistical analysis

Results are expressed as mean ± SEM. The statistical analysis was performed by unpaired t-test according to a completely randomized design, using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). *P* values < 0.05 were taken to indicate significance.

Results

Table 1 shows the effect of dietary supplementation with *A. fourcroydes* powder on growth performance of broiler rabbits. Viability was excellent for both treatments (100 %), also the experimental treatment increased the final BW, ADG and ADFI when was compared with control diet, although the FCR was not affected by the effect of the treatments (P>0.05).

Table 1: Effects of dietary supplementation with dried-stem powder of *A. fourcroydes* on growth performance of broiler rabbits at 95-d old

	Treatments			
Items (n=40 rabbits)	**Control**	***A. fourcroydes* powder**	**SEM ±**	***P* value**
Finish BW, g	2,395.69	2,468.13	13.025	<0.001
ADFI, g/d	121.42	123.40	0.425	0.031
ADG, g/d	27.12	28.33	0.229	0.022
FCR	4.48	4.36	0.031	0.323
Viability, %	100	100		

SEM= standard error of the mean; BW= body weight, ADFI= average daily feed intake, ADG= average daily gain, FCR= feed conversion ratio.

Table 2 shows that dietary supplementation with *Agave fourcroydes* powder had no significant effect (P>0.05) on the edible portion yields and chemical composition, colorimetry, pH, and sensory quality of *Longissimus dorsi* muscle in rabbit broilers.

Table 2: Effect of dietary supplementation with dried-stem powder of *Agave fourcroydes* on carcass traits of broiler rabbits at 95-d old

Items (n=20 rabbits)	Treatments		SEM ±	*P*-value
	Control	*A. fourcroydes* powder		
Edible portions (%)				
Carcass	57.08	56.55	1.073	0.734
Fore legs	16.44	15.54	0.753	0.420
Hind legs	34.13	32.86	1.291	0.505
Ribs	23.11	24.72	1.688	0.519
Chemical composition (%)				
Dry matter	32.87	33.57	0.492	0.541
Crude fat	3.53	3.06	0.283	0.089
Ashes	0.92	1.33	0.170	0.148
Crude protein	23.44	23.22	0.481	0.447
Colorimetry				
*L**	52.05	51.18	1.173	0.614
*a**	5.61	6.03	0.327	0.517
*b**	1.78	1.36	0.189	0.772
pH, 24 h post-mortem	5.41	5.38	0.042	0.665
Sensory quality				
Aroma	Normal	Normal		
Juiciness	Normal	Normal		
Tenderness	Normal	Normal		
Color	Normal	Normal		

SEM= standard error of the mean; *L**: lightness; *a**: redness; *b**: yellowness.

Similarly, dietary supplementation with *A. fourcroydes* did not indicate notable differences ($P>0.05$) (Table 3) for the relative weight of the organs, intestinal morphometry and pH of the digestive system, except for the cecum pH, which decreased due to the use of *A. fourcroydes* ($P<0.05$). Also, this natural product (*A. fourcroydes*) increased the count of viable mesophilic bacteria and cecal lactic acid bacteria ($P<0.05$).

Table 3: Effect of dietary supplementation with dried-stem powder of *Agave fourcroydes* on organ weights, morphometry, and gut pH of broiler rabbits at 95-d old

Items (n=20 rabbits)	Treatments		SEM ±	*P*-value
	Control	*A. fourcroydes* powder		
Relative weight (%)				
Liver	2.38	2.36	0.137	0.941
Heart	0.30	0.29	0.019	0.529
Spleen	0.06	0.05	0.011	0.826
Stomach	4.27	3.97	0.404	0.432
Small intestine	2.15	2.41	0.189	0.350
Large intestine	9.30	8.66	0.803	0.200
Cecum	7.48	7.03	0.785	0.240
Gut morphometry (cm)				
Small intestine	272.83	268.66	5.625	0.681
Large intestine	113.00	110.16	4.225	0.646
Cecum	47.50	47.83	1.267	0.856
pH				
Stomach	5.94	5.54	0.249	0.285
Small intestine	6.93	6.90	0.006	0.798
Cecum	6.77	6.44	0.018	0.046
Colon	6.90	6.80	0.113	0.544
Cecum (CFU/ml)				
Mesophilic viable bacteria	10.42	11.6	0.309	0.021
Lactic acid bacteria	6.36	8.05	0.520	0.044

SEM= standard error of the mean.

Dietary supplementation with 1.5% *A. fourcroydes* reduced ($P<0.05$) the serum concentration of glucose, total lipids, total cholesterol, triacylglycerides, HDL and LDL, while the concentration of ureic nitrogen, creatinine and VLDL showed no differences ($P>0.05$) among treatments (Table 4).

Table 4: Effect of dietary supplementation with dried-stem powder of *Agave fourcroydes* on blood biochemistry and atherogenic index of broiler rabbits at 95-days old (mg/dL)

Items (n=20 rabbits)	Treatments		SEM ±	*P*-value
	Control	*A. fourcroydes* powder		
Ureic nitrogen	39.20	37.00	0.906	0.124
Glucose	129.80	104.20	1.338	<0.001
Creatinine	0.98	0.92	0.150	0.091
Total lipids	512.00	494.80	3.077	0.004
Total cholesterol	213.60	192.80	2.302	<0.001
Triacylglycerides	180.20	163.80	2.447	<0.001
HDL	65.44	53.60	1.392	<0.001
LDL	184.60	102.82	2.056	<0.001
VLDL	36.40	35.20	0.739	0.284
Atherogenic index	2.82	1.93	0.048	<0.001

SEM= standard error of the mean; HDL= high-density lipoproteins, LDL= low density lipoproteins, VLDL= very low-density lipoproteins.

Discussion

The use of new feeds and additives in the diets of experimental animals causes changes in morphophysiology, immune response and microbiology. Being more accentuated in rabbits, with characteristic of a non-ruminant herbivore[9]; that is why the viability can show in the first instance the biological effectiveness of these products. In this sense, *Agave fourcroydes* as a nutraceutical additive did not cause morbidity and mortality in rabbits; similar results were found in a previous experiment[7]. Therefore, Ayala *et al*[3] and Abd El-Hack *et al*[5] indicated that natural products have no residual effects in animal products.

Furthermore, it appears that the organoleptic characteristics of *A. fourcoydes* powder contributed to an increase in feed intake of 1.98 g/d/rabbit in relation to the control. According to Iser *et al*[6] the *A. fourcroydes* powder, have a moderately sweet flavor due to the presence of fructans and fructose, this could stimulate feed intake, without affecting the feed conversion ratio. Likewise, Bovera *et al*[15] reported a higher feed intake in rabbits, due to the effect of MOS (mannan-oligosaccharides) compared to the control group.

Moreover, a higher feed intake with 1.5 % of *A. fourcroydes* could increase the body weight in this treatment, due to the presence of beneficial secondary metabolites and fructans in the diet, which modified the animal response as observed in Table 1. The fructans found in this natural product (*A. fourcoydes*) increase the population of lactic acid bacteria, which causes a competitive exclusion, with favorable influences on body weight[1]. On the other hand, the possible action of secondary metabolites on the beneficial intestinal microbiota of rabbits, could improve the absorption of nutrients, weight gain and therefore the final body weight[5]. Some studies[16,17], found a positive relationship between the incorporation of small concentrations of beneficial secondary metabolites in the diets and the final body weight.

Currently, the *Longissimus dorsi* (LD) muscle is taken as a reference to assess the composition and meat quality[11]. *Agave fourcroydes* as a nutraceutical additive did not affect the protein, fat and ash content of rabbit meat. Dalle-Zotte *et al*[18] indicated protein values (23 to 23.1 %) similar to this research. The fat values in the LD muscle (3.53 to 3.06 %) are within the permissible range for this species, similar to that published by Carrilho *et al*[19], who reported levels of 3.7 to 4.3%. The pH value is directly related to the maturation and color of the meats[3]. According to Składanowska-Baryza *et al*[20], the evolution of post-mortem pH in meat affects luminosity and tenderness. In rabbits, the pH ranges range from 5.3 to 6.4[21], similar to this study. Also, Vázquez *et al*[22], considered the most important chromatic coordinates in meat: *L* * (lightness), *a* * (red tones) and *b* * (yellow tones). There are many factors that influence the value of these indicators, such as muscle type, pH, age, breed, myoglobin content, method of slaughter and feeding[13]. In this sense, it was reported similar values of *a** (5.53), although low values of *b** (0.85) than those shown in Table 2[23].

On the other hand, *Agave fourcrydes* powder as nutraceutical additives in diets did not alter ($P>0.05$) the sensory quality of the LD muscle of fattening rabbits (Table 2), a result that is considered positive, since an alteration in these parameters decreases the choice of this product by the consumer and affects significant economic losses. Apparently, the presence of beneficial fructans and secondary metabolites in the diets[6] due to supplementation with *A. fourcroydes* did not cause abnormalities in rabbit meat.

The results in the relative weight of the liver, heart, and spleen of rabbits (Table 3), showed that the *Agave fourcroydes* stem meal did not affect the organic functions of the rabbits, verified by the growth performance of the rabbits in this group. Similar results were reported for the viscera relative weight, when using a dry extract of *A. fourcroydes*

in laboratory animals[24]. However, in several works[25,26] when using nutraceutical feeds, reported variable weights in the viscera. Another interesting fact is that the relative weight of the spleen did not increase (P>0.05) when *A. fourcroydes* was supplemented on rabbit diets. The increase in the weight of the immune organs is not always associated with increased immunological activity and a productive response[22], as observed in this study, that T1 improved performance, without influence on the relative weight of this immune organ.

In rabbits, studies have shown that the physical-chemical characteristics of feed (mainly high concentrations of NDF) modify the weight and intestinal morphometry due to the greater permanence of the food chyme in these portions[9]. In this sense, *A. fourcoydes* as a nutraceutical additive has a low content of NDF, DAF and LAD[6] and its dietary supplementation did not cause significant changes in GIT (Table 3). Likewise, Mourão *et al*[27] found no variations in the relative weight of the digestive gitorgans in rabbits when they used fructooligosaccharides as a prebiotic supplement. It should be noted that the GIT of rabbits is an organ system, which reacts very sensitively due to its anatomical specialties against strong alterations[28].

Moreover, fructans stimulate the proliferation of beneficial microorganisms, mainly lactic acid bacteria (LAB)[29]. An increase in LAB may influence a favorable competitive exclusion at a GIT level in the rabbits under study, which could increase the inhibition of the proliferation of pathogenic microorganisms[30]. Also, the secondary metabolites, such as tannins, coumarins, reducing carbohydrates and flavonoids identified in the *A. fourcroydes* by having a proven antimicrobial effect[6], which could reduce intestinal pathogenic bacteria, such as *E. coli*, *Clostridium* spp. and *Salmonella* spp. and cause a favorable competitive exclusion, due to the greater proliferation of LAB. Dietary supplementation with 1.5% *A. fourcroydes* caused a decrease (P<0.05) of the cecal pH, perhaps due to the fact that the cecal lactic acid bacteria in rabbits totally degrade the fructans[24]. Authors as Pinheiro *et al*[31], who used diets rich in fructans found similar responses in the cecal pH of rabbits.

Apparently, the dietary supplementation of *A. fourcroydes* did not decrease the protein efficiency of the diet due to the values of blood urea nitrogen[32]. Many reports indicate that feeds rich in fructans such as *A. fourcoydes* lower serum glucose by increasing the secretion of glucagon-like peptide 1 (GLP 1) in endocrine L cells in the intestine, authors have found similar results when using extracts from *Agave fourcroydes* in the diets of laboratory mice[24]. This natural product was shown to have a significant hypoglycemic

effect, since it decreased serum glucose by 25 mg/dL compared to the control. Likewise, perhaps, the presence of secondary metabolites (especially polyphenols) in *A. fourcroydes* could influence serum glucose concentration due to the astringent effect of these metabolites (main polyphenols)[6], which cause slow intestinal release and maintenance of dietary glucose.

A relevant fact in this study is that the addition of *A. fourcroydes* has an important hypolipidemic effect. The high concentration of fructans and the presence of beneficial secondary metabolites in *A. fourcroydes*, as well as a larger BAL population and better intestinal health[7], could have influenced the decrease in serum cholesterol by 21 mg/dL with respect to the control. Perhaps this caused a decrease in LDL by 82 mg/dL, compared to the control. Also, triacylglycerides decreased due to the effect of *A. fourcroydes* powder by 17 mg/dL compared to the control. The results showed that dietary supplementation with *A. fourcroydes* decreased both lipoproteins (LDL and HDL) (Table 4). However, *A. fourcroydes* powder reduced the atherogenic index by 0.89 compared to the basal diet. Currently, there are no defined patterns of atherogenic indices for rabbits. However, a decrease in this index should favor the health of these growing animals[33].

Conclusions and implications

Dietary supplementation with *Agave fourcroydes* powder promoted better growth, with a decrease in cecal pH and an increase in the count of cecal lactic acid bacteria, in addition to reducing harmful lipids (cholesterol, triacylglycerides and LDL), the atherogenic index and serum glucose, without significant changes in the relative weight of the edible portions, digestive organs and chemical composition and sensory quality of the *Longissimus dorsi* muscle.

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