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Article

Economic and productive impact of an herbal mixture with choline derivatives on rabbit production

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Abstract:

The use, as well as the productive and economic effect of herbal compounds on rabbit production has been little studied, for this reason, the objective was to evaluate the effect of a polyherbal mixture rich in choline conjugates based on *Trachyspermum ammi*, *Achyranthes aspera*, *Azadirachta indica* and *Citrullus colocynthis*, on the economic and productive response of meat rabbits. For this, 40 New Zealand X California rabbits (30-d old) were used,

which were randomized into five groups (0.0, 200, 400, 600 and 800 mg kg^{-1} of polyherbal DM, BioCholina®), the experiment lasted 34 days. The productive response, meat quality and economic indicators were evaluated. A completely randomized design with an arrangement of orthogonal polynomials was used to determine linear and quadratic effects, with a significance level of $P<0.05$. The results in terms of production parameters were similar between treatments, except for feed consumption ($P=0.006$) and feed conversion ($P=0.005$) with a linear effect at higher concentration of the polyherbal. The inclusion of the polyherbal increased pH in meat (linear, $P=0.004$) and coordinate b^* (linear, $P=0.009$), it was observed that the treatment with 200 mg showed the best economic indicators, improving the income-expense ratio by 9 percentage units. It is concluded that the addition of polyherbal mixtures based on natural choline conjugate did not improve the productive variables, however, a favorable economic trend is marked with the addition of 200 mg kg^{-1} of DM.

Key words: Economic analysis, Choline conjugates, Phytoadditives, Nutraceuticals, Meat quality.

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Introduction

Animal production presents productive and economic challenges that must be addressed through methods and techniques that do not represent health risks, which is why the use of natural additives considered phytobiotic (phytogenic) that can be included in the diet of animals in production[(1)] is proposed, with the intention of improving productive yield, carcass quality, and that they generate acceptable economic indicators[(2)]. These additives include herbs, essential oils and extracts[(3)], which contain bioactive compounds (secondary metabolites) such as alkaloids, phenols, terpenoids, steroids, tannins, saponins, phenolic compounds (flavonoids, flavones, isoflavones), anthocyanins, lignans, stilbenes, coumarins, carotenoids (tetraterpenes), quinones, among others[(4)]. Other metabolites exert various functions such as antimicrobial, antiparasitic, antioxidant, immunostimulant[(5)], antifungal, anti-inflammatory, antiulcer, antiviral, anticancer agents, and, in animal production, behave as appetite stimulants and growth promoters[(6)], since they can act on the metabolism of the intestine microbiota, inhibiting the replication of specific pathogenic microorganisms, as well as stimulating the production of endogenous digestive enzymes, which benefit health[(7;8)].

There are several reports that demonstrate the use of herbal mixtures rich in choline derivatives in animal production, where the following has been evaluated: the response in

liver and productive function in broilers[9], the impact on rumen fermentation in lambs[8,10], oxidative stress and biochemical changes in dairy cows[11], gene expression and immunostimulant effect in female calves[1], blood analytes in lambs as indicators of lipid metabolism[12].

Choline derivatives (conjugate) are essential nutrients for growth and productive yield of animals; this is because choline is involved in various cellular functions. Usable forms of choline are phosphatidylcholine, lysophosphatidylcholine and sphingomyelin, which are lipid-soluble (components of all cell membranes and have a central role in lipid metabolism and cell signaling), free choline and choline metabolites, acetylcholine (ACho), betaine, glycerophosphocholine and phosphocholine that are soluble in water[13]; likewise, it acts as a donor of the methyl group, after oxidizing to betaine, to convert homocysteine into methionine in the transmethylation pathway in the liver to prevent the accumulation of fat[14].

Due to the multiple functions and benefits, natural choline is an alternative for animal production as an additive to the use of synthetic choline chloride or other additives with side effects and high costs of addition[15], for this reason, the polyherbal mixture Biocholine® (source of low hygroscopicity phosphatidylcholine), made up of the plants *Trachyspermum ammi*, *Achyranthes aspera*, *Azadirachta indica*, *Citrullus colocynthis* and *Andrographis paniculata*[16], can be a productive and economically profitable alternative. That is why the objective of the present study was to evaluate the economic and productive impact of the addition of a polyherbal mixture in choline conjugates added to the diet of rabbits.

Material and methods

Animals and diet

The research was conducted under the guidelines approved by the Academic Committee of the Department of Animal Science of Ethics, Biosafety and Animal Welfare of the UAEM Amecameca University Center of the Autonomous University of the State of Mexico. The experiment was conducted in the rabbit metabolic area.

Forty weaned kits (30 days old), California X New Zealand breed (506.52 ± 120.47 g), were used, which were assigned in individual cages in five treatments (n= 8); with levels of addition of the polyherbal mixture at 0.0, 200, 400, 600 and 800 mg kg^{-1} of dry matter (DM) (BioCholina Powder®, Technofeed Mexico), an additive composed of *Trachyspermum ammi*, *Achyranthes aspera*, *Azadirachta indica*, *Citrullus colocynthis* and *Andrographis paniculata*), which contains 16 g kg^{-1} of total choline conjugates[16], the five groups being homogeneous in terms of weight, which was considered as initial weight (P>0.05).

The experimental diet was formulated to meet the nutritional requirements of fattening rabbits[17] (Table 1), however, choline requirements were kept below (1,130 mg kg^{-1}). The polyherbal formula was added to the formulated balanced feed, mixed and subsequently pelletized to offer it to rabbits *ad libitum*, as well as clean and drinking water. The experiment lasted 34 days, with seven days of adaptation, the diets of the treatments were isoenergetic and isoproteic.

The following productive variables were determined, initial weight (0d), final weight (34d) and daily weight gain, as well as the record of the feed offered and rejected, to obtain voluntary consumption on a wet basis and its subsequent adjustment to dry matter; in addition, the feed conversion (kilos of feed to obtain one kilogram of live weight) was obtained.

Table 1: Ingredients (g kg^{-1}), polyherbal mixture (mg kg^{-1} of feed) and chemical composition of experimental diets

	Polyherbal mixture (mg kg^{-1} DM)				
Ingredients	**Control**	**200**	**400**	**600**	**800**
Wheat bran	33	33	33	33	33
Corn grain	19	19	19	19	19
Oat hay	19	19	19	19	19
Soybean meal (44% CP)	17	17	17	17	17
Alfalfa hay	9	9	9	9	9
Vegetable oil	2	2	2	2	2
Saccharomyces cerevisae	1	1	1	1	1
Polyherbal mix	0.0	200	400	600	800
Crude protein, %	17.9	17.9	17.9	17.9	17.9
Digestible energy, Mcal kg^{-1} DM	2.85	2.85	2.85	2.85	2.85
Crude fiber, %	12.6	12	12	12	12
Neutral detergent fiber, %	31.3	31.3	31.3	31.3	31.3
Acid detergent fiber, %	15.9	15.9	15.9	15.9	15.9
Choline, mg kg^{-1} DM	1127	1130	1133.2	1136.4	1139.6

NCR, 1977.

Sample collection and analysis

At 65 days of age of the rabbits, the slaughter was carried out, with previous fasting of 24 h. The management of the carcasses followed the methodology proposed by Peiretti and Mineri[18]. During the slaughter, the weight of the hot carcass, the gastrointestinal tract including spleen and mesenteric tissue, liver and kidneys (abdominal organs), as well as heart and lungs (thoracic) were recorded. The carcasses were stored at 4 °C for 24 h and weighed again to obtain the weight of the cold carcass; to perform the meat quality measurements, the *Longissimus dorsi* muscle was dissected in its lumbar portion[19].

Meat quality

Analyses were performed on the *Longissimus dorsi* muscle at 24 h *postmortem*; measurements were made in duplicate for each of the variables. The pH was taken at the level of the 5th lumbar vertebra with a penetration potentiometer (Hanna instruments H199163)[20]. Color measurements were taken with a colorimeter (Konica Minolta, trichromatic) and readings were reported as: *L** (luminosity), *a**(red), *b** (yellow), used to obtain C* (chroma) and H* (Hue)[21]. The water retention capacity (WRC) was determined through loss of water by dripping and by pressure[22] and also the loss of water by cooking[23].

To determine the economic impact, the cost of the kit, cost of each ingredient of the diet, including the herbal mixture per treatment dose, were considered, with prices of 2020; all these as expenses of the activity. For income, the sale price of live animals and carcass was calculated based on the local market; once the income and expenses were obtained, the profit (income–expenses) was obtained for the live animal, in hot and cold carcass; finally, the income-expense ratios (income-expenses) were calculated, which allowed to know the rate of return in percentage units, where it is indicated that an income-expense rate less than 1 represents losses, equal to 1 indicates that there were no profits, while rates greater than 1 are indicative of profits. All economic values are indicated in US dollars at an exchange rate of $1USD to $20.03MX dated February 10, 2021.

Statistical analysis

The results were analyzed with a completely randomized design, using the R[24] software, using initial weight as a covariable for the productive variables and using orthogonal

polynomials to obtain linear and quadratic effects to evaluate the effects of the polyherbal additive[25] with a significance level of $P<0.05$.

Results

The addition of the polyherbal mixture showed no significant effects ($P>0.05$) on final weight, daily weight gain, hot and cold carcass weight (Table 2). In contrast, a linear effect is observed on feed consumption ($P=0.006$) and feed conversion ($P=0.005$).

Table 2: Productive test and characteristics of the carcass of rabbits added with a polyherbal mixture (g)

	Polyherbal mixture (mg kg^{-1} DM)					SEM	*P*-value	
Item	**0.0**	**200**	**400**	**600**	**800**		**Linear**	**Quadratic**
Initial weight	513.0	516.5	513.8	498.0	481.1	25.92	0.52	0.71
Final weight *	1444.3	1505.5	1461.5	1443.6	1429.3	38.25	0.44	0.38
Daily weight gain	27.7	30.0	28.3	27.2	26.3	1.85	0.32	0.41
Feed intake *	82.9	87.6	88.6	89.3	91.1	3.22	0.006	0.55
Feed conversion	3.01	2.97	3.18	3.2	3.4	0.12	0.005	0.52
Hot carcass weight *	777.3	845.6	794.4	773.1	769.6	59.51	0.63	0.61
Cold carcass weight *	749.7	816.1	764.7	743.0	739.7	58.88	0.61	0.62
Gastrointestinal tract	340.2	338.5	348.0	335.5	288.7	26.29	0.14	0.19
Heart and lungs	22.5	20.7	23.1	22.7	20.8	2.17	0.84	0.70
Liver	35.5	35.5	39.0	35.8	35.2	2.24	0.68	0.21
Kidneys	12.6	12.6	13.0	12.7	12.5	0.92	0.95	0.75

SEM= standard error of the mean.

* The initial weight was considered as covariable ($P<0.05$)

With respect to the weight of the hot and cold carcass, as well as the weight of the gastrointestinal tract, liver, kidneys and thoracic organs (heart and lungs), no significant effect was observed (P>0.05).

The results on meat quality are shown in Table 3, where it can be seen that the pH showed a linear (P=0.004) and quadratic (P=0.009) effect in the groups added with the polyherbal mixture.

As for the trichromatic coordinates of the meat, no effects (P>0.0.5) were observed in L^*, a^* and Hue angle, not so for b^* and chroma that presented a linear effect (P=0.009 and 0.03), in the water retention capacity, no significant differences were observed (P>0.05).

Table 3: Quality characteristics of the *Longissimus dorsi* muscle obtained from rabbits fed a polyherbal mixture

	Polyherbal mixture (mg kg^{-1}DM)					**SEM**	***P*-value**	
Item	**0**	**200**	**400**	**600**	**800**		**Lin**	**Quad**
pH	5.5	5.6	5.8	5.6	5.8	0.06	0.004	0.09
L^*	51.3	54.3	51.9	57.0	53.5	1.45	0.12	0.31
a^*	3.8	4.2	4.0	4.3	4.8	0.53	0.20	0.74
b^*	3.0	4.3	3.5	4.7	4.6	0.42	0.009	0.59
Chroma	4.9	6.1	5.4	6.4	6.7	0.58	0.03	0.99
Hue Angle	180.6	180.7	180.7	180.8	180.7	0.063	0.13	0.22
Drip loss, %	3.7	3.5	3.8	4.0	3.9	0.016	0.15	0.99
Cooking loss, %	33.9	34.9	33.6	34.4	34.1	0.002	0.39	0.50
Pressure loss, %	20.3	17.5	20.9	20.0	20.0	0.000	0.86	0.81

SEM= standard error of the mean; Lin= linear, Quad= quadratic.
L^*: luminosity; a^*: it tends to red; b^*: it tends to yellow

In the economic analysis (Table 4), an increase in costs as the addition of the polyherbal mixture increased was observed, both in costs per feed and per animal, finding the lowest costs in the control treatment, this derived from the cost of the additive. Regarding income per live animal, in hot and cold carcass, it was obtained that the treatment with the addition of 200 mg kg^{-1} presented higher incomes, a situation that is related to the productive response, since this group was the one with the highest weight.

With respect to the profit and the income-expense ratio, it was observed that the addition of 200 mg kg^{-1} gives the maximum value, reaching the highest profitability in the cold carcass, this being 1.38 (for each unit of expense, the unit is recovered and a profit of 0.38 units is obtained).

Table 4: Economic analysis of rabbits fed a polyherbal mixture

	Polyherbal mixture (mg kg^{-1} DM)				
Item	**0**	**200**	**400**	**600**	**800**
Feed cost, $	0.7688	0.8152	0.8272	0.8362	0.8557
Cost per animal, $	2.7658	2.8122	2.8242	2.8332	2.8527
Income per live animal, $	3.2755	3.4767	3.3180	3.2026	3.0908
Income per hot carcass, $	3.4927	3.7993	3.5696	3.4737	3.4578
Income per cold carcass, $	3.5556	3.8706	3.6270	3.5242	3.5082
Profit per live animal, $	0.5097	0.6645	0.4937	0.3694	0.2381
Profit per hot carcass, $	0.7264	0.9885	0.7453	0.6405	0.6055
Profit per cold carcass, $	0.7898	1.0584	0.8027	0.6909	0.6555
Income-expense ratio live animal	1.18	1.24	1.17	1.13	1.08
Income-expense ratio hot carcass	1.26	1.35	1.26	1.23	1.21
Income-expense ratio cold carcass	1.29	1.38	1.28	1.24	1.23

Discussion

Productive response

According to the results obtained, there were no significant effects on feed consumption and daily weight gain due to the addition of polyherbal mixtures, despite the fact that these additives are considered as phytogenic additives with the characteristic of stimulating the production of digestive enzymes, such as trypsin and amylase, with the ability to optimize the absorption of nutrients and consequently improve productive and carcass responses[6], however, in this study, these benefits were not observed, a situation similar to that presented

by other authors[12], in a study carried out in lambs fed choline conjugates at doses of 0 and 4 g day^{-1}, no better response was found in the added treatment with respect to the control on final weight (28.63 – 28.68 kg) or daily weight gain (106.44 – 107.98 kg). Similarly, in two studies carried out in rabbits using various plants, they did not observed improvements in production parameters despite being plants rich in secondary metabolites[26,27], since it is reported that the metabolites present are not sufficient to modify the microbiota or the conditions of the gastrointestinal tract.

On the other hand, the relationship between feed conversion and weight gain was not affected by the doses of polyherbal mixtures, values that agree with what was reported by another paper[10], an study in which there was a linear effect (P=0.07) in conversion but not in weight gain (P>0.10) in growing lambs, using a polyherbal formula, as well as in the results of Selvam *et al*[28] in chickens fed an herbal mixture (0.0, 500 and 1,000 g^{-1} t^{-1} of feed), and they obtained values of 1.58 in the control treatment and of 1.47 and 1.48 in the added groups.

The addition of polyherbal mixtures showed a positive effect on the variables of feed consumption and daily weight gain, since rabbits with the lowest body weight were in the control group (27.7 g d^{-1}), which is deficient in choline with 1,130 mg d^{-1}, when the minimum requirement is 1,130 mg kg^{-1} DM. In this regard, in studies where choline deficiency has been experimentally induced, growth retardation, anemia, muscular dystrophy and death have been observed[29], a situation that is confirmed in this experiment.

Although the active mechanism of choline chloride is still unclear, excess choline has been shown to negatively affect animal yield; one of the hypotheses that may explain the results obtained is that, when exceeding the ability to metabolize choline at the cellular level, an accumulation of phosphocholine occurs, a situation that could be occurring in treatments with 600 and 800 mg (27.2 and 26.3 g d^{-1} respectively), which are above the necessary requirement for rabbits at this stage of growth[16].

An experiment conducted on quails at doses of 1,000, 1,500, 2,000 and 2,500 mg kg^{-1} of choline showed a greater weight gain between d 7 to 21 of age (1.95, 2.10, 2.98, 2.48 g), as well as better conversion (5.29, 5.44, 3.68, 4.35); this situation contrasts with what was observed in the present research, probably because the addition of polyherbal mixtures could have modified the digestive physiology, acting on the cecal microbiome, since the digestive system of the rabbit is adapted to the fermentation of plant epithelial cells in the cecum[30], while in birds, these digestive fermentation processes do not occur.

Although in this study no hematological analyses were performed to know the hepatic functioning due to the addition of polyherbal mixtures, various studies have observed the variation of the response when using herbal formulas; in a research[31], they found that liver weight had no relationship in the production of specific enzymes (ALT and AST), as they

showed no changes between the groups when choline (1,000 mg kg^{-1}) was added as a lipotropic agent in broilers, likewise[9], they observed a decrease in serum concentrations of AST in broilers fed choline (1 g kg^{-1}) and lectin (0.5 g kg^{-1}). It is worth mentioning that the use of various plants participates in the metabolism of lipids and cholesterol[32], a situation that modifies the general metabolism of the animal and therefore the productive response, it is possible that the plants that make up the polyherbal formula have effects on lipids and these in turn can modify the absorption of nutrients.

Meat quality

According to the pH results obtained, significant differences were found between the treatments added with polyherbal mixtures compared to the control ($P<0.05$), presenting values ranging from 5.5 for the control and 5.8 as a maximum for the added groups. These results are consistent with what was reported by others[33], where they found pH values of 5.8 for meat of rabbits fed different parts of the *Tithonia tubaeformis* plant. The optimal pH at 24 h *postmortem* for glycolytic muscles, such as the *Longissimus dorsi* muscle of the rabbit, is between 5.3 and 6.0[32]; this parameter is one of the most important indicators for assessing meat quality, as it is associated with appearance, color (myoglobin chemical reactions) and water absorption[34].

The parameters obtained of the variable *b** of meat color are lower (between 3 and 4.7) than those reported[35] in rabbits fed flavonoids contained in alfalfa, at doses of 0.0, 400, 800, 1,200 mg kg^{-1} and find values that range between 6.1 and 6.6. As mentioned by Selvam *et al*[28], where they reported values of 8.34 and 10.16 for variable *b* of rabbits fed different parts of the *Tithonia tubaeformis* plant. The coordinate *b** that represents the yellow pigment in meat, this value was affected in the treatments with the addition of the polyherbal mixture, which could be due to a series of nonenzymatic browning reactions that occur in the oxidation products of lipids and amines in the phospholipid head groups or the amine in the protein[36]. Likewise, the presence of antioxidant compounds such as phenols and flavonoids contained in polyherbal mixtures could be responsible for the color differences observed. The results obtained in this experiment in terms of color indicate a meat with a slight coloration that tends to brown and is nonexudative, a situation that is not desirable since rabbit meat is pale nonexudative[37].

In several studies[26,27] that involved the use of plants in the feeding of rabbits, it was observed that the incorporation at low levels of plants with high antioxidant potential and with phenolic compounds, they do not have the ability to modify the quality of the meat, a situation similar to that reported in this experiment, this because the volumes added are low.

Economic impact

The addition of 200 mg kg^{-1} DM of the polyherbal formula generated the highest live weight and the best feed conversion in this experiment, such a situation was reflected in the economic indicators; when adding this dose, the greatest profits and income-expense ratios were obtained, since it can be indicated that it was the optimal dose for these animals. In one study[38], it was observed that the addition of plants with immunostimulant potential generated economic benefits in the production of rabbits, a situation similar to that reported in this research; this effect may be due to the fact that polyherbal formulas contain a large number of metabolites, and these generate beneficial modifications in the gastrointestinal tract, or in the absorption of nutrients, a situation that generates better feed conversions. In the same sense, in a study conducted in dairy cattle, it was observed that the addition of a polyherbal formula rich in choline improved economic indicators due to the fact that milk production was improved and the number of pathology events was decreased[39].

Conclusions and implications

The addition of polyherbal mixtures based on natural choline conjugate did not improve the productive variables; however, a favorable economic trend is marked with the addition of 200 mg/kg of dry matter, due to a positive effect on feed conversion. It is recommended to continue studying the use of natural choline in rabbits, at doses less than 200 mg kg^{-1}, since in this research, the possible benefits were found in this group, as well as maintaining the recommended requirements of choline for optimal storage of vitamins, avoiding inefficiencies in absorption or dietary metabolism, and ensuring the physiological state of rabbits.

Literature cited:

1. Díaz GC, Méndez OET, Martínez GD, Gloria TA, Hernández GPA, Espinosa AE, *et al*. Influence of a Polyherbal Mixture in Dairy Calves: Growth Performance and Gene Expression. Front Vet Sci 2021;7:623710.

2. Madhupriya V, Shamsudeen P, Manohar GR, Senthilkumar S, Soundarapandiyan V, Moorthy M. Phyto feed additives in poultry putrition: A review. Int J Sci Environ Technol 2018;7(3):815-822.

3. Salajegheh A, Salarmoini M, Afsharmanesh M, Salajegheh MH. Growth performance, intestinal microflora, and meat quality of broiler chickens fed lavender (*Lavandula angustifolia*) powder. J Liv Sci Technol 2018;6(1):31-38.

4. Irchhaiya DR. Anumalik Y, Nitika G, Swadesh K, Nikhil G, Santosh K, *et al*. Metabolites in plants and its classification. World J Pharmacy Pharmaceut Sci 2015;4(1):287-305.

5. Syahidah A, Saad CR, Daud HM, Abdelhadi YM. Status and potential of herbal applications in aquaculture: A review. Iran J Fisher Sci 2015;14(1):27-44.

6. Dalle-Zotte, A, Celia C, Szendrő Z. Herbs and spices inclusion as feedstuff or additive in growing rabbit diets and as additive in rabbit meat: A review. Livest Sci 2016;189:82-90.

7. Soliman WSED. Trial on using of some herbal extracts as promising immunoprophylaxis feed additives in cultured Oreochromis niloticus. Egyp J Vet Sci 2017;48(2):53-60.

8. Razo OPB, Mendoza MGD, Silva GV, Osorio TAI, González SJF, Hernández GPA, *et al*. Polyherbal feed additive for lambs: effects on performance, blood biochemistry and biometry. J Appl Anim Res 2020;48(1):419-424.

9. Khosravinia H, Chethen PS, Umakantha B, Nourmohammadi R. Effects of lipotropic products on productive performance, liver lipid and enzymes activity in broiler chickens. Poultry Sci J 2015;3(2):113-120.

10. Ortega ANI, Mendoza GD, Martínez GJA, Bárcena GR, Buendía RG. Impact of a Polyherbal mixture (*Withania somnifera, Ocimum sanctum, Tinospora cordifolia and Emblica officinalis*) on lamb growth and ruminal fermentation. Int J Agri Biol Sci 2020;32(12):864-870.

11. Koujalagi S, Chhabra S, Randhawa SNS, Singh R, Randhawa CS, Kashyap N. Effect of herbal bio choline supplementation on oxidative stress and biochemical parameters in transition dairy cows. Pharma Innov J 2018;7(4):842-847.

12. Rodríguez-Guerrero V, Lizarazo AC, Ferraro S, Suárez N, Miranda LA, Mendoza GD. Effect of herbal choline and rumen-protected methionine on lamb performance and blood metabolites. S Afri J Anim Sci 2018;48(3):427-434.

13. De Veth MJ, Artegoitia VM, Campagna, SR, Lapierre H, Harte F, Girard CL. Choline absorption and evaluation of bioavailability markers when supplementing choline to lactating dairy cows. J Dairy Sci 2016;99(12):9732-9744.

14. Khose KK, Manwar SJ, Gole MA, Ingole RS, Rathod PR. Efficacy of herbal choline as a replacement of synthetic choline chloride in diets on growth performance of broilers. J Lives Res 2018;8(10):313-322.

15. Calderano AA, Nunes RV, Rodrigueiro RJB, César RA. Replacement of choline chloride by a vegetal source of choline in diets for broilers. Ciên Anim Bra 2015;16(1):37-44.

16. Martínez-Aispuro JA, Mendoza GD, Cordero-Mora JL, Ayala-Monter MA, Sánchez-Torres MT, Figueroa-Velasco JL, Gloria-Trujillo A. Evaluation of an herbal choline feed plant additive in lamb feedlot rations. Rev Bra Zoot 2019;48.

17. NRC. Nutrient Requirements of Rabbits. National Academy of Sciences, Washington, DC. 1977.

18. Peiretti PG, Meineri G. Effects on growth performance, carcass characteristics, and the fat and meat fatty acid profile of rabbits fed diets with chia (*Salvia hispanica* L.) seed supplements. Meat Sci 2008;80(4):1116-1121.

19. Blasco A. Ouhayoun J. Harmonization of criteria and terminology in rabbit meat research. Revised proposal. World Rabbit Sci 1996;4(2):93-100.

20. Hajji H, Joy M, Ripoll G, Smeti S, Mekki I, Gahete FM, Atti N. Meat physicochemical properties, fatty acid profile, lipid oxidation and sensory characteristics from three North African lamb breeds, as influenced by concentrate or pasture finishing diets. J Food Compos Anal 2016;48:102-110.

21. Wang W, Chen J, Zhou H, Wang L, Ding S, Wang Y, Li, A. Effects of microencapsulated *Lactobacillus plantarum* and f*ructooligosaccharide* on growth performance, blood immune parameters, and intestinal morphology in weaned piglets. Food Agric Immunol, 2018;29(1):84-94.

22. Celi P, Cowieson AJ, Fru-Nji F, Steinert RE, Kluenter AM, Verlhac V. Gastrointestinal functionality in animal nutrition and health: new opportunities for sustainable animal production. Anim Feed Sci Tech 2017;234:88-100.

23. Liu H, Li K, Mingbin L, Zhao J, Xiong B. Effects of chestnut tannins on the meat quality, welfare, and antioxidant status of heat-stressed lambs. Meat Sci 2016;116:236-242.

24. Mirman D. Growth curve analysis and visualization using R. Chapman & Hall/CRC. The R Series. CRC Press. Boca Raton, FL, USA. 2014.

25. Steel RG, Torrie JH, Dickey DA. Principles and procedures of statistics. A biometrical approach. 2011.

26. Ayala MM, Zepeda BA, Soto SS. Dietary supplementation effects with *Ruta graveolens* on performance, carcass traits and meat quality on rabbits. Rev Mex Cien Pecu 2020; 11(4):1220-1230.

27. Zepeda BA, Martínez MA, Simental SS. Carcass and meat quality of rabbits fed *Tithonia tubaeformis* weed. Rev Bras Zootec 2019;48.

28. Selvam R, Saravanakumar M, Suresh S, Chandrasekeran CV, Prashanth DS. Evaluation of polyherbal formulation and synthetic choline chloride on choline deficiency model in broilers: implications on zootechnical parameters, serum biochemistry and liver histopathology. Asian-Australasian J Anim Sci 2018;31(11):1795.

29. Halls AE. Nutritional requirements for rabbits. Nutreco Canada Inc. Canada 2010. Accessed Jan 4, 2021 https://www.researchgate.net/profile/Rana-Al-Difaie/post/What-the-nutrient-requirement-for-rabbit-during-pregnancy/attachment/59d64eb079197b80779a7fc8/AS%3A493952089899008%401494778763533/download/nutritional-requirements-of-rabbits.pdf.

30. Fortun-Lamothe L, Boullier S. A review on the interactions between gut microflora and digestive mucosal immunity. Possible ways to improve the health of rabbits. Livestock Sci 2007;107(1):1-18.

31. Javed I, Iqbal Z, Rahman ZU, Khan FH, Muhammad F, Aslam B, Ali L. Comparative antihyperlipidaemic efficacy of *Trachyspermum ammi* extracts in albino rabbits. Pakistan Vet J 2006;26(1):23.

32. Rezaeipour V, Aghayar F, Bozorgnia A, Norozi M, Zakaria H. Effects of feeding strategies and supplemental lipotropic factors on growth performance, ascites-related indices, serum metabolites and meat quality in broiler chickens reared at high altitude. JAPS, J Anim Plant Sci 2019;29(1):25-32.

33. Zepeda BA, Martínez MA, Simental SS. Carcass and meat quality of rabbits fed *Tithonia tubaeformis* weed. Rev Bras de Zootec 2019;48:1-10.

34. Hulot F, Ouhayoun J. Muscular pH and related traits in rabbits: a review. World Rabbit Sci 1999;7(1).

35. Kozioł K, Maj D, Bieniek J. Changes in the color and pH of rabbit meat in the aging process. Med Weter 2015;71(2):104-108.

36. Dabbou S, Gasco L, Rotolo L, Pozzo L, Tong JM, Dong XF, Gai F. Effects of dietary alfalfa flavonoids on the performance, meat quality and lipid oxidation of growing rabbits. Asian-Australasian J Anim Sci 2018;31(2):270.

37. Wang Z, He Z, Li H. The effect of repeated freeze-thaw cycles on the meat quality of rabbit. World Rabbit Sci 2018;26(2):165-177.

38. Pulido HS, Espinosa AE, Hernández GPA, Mendoza MGD. Análisis productivo y económico de la adición de fórmula poliherbal en conejos en finalización. En: Herrera CJ, *et al*. Avances de la investigación sobre producción animal y seguridad alimentaria en México. Ed. Universidad Michoacana de San Nicolás de Hidalgo. 2018:991-995.

39. Ortega ANI, Mendoza MGD, Bárcena GR, Hernández GPA, Espinosa AE, Martínez GJA, Gloria TA. Economic impact of polyherbal mixtures containing choline, lysine and methionine on milk production and health of dairy cows. Emirates J Food Agric 2020;864-870.