https://doi.org/10.22319/rmcp.v10i3.4714

Article

Dietary supplementation of inulin or flavomycin and type of cut of rabbit meat: changes on fatty acid profile and sensorial characteristics

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Abstract:

The demand for meat from animals raised with the minimum use of antibiotics is growing. Also, the use of prebiotics, antibiotics, type of cut of meat to modify the fatty acid profile and the effects on consumer preferences are still not clear. The present study investigated the fatty acid profile, the health, and risk fatty acid indices and the consumer sensory evaluation of rabbit's meat fed inulin and flavomycin as additives. Forty-eight (48) New Zealand rabbits were randomly arranged into 4 treatments of 12 animals each. The control group did not receive antibiotic or inulin supplementation. The second group was supplemented with inulin (2.5 g of inulin/kg of feed) while the third group received flavomycin as supplement (0.1 g of flavomycin/kg of feed). The fourth group received both inulin and flavomycin. Inulin addition in rabbit's diet increases beneficial fatty acids (CLA, *P*=0.0001; and n3-PUFA, *P*=0.0001) and enables a better health-promoting index (*P*=0.0004) while reducing the atherogenic (*P*=0.001) and thrombogenic indices (*P*=0.042) of meat. The type of cut of meat (loin, fore legs and hind legs) had a minor impact on changing the fatty acid profile. In contrast, inulin or flavomycin addition showed larger modifications than type of cut of meat on this respect. Flavomycin reduced hedonic properties of meat (taste, *P*=0.0001; color, *P*=0.01; and aroma, *P*=0.0001). Loin tended to be the most preferred cut of meat (*P*=0.01). Inulin is a good alternative to avoid the utilization of antibiotics in rabbit's feeding.

Key words: Fatty acids, Growth promoter, Prebiotic, CLA, Aroma, Meat rabbit.

Received: 04/12/2017

Accepted: 16/05/2018

Introduction

In recent years, increasing interest in the lipid composition of meat and in the impact of fatty acids on human health has occurred. Rabbit's meat is highly valued for its nutritional properties due to the high content of protein with excellent biological value(1,2), low fat content with low amounts of cholesterol(2). There is considerable interest in increasing the levels of polyunsaturated fatty acid (PUFA), especially n-3 fatty acids in animal products to enhance the functional properties of foods while promoting human health(3,4).

Diet is a crucial way to modify the fatty acid profile of rabbit's meat(5). The addition of functional substances to animal's diet can generate favorable response in the meat quality increasing the content of polyunsaturated n-3 fatty acids(6). Fatty acids n-3 consumption may help to balance the n-6/n-3 ratio, which might impact on the prevention of cardiovascular diseases, hypertension, diabetes, arthritis, osteoporosis, among other diseases(2,4). Moreover, the close relationship between diet and health has modified consumer's habits, with an increasing demand for products that not only meet nutritional needs but also healthy food choices(7). Food research linked to human health has included cholesterol and fatty acid profile to represent foods as promoters or threats to health(8-11). Rabbit's meat is a good source of n-6 fatty acids and is a limited source of n-3 fatty acids as well as eicosapentaenoic fatty acid (EPA) and docosahexaenoic fatty acid (DHA)(12). Due to this low n-3 fatty acids content, the n-6/n-3 ratio in rabbit meat is high with values ranging from 7 to 11(12,13). Therefore, lowering the n-6/n-3 ratio in rabbit's meat is highly desirable.

Flavomycin is an antibiotic frequently used in pig, poultry, and cattle farming(14,15). However, the use of antibiotics as growth promoters in animal production has been associated with bacterial resistance in humans. The European Union banned the use of antibiotics as additives in 2006. These circumstances have stimulated the study of alternative products, such as inulin, a prebiotic fiber composed of a chain of fructose units with a terminal of glucose(16). Many studies have shown that fructooligosaccharides supplementation have advantageous effects in humans and animals. Supplementation promotes the maturation of the gastrointestinal tract of suckling rabbits, i.e. gastric pH gradually reach lower values if young rabbits are supplemented with prebiotics. Lower pH (around 2.0) promotes enzyme activity (amylase and proteases) in comparison to higher gastric pH values (around 4.0). Further, complementary effects of supplementation comprise better immune response and enhancement of the growth (number) and permanency of beneficial microbiota including *Bifidobacterium* and *Lactobacillus*(17) in the gut while reducing the risk of pathogenic infections(18). Inulin added to rabbit and poultry diets reduces the total deposition of fat in the body, lowers abdominal fat and modifies the cholesterol, lowers triglyceride values and lowers lipoprotein concentrations(19). Therefore, the meat of rabbits fed inulin may be a good alternative for human consumption, because inulin adjusts microbiota metabolism, induces the synthesis of favorable compounds, and effectively increases desirable nutrients to maintain human wellbeing status. In contrast, the utilization of flavomycin in emerging economies as in Mexico is not prohibited despite consumers from such regions of the globe are increasing their preference for products free of antibiotics.

Sensory evaluation and preference of consumers can be modified by the addition of ingredients in the animal diets that change the hedonic properties of animal products. Also, different cuts of meat may drive the consumer preferences, owing to variations on physical and specific sensory properties of animal muscles. Therefore, consumer preferences can shift the markets according to specific demands. Therefore, it was tested inulin and flavomycin and their combination to investigate the potential benefits on nutritional aspects and the consumer preferences of non-antibiotic *versus* antibiotic agents' utilization. Though flavomycin has an impact on disease prophylaxis, the present study did not focus on those effects. The objectives of this study were to evaluate the dietary supplementation of inulin or flavomycin as well as the type of cut of meat (loin, fore legs and hind legs) on the profile of long fatty acids and on the sensorial characteristics and consumer preference of rabbit's meat.

Material and methods

Experimental setup

Forty-eight (48) New Zealand rabbits (female= 24/male= 24) 40 d old (790 ± 150 g) were randomly arranged into four treatments of 12 animals each. Rabbits were obtained from the experimental farm “Granja Veracruz” at the Universidad Nacional Autónoma de México (UNAM). The study lasted 57 d, the first 15 d of the trial were used for adaptation to the animal management, the housing and the experimental diets. The ingredients and composition of the diets for each treatment are displayed in Table 1. The diets covered the necessary nutritional needs indicated for the species. Each treatment was prepared by mixing the individual ingredients. The control group (CG) did not receive antibiotic (Flaveco40 ECO-Animal Health) or inulin (IPS Raftifeed, Megafarma-Orafti) supplementation. The second group (I+) was supplemented with 2.5 g of inulin/kg of feed. The third group (F+) received 0.1 g of flavomycin/kg of feed. The fourth group (IF) received both inulin and flavomycin doses as previously discussed (2.5 g of inulin/kg and 0.1 g of flavomycin/kg of feed). Rabbits were housed individually in stainless steel cages. Food and water were provided *ad libitum* throughout the research period. Protocols for animal housing, management and sampling were approved by the Institutional Animal Care and Research Advisory Committee (Comité de Investigación en Animales-CINVA) at the INCMNSZ under the registration number NAN-059-09-10-1. Rabbits were slaughtered following the guidelines of the Official Mexican Standard of methods for slaughter domestic and wild animals (NOM-033-SAG/ZOO-2014).

Table: Ingredients and chemical composition of the diet (g/kg)

Ingredients	Control	Inuline (I)	Flavomycin (F)	IF
Corn	116	116	116	116
Wheat bran	251	251	251	251
Soybean meal	129	129	129	129
Soybean oil	31	31	31	31
Alfalfa	438	438	438	438
Calcium phosphate	10	10	10	10
Vitamin and mineral Premixa	a	a	a	a
Antioxidant BHTb	0.01	0.01	0.01	0.01
Potassium sorbate fungicide	0.01	0.01	0.01	0.01
Inulin	0	2.5	0	2.5
Flaveco 40®c	0.0	0.0	0.1	0.1
Coccidiostatd	0.5	0.5	0.5	0.5
Binderd	20	20	20	20
Sodium chloride	5	5	5	5
Chemical composition				
Crude protein, g/kg	169	169	168	171
NDF, g/kg	525	530	517	514
ADF, g/kg	225	202	193	250
Ether extract, g/kg	39	30	29	42
Gross energy, MJ/kg	10.8	13.5	11.7	12.8

[a]Mixture of vitamins and minerals content in grams per kilogram: vit $_A$ 32 000 UI, vit D_3 4000 UI, vit E 100 g, vit K_3 4g, vit B_1 8.0 g, vit B_2 8.0 g, vit B_6 8.0 g, vit B_{12} 40 g, Biotin 200 mg, Panthotenic acid 40 g, Iron 4 g, Copper 6 g, Cobalt 1 g, Zinc 60 g, Manganese 43 g, Iodine 32 mg and Selenium 8 mg. (BASF, Mexico).

[b]BHT, hidroxitolueno butilado;

[c]Flaveco 40 contains 40 g of flavophospholipol per kg.

[d]Coccidiostat robenidine hydrochloride at 33 ppm (Alpharma AS).

[e]Carboxymetil cellulose.

Collection and preparation of samples

The rabbits were slaughtered by cervical dislocation, following the recommendation of the NOM-062-ZOO-1999 (Mexican Official Norm - NOM, 2001). The hot carcasses were placed in a ventilated area for 1 h before being cut to get the three meat cuts (loin, fore legs and hind legs) per single rabbit on each of the four treatments according to the guidelines of Blasco and Ouhayoun[20]. Cuts of meat were individually packed into hermetically sealed plastic bags and stored at -18 °C until evaluation. For fatty acid evaluation, the experimental unit was the cut of meat (loin, fore legs and hind legs) of six animals within the treatments. The rest of cuts of meat from the animals of each treatment (six rabbits) were used for sensorial evaluation. The experimental unit was the pool of the same cuts of meat of two animals to obtain enough material for 10 panelists.

Chemical analysis of diets

Crude protein was measured by Kjeldahl nitrogen analysis AOAC cod 976.05[21]. The neutral detergent fiber (NFD) and acid detergent fiber (ADF) contents were determined according to the Ankom method, following the protocol of the manufacturer, using model F-57 filter bags (Ankom Technology, NY, USA). The ether extract was extracted by anhydrous diethyl ether using a Soxhlet apparatus AOAC 920.15 and 963.39[21]. Energy was calculated for each diet in a Parr calorimeter model 1241 (Parr Instrument Company, IL, USA), following the protocol of the manufacturer[22].

Fatty acid extraction and determination of methyl-esters fatty acids (FAME) and conjugated linoleic acid (CLA)

Fatty acids were extracted from the meat using a 2:1 chloroform-methanol mixture[23] and a gravimetric calculation according to the official method 696.33, AOAC[21]. Fatty acid methyl esters (FAME) were quantified by gas chromatography-GC (Varian, Inc., Palo Alto, CA, USA) using a CP-3380 chromatograph equipped with a split injector, FID, and an autosampler CP 8400, in a DB 23 column (30 m×0.25 mm inner diameter; Varian, Inc., Palo Alto, CA, USA) with a film thickness of 0.25 μm. Nitrogen was used as the carrier gas at a flow rate of 30 ml/min. The column temperature was held for 1 min at 120 °C, then increased at a rate of 10 °C/min to 200 °C, and finally at 5 °C/min to 230 °C. The injector and FID temperatures were 250 °C and 300 °C, respectively. The volume of injection was 1 μL. Integration of each fatty acid was performed with a Varian Star Chromatography Workstation Software version 4.51. Identification of the peaks was made on the basis of retention times of standard methyl esters of each individual fatty acid (FAME mix C4-C24 no. 18919-1 AMP; Sigma-Aldrich Inc., St. Louis, MO, USA). Conjugated linoleic acid (CLA) in particular was identified using a CLA methyl ester standard with a mixture of cis- and trans-9,11-, and -10,12- octadecadienoic acids (Cat.

no. O5632. Sigma-Aldrich Co., USA). Myristoleic acid (C: 14 9-tetradecenoic acid; Cat. no. M3525 Sigma-Aldrich Co., USA) was added to the methylated meat fat samples prior to GC analysis and using an internal standard. We only evaluated the long chain fatty acids because of their high relevance on human health while volatile fatty acids were not considered in the present study. Results were reported as the percentage of long chain fatty acids (g/100 g).

Health and risk indices (AI, TI and HPI)

Atherogenic (AI) and thrombogenic (TI) indices, were calculated according to Ulbricht and Southgate[9] using the following formulas: AI = C12:0 + (4 x C14:0) + C16:0 / n-6 PUFA + n-3 PUFA + MUFA. TI = C14:0 + C16:0 + C18:0 / (0.5 MUFA) + (0.5 n-6 PUFA) + (3 n-3 PUFA) + (n-3 PUFA/ n-6 PUFA). Health promoting index (HPI) was calculated according to the recommendation of Chen *et al*[10] : HPI= n-6 PUFA + n-3 PUFA + MUFA / C12:0 + (4x C14:0) + C16:0.

Consumer sensory panel evaluation

Twelve hours before the sensorial evaluation's day, cuts of meat were defrosted at a refrigeration temperature (4 °C). Further, cuts of meat were chopped (dices, 2 x 1 x 1 cm) and later were pooled within each treatment. The cuts of meat of the four treatments were cooked using water (1:1 w/v) at an intern temperature of 71 °C using conventional pots with lids (diameter 25 cm and volume of 4 L) for 60 min. The four pots were heated simultaneously (100 °C) on a gas stove (IEM). No additive was added during the cooking process. The cooked samples (2 cm^3/12 g and pH 5) were offered on disposables plastic white plates. The evaluation was performed at the sensorial evaluation laboratory of the INCMNSZ. A mild-white light was used at the individual evaluation rooms[24]. A total of 30 untrained panelist (male and women 15:15) generated 1,080 results (30 panelist x 4 treatments x 3 types of cut of meat x 3 meat sensory characteristics). Double-blind evaluation was carried out. To score the preference of taste, color and aroma, a scale from 1 to 4 was used (4= like it very much; 3= like it; 2= do not like; 1= dislike). Plain water and a slice of white bread was offered to clean and eliminate residuals between samples.

Statistical analysis

The data was processed by ANOVA using the Proc GLM[25]. The model utilized was:

$$\mathbf{Y}_{ij} = \boldsymbol{\mu} + \mathbf{AA}_i + \mathbf{PM}_j + \mathbf{AA}_i \times \mathbf{PM}_j + \mathbf{e}_{ij};$$

Where:

Y is the target variable;
μ is the mean;
$\mathbf{AA}_i$ = Additive agent $_i$ [Inulin (I+), Flavomycin (F+) and Inulin plus flavomycin (IF)];
$\mathbf{PM}_j$ = Piece of meat $_j$ (loin, fore legs and hind legs);
e = experimental error.

Differences were stablished with Tukey test (α= 0.05).

Results

Table 1 shows the chemical composition of the different diets. No differences were observed between diets in any of the analized variables. Total protein content of the four diets was on average 169 ± 1 g/kg, whereas NDF and ADF were 522 ± 7 and 218 ± 26 g/kg, respectively. The content of ether extract did not differed significantly among treatments. Also, inulin did not modify the gross energy of the diets in which this ingredient was added (I+ and IF).

Fatty acid concentration of rabbit's meat was influenced by the addition of inulin or flavomycin as well as the type of cut of meat (Table 2). However, feeding treatment showed to have more impact on fatty acid concentration than type of cut of meat on this respect and there were few interactions of these two main factors. For example, no significant effect was observed for myristic acid (C14:0) because of the type of cut of meat; however, I+ and IF treatments were lower in the values of this fatty acid, due to the diet effect (*P*=0.0001). Palmitic (C16:0) acid was numerically higher in CG, moreover was only different from the fore legs when rabbits were fed F+ and IF (*P*=0.004). Differences in heptadecanoic acid (C17:1) in all feeding treatments and cut of meat were not detected, except for loin (F+) and fore legs (IF) with an effect type of cut of meat (*P*=0.04). Stearic acid (C18:0) increased when rabbits were fed with F+ and the same results were observed when flavomycin was combined with inulin. Flavomicine (F+)

influenced the increase in stearic acid (C18:0) content in the three types of cut of meat (loin, fore legs and hind legs), however, the hind legs in F+ and IF showed the maximum value while the lowest was for CG. Oleic acid (C18:1) concentration was highly influenced by feeding treatment, type of cut of meat, and their interaction (P=0.02; P=0.01, P=0.001). The concentration of α-linolenic acid (C18:3) was highest when rabbits were fed I+, in contrast, IF reported the lowest value of this fatty acid. Eicosanoic acid (C20:0) in fore legs from rabbits fed F+ was higher than the rest of types of cut of meat in all treatments (P=0.007). Arachidonic acid (C20:4 n-6) was eleven times (3.4 %) higher in hind legs from IF group than fore legs from CG (0.3 %). This outcome was affected by the two main factors and their interactions (P=0.001; P=0.0001; P=0.0003).

Table 2: Percentage of long chain fatty acids (g/100 g) of cooked rabbit's meat influenced by the adittion of inulin and flavomycin and type of cut of meat (n=6)

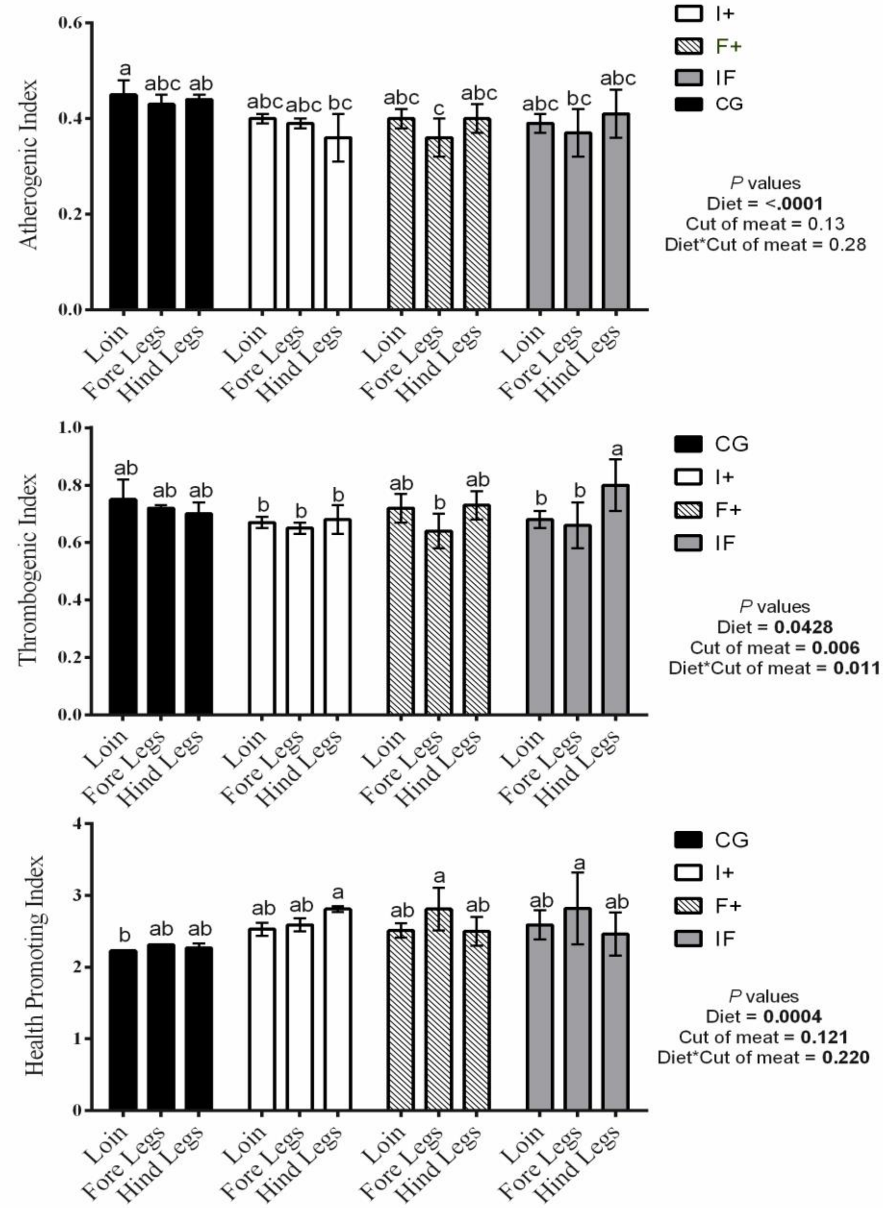
	Control			Inulin (I)			Flavomycin (F)			IF				P value		
	Loin	Fore legs	Hind legs	Loin	Fore legs	Hind legs	Loin	Fore legs	Hind legs	Loin	Fore legs	Hind legs	SEM	Diet	Cut of meat	Diet x Cut of meat
C 12:0	0.07	0.08	0.14	0.11	0.17	0.09	0.12	0.1	0.23	0.16	0.13	0.08	0.01	0.47	0.74	0.11
C14:0	1.53^{a}	1.6^{a}	1.7^{a}	1.34^{ab}	1.2^{ab}	0.8^{b}	1.4^{a}	1.6^{a}	1.5^{a}	1.4^{a}	1.3^{ab}	1.2^{ab}	0.04	<0.0001	0.27	0.08
C 16:0	21.35^{a}	20.5^{a}	21.7^{a}	20.23^{ab}	20.2^{abc}	20.2^{abc}	19.2^{abc}	17.2^{bc}	19.2^{abc}	19.5^{abc}	17.08^{c}	19.5^{abc}	0.2	<0.0001	0.004	0.49
C 16.1 n-9	1.98	1.5	1.7	1.42	1.9	2.03	1.4	1.3	1.5	1.2	1.3	1.4	0.07	0.001	0.43	0.14
C 17:1	0.54^{ab}	0.5^{ab}	0.6^{ab}	0.62^{ab}	0.5^{ab}	0.6^{ab}	0.5^{b}	0.7^{ab}	0.6^{ab}	0.6^{ab}	0.8^{a}	0.6^{ab}	0.02	0.10	0.04	0.27
C 18:0	6.72^{cd}	6.8^{bcd}	6.5^{d}	6.9^{bcd}	7.07^{abcd}	7.6^{abcd}	8.12^{ab}	8.1^{ab}	8.3^{a}	7.5^{abcd}	7.9^{abc}	8.3^{a}	0.11	<0.0001	0.16	0.50
C:18.1 n-9	33.62^{ab}	35.2^{a}	36.3^{a}	37.4^{a}	36.5^{a}	34.3^{ab}	34.3^{ab}	35.9^{a}	33.6^{ab}	36.8^{a}	33.5^{ab}	30.3^{b}	0.41	0.02	0.01	0.001
C18:2 n-6	19.65^{c}	20.8^{abc}	20.9^{abc}	20.7^{abc}	20.3^{bc}	20.9^{abc}	21.33^{abc}	23.02^{a}	22.7^{ab}	21.2^{abc}	20.3^{abc}	20.5^{abc}	0.23	0.0003	0.37	0.25
C 18:3 n-6	0.03	0.02	0.07	0.11	0.03	0.05	0.006	0.1	0.03	0.02	0.03	0.02	0.01	0.27	0.98	0.03
C18:3 n-3	3.45^{ab}	3.6ab	4.03^{ab}	3.8^{ab}	4.2^{a}	3.8^{ab}	3.5^{ab}	3.6^{ab}	3.4^{b}	3.4^{b}	3.3^{b}	2.5^{c}	0.06	<0.0001	0.07	0.0003
C 20:0	0.51^{b}	0.6^{b}	0.6^{b}	0.72^{b}	0.7^{b}	0.5^{b}	0.5^{b}	1.2^{a}	0.7^{b}	0.6^{b}	0.7^{b}	0.6^{b}	0.03	0.09	0.007	0.0005
C 20:4 n-6	1.93^{abc}	0.3^{d}	1.3^{cd}	0.83^{cd}	1.1^{cd}	2.9^{ab}	1.4^{bcd}	0.5^{cd}	1.4^{bc}d	1.2^{cd}	1.8 ^{abcd}	3.4^{a}	0.13	0.001	<0.0001	0.0003
C 20:5 n-3	0.14^{ab}	0.06^{abc}	0.07^{abc}	0.12^{ab}	0.1^{abc}	0.12^{abc}	ND	0.06^{bc}	ND	0.2^{a}	0.2^{a}	0.14^{ab}	0.01	<0.0001	0.36	0.24
C 22:6 n-3	0.07^{ab}	0.04^{ab}	0.03^{ab}	0.04^{ab}	0.05^{ab}	0.07^{ab}	ND	0.02^{ab}	ND	0.05^{ab}	0.1^{a}	0.09^{a}	0.01	0.0001	0.46	0.20
SFA	30.18	29.7	30.6	29.34	29.2	29.2	29.3	28.1	29.9	29.07	27.04	29.7	0.33	0.12	0.06	0.77
MUFA	36.14^{ab}	37.3^{a}	38.5^{a}	39.4^{a}	39.03^{a}	36.9^{ab}	36.12^{ab}	37.9^{a}	35.7^{ab}	38.7^{a}	35.5^{ab}	32.3^{b}	0.42	0.008	0.03	0.004
PUFA	25.36	24.9	26.4	25.5	25.7	27.8	26.2	27.2	27.5	26.01	25.8	26.7	0.34	0.18	0.04	0.90
n -3 PUFA	3.7^{ab}c	3.7^{ab}c	4.14^{a}b	3.9^{abc}	4.3^{a}	3.9^{abc}	3.5^{bcd}	3.6^{abc}	3.4^{cd}	3.7^{abc}	3.6^{abc}	2.7^{d}	0.06	<0.0001	0.055	0.0005
n-6/n-3	5.9^{bcd}	5.8^{cd}	5.4^{cd}	5.5^{cd}	4.9^{d}	6.14^{bc}d	6.6^{bc}	6.5^{bc}	7.2^{b}	6.2 ^{bcd}	6.3^{bcd}	8.9^{a}	0.12	<0.0001	<0.0001	<0.0001
SFA/ PUFA	1.21	1.2	1.2	1.2	1.1	1.05	1.121	1.03	1.09	1.12	1.073	1.11	0.02	0.03	0.40	0.80
n-6 PUFA	21.6	21.2	22.2	21.6	21.4	23.9	22.7	23.6	24.2	22.4	22.2	23.9	0.31	0.03	0.008	0.84
CLA	5.3^{abc}	3.9^{abc}	4.09^{abc}	8.3^{a}	6.04^{abc}	7.8^{ab}	2.6^{c}	1.9^{c}	3.1^{c}	2.9^{c}	4.05^{abc}	3.9^{bc}	0.37	<0.0001	0.40	0.60

SFA= saturated fatty acids; PUFA= polyunsaturated fatty acids; MUFA= monounsatured fatty acids; CLA= conjugated linoleic acid isomers (cis-9, trans-11; trans-9, cis-11; trans-10, cis-12; mg g-1 of fat); SEM= standard error of the mean; ND= not detected.

^{a,b,c,d} Means with different letters within the same row are significantly different (P<0.05).

In the analysis of SFA and PUFA the results indicated that they were not modified by inulin, flavomycin or the type of cut. MUFA content was affected by feeding and type of cut of meat and showed significant interaction (P= 0.008; P= 0.03 and P= 0.004). Moreover, the n3-PUFA and monounsaturated fatty acids (MUFA) content increased with the inulin adittion and in CG. The ratio n6/n3 decreased in the CG and I+ with respect to F+ and IF, but only hind legs from IF was different from the rest (*P*= 0.0001). This is more evident when we analized the effects by cuts of meat; i.e., inulin decreased the n-6/n-3 ratio in fore legs (4.9) while IF increased this value in hind legs (8.9 %). CLA content (8.3 %) of loin from animals fed with inulin was three times higher than the average of the three cuts of meat (2.5 %) from rabbits fed with flavor. Atherogenic and health promoting indices were influenced by feeding while thrombogenic, was influenced by feeding and type of cut of meat factors (Figure 1).

Figure 1: Atherogenic, thrombogenic and health promoting indices of rabbit's meat influenced by the adittion of inulin or flavomycin and type of cut of meat (n= 30)



AI = atherogenic index [C12:0 + (4 x C14:0) + C16:0 / n-6 PUFA + n-3 PUFA + MUFA].

TI = thrombogenic index [C14:0 + C16:0 + C18:0 / (0.5 MUFA) + (0.5 n-6 PUFA) + (3 n-3 PUFA) + (n-3 PUFA/ n-6 PUFA)].

HPI = health promoting index [n-6 PUFA + n-3 PUFA + MUFA / C12:0 + (4 x C14:0) + C16:0].

[a,b,c] Means with different letters are significantly different (P<0.05).

CG = Control group; I+= 2.5 g of inulin/kg; F+= 0.1 g of flavomycin/kg; IF= inulin and flavomicyn.

Fore legs from control group was the most preferred cut of meat (taste= 3.4, color= 3.2 and aroma= 3.3) within all analyzed treatments. In contrast, hind legs from F+ and IF (taste= 2.47 and 2.47; color= 2.90 and 2.50; aroma= 2.53 and 2.57, respectively) were the least preferred cut of meat of all samples evaluated.

Discussion

Inulin addition to animal diet did not change the gross energy of the diets in which this ingredient was added (I+ and IF). In another study where inulin was used, the protein and fat digestibility increased while the gross energy of diet remained unchanged[26]. Though, inulin is a source of carbohydrates, the percentage of inclusion of inulin in both studies was not enough to modify the gross energy values of diets. In the present study, although ADF content slightly decreased while NDF increased in I+ treatment, no statistical differences were detected.

Feeding treatment modified the fatty acid profile as in other studies[27,28]. The fatty acid profile in monogastric animals is almost a direct reflection of their dietary fatty acids. In the present study, inulin (I+) which is a linear polymer and oligomer of fructose with a terminal of glucose, favored the increase of fatty acids such as oleic (C18:1), α-linolenic (C18:3) and DHA (C22:6) while reducing the content of myristic acid (C14:0). It has been shown that cecal fermentation of rabbits can be modified with the use of inulin, by shifting the precursor of n-6 fatty acids (linoleic acid, C18:2) to α-linolenic acid (C18:3), the n-3 precursor, stimulating the production of healthier unsaturated fatty acids[27]. In contrast, the antibiotic flavomycin inhibited the viability and growth of not only pathogen microbiota but also the beneficial microbiota along the gut and caecum[29]. According to Bovera *et al*[30], endogenous production of saturated fatty acids as palmitic (C16:0) and stearic (C18:0) made it impossible to lower the values with the addition of inulin. Flavomycin alone and inulin plus flavomycin supplemented together, decreased the concentration of C16:0 when these ingredients were added. In contrast, flavomycin alone and Inulin plus flavomycin supplemented together, increased the concentration of C18:0. Divergent effects on particular fatty acids are possible, because gut and cecal microorganisms are capable of hydrogenating unsaturated fatty acids into more saturated ones, or vice versa through elongation (addition of two-carbon units to the carboxyl ends) and desaturation (introduction of double bonds into the long-chain acyl CoAs) of palmitic acid as discussed by other authors[29,30]. This explains why in the present study, F+ and IF had lower value of palmitic acid (C16:0) than I+ and CG. In contrast to palmitic acid (C16:0), stearic acid (C18:0) increased in F+ and IF and decreased in I+ and CG. The possible explanation is that microbial population is altered, yielding distinct microbiota composition, further, modifying the metabolites produced. Likely, palmitic acid is elongated to stearic acid more rapidly in F+ and IF than in I+ and CG as shown in Table 2.

Coprophagia of soft feces performed by rabbits mainly at diurnal cycles, can increase PUFA concentration in meat(31). However, in the present study, no differences were observed in the total PUFA content. Moreover, I+ increased CLA (C18:2 cis-9, trans-11), total PUFA-n3 and MUFA as palmitoleic acid (16:1), which were able to positively affect the HPI while reducing the atherogenic and thrombogenic indices. Consequently, the n-6/n-3 ratio decreased in the CG and I+ with respect to F+ and IF. In contrast, CG showed the highest TI and AI indices and a reduced HPI, possibly due to the high content of SFA as myristic (C14:0), palmitic (C16:0) and the reduced content of PUFA. These results are in accordance to those found by Bovera *et al*(30), when feeding rabbits a prebiotic mannanoligosccharide additive (at 0.5, 1.0 and 1.5 of g/kg of diet), which resulted in a reduction in the TI but no differences were detected in the AI. In the present study, inulin inclusion positively affected AI and TI indices in comparison to CG, but the addition of inulin did not decrease the AI and TI indices in relation to F+ and IF. Therefore, it would be prudent to boost the concentration of desirable fatty acids such as conjugated linoleic acid isomers (CLA), α-linolenic, EPA and DHA to intensify this trend. The inclusion of rich sources of n-3 fatty acids in the rabbit diets e.g. oils and meals from oleaginous, would be good option for this purpose(13,28). The occurrence of these metabolites in rabbit meat may contribute to its shelf life and improved human health as was reported in other animal products(11,32-34). Daily intake recommendation for eicosapentanoic (C20:5) and docosahexanoic (C22:6) acids, which belong to the family n3, is 500 mg to maintain a healthy cardiovascular system in humans. The intake of rabbit meat fed from CG, I+, F+ and IF contribute to the n-3 fatty acids daily ingestion of about 145, 165, 121 and 110 mg/100 g of meat, corresponding to 29, 33, 24 and 22 % of the recommended daily allowance, respectively(35). Analyzing the contribution of n-3 fatty acids by cuts of meat; loin, fore legs and hind legs would contribute to 135, 144 and 126 mg/100g of meat, corresponding to 27, 28 and 25 % of the recommended daily allowance, respectively.

The n-3-PUFA and MUFA content slightly increased with the inulin addition and the health promoting index while reducing the atherogenic and thrombogenic indices, thus indicating smaller feasibility to cause an atherogenic and a thrombogenic event. This may be due to the caecotrophes ingestion by rabbits that increase the recycle of nutrients, i.e., rabbits eating soft faeces, increased the availability of unsaturated fatty acid in the diet(29). In that process, non-utilized lipids, which escape from intestinal digestion, are hydrogenated/dehydrogenated in the ceacum by the microbial population and then liberated to the lower part of the hindgut, further, the rabbits ingest them through the caecotrophes. Likely, inulin may exert the metabolic pathway to produce unsaturated fatty acids. This explains why CG showed the highest thrombogenic and atherogenic indices and a reduced health-promoting index. Also, the utilization of inulin by some bacteria as a food source, produced short chain fatty acids, which might favor the acidification of the gut and ceacum environment, facilitating the colonization of beneficial lactic acid bacteria and hampering the presence and activity of potential pathongens(15,29).

In respect to cuts of meat influence on fatty acid profile, no clear difference was found among cuts for SFA, MUFA and PUFA. Petracci *et al*[6] found that the content of n-3 PUFA in rabbit's fed 3%, 6% and 9% linseed diets, increased more in leg (2.4, 6.0, 8.5 11.0 % of the total fatty acids) than in loin (2.1, 4.6, 6.8 and 8.85 % of the total fatty acids). Leg pieces tended to increase the PUFA amount than loin. These results are higher than those found in the present study, where n-3 PUFA averaged 3.7 % for loin, fore legs and hind legs. The authors concluded that the large percentage of linseed (up to 9 %), which is a rich source of PUFA, probably increased the α-linolenic acid (18:3) and further the n-3 fatty acids content. The lower concentration of α-linolenic acid (18:3) in the present study compared to the higher content in the study reported by Petracci[6], helps to explain these differences. Moreover, their results are similar to the present study findings, because hind legs showed the highest concentration of PUFA compared to loin and fore legs.

Currently, no studies have assessed consumer's sensory evaluation of rabbit meat fed a functional supplement *versus* an antibiotic as growth promoter. However, it is feared that over use of antibiotics employed as growth promoters in animal feeding may cause bacteria and microorganism resistance. In the current investigation, flavomycin reduced the preferences of panelists in terms of taste, color, and aroma of meat. Because of flavomycin modifying the thickness of intestinal wall, constrained intestinal bacteria growth by inhibition of peptidoglycan biosyntesis, and increase the absorption of nutrients[15,16], these might substantially modify nutrient transport to animal blood stream and to animal tissues. Therefore, nutrient availability and lipid deposition changes triggered by flavomicyn might help to explain at some extent those differences[19]. Diet is a major driver for sensorial modifications of animal products[28,32]. Inulin may favor the hedonic traits as shown by Mendez-Zamora *et al*[36] who evaluated the inclusion of 15% (dry weight basis) of inulin as a flavor additive in sausages. In that study, inulin improved the color and the overall acceptance of sausages by consumers. These results suggest that inulin added to the diet would have similar effects in the rabbit meat apart from the prebiotic benefit by increasing hedonic properties. However, this effect was not evident when inulin was mixed with flavomycin.

Table 3: Sensorial evaluation and consumer preference of rabbit's meat influenced by the addition of inulin or flavomycin and type of cut of meat (n = 30)

	Control			Inulin (I)			Flavomycin (F)			IF			SEM	*P* value		
	Loin	Fore legs	Hind legs	Loin	Fore legs	Hind legs	Loin	Fore legs	Hind legs	Loin	Fore legs	Hind legs		Diet	Cut of meat	Diet x Cut of meat
Taste	3.3^{abc} ±0.52	3.46^{a} ±0.57	2.97^{abcd} ±0.80	3.03^{abcd} ±0.41	2.73^{cd} ±0.63	2.90^{abcd} ±0.54	3.03^{abcd} ±0.66	2.87^{bcd} ±0.73	2.47^{d} ±0.68	3.43^{abc} ±0.67	2.80^{cd} ±0.92	2.47^{d} ±0.73	0.007	<0.0001	0.01	0.0003
Color	3.10^{ab} ±0.60	3.23^{a} ±0.56	2.90^{ab} ±0.75	3.0^{ab} ±0.45	2.83^{ab} ±0.74	3.03^{ab} ±0.71	3.0^{ab} ±0.78	2.77^{ab} ±0.72	2.90^{ab} ±0.71	3.30^{a} ±0.65	2.83^{ab} ±0.91	2.50^{b} ±0.90	0.008	0.01	0.01	0.18
Aroma	3.13^{ab} ±0.43	3.30^{a} ±0.70	3.06^{abc} ±0.73	3.03^{abcd} ±0.49	2.77^{bcd} ±0.56	3.0^{abcd} ±0.45	3.13^{ab} ±0.50	2.80^{abcd} ±0.71	2.53^{d} ±0.73	3.10^{ab} ±0.54	2.83^{abcd} ±0.74	2.57^{cd} ±0.67	0.006	<0.0001	0.33	0.03

^{a,b,c,d} Means with different letters within the same row are significantly different (P<0.05). SEM= standard error of the mean.

When main effects (feeding and cuts of meat) were tested, loin tended to be the most preferred cut in all sensorial traits evaluated, but when specific effects of feeding treatment by single cut of meat was evaluated; fore legs from CG was the most preferred piece among all cuts evaluated. Though we did not evaluate rheological properties, they may play an important role for this result, where texture, hardness, tenderness, springiness and chewiness between pieces of meat are determinants for consumer's preferences[2].

Conclusions and implications

Inulin addition in rabbit's diet increases beneficial fatty acids (CLA and n3-PUFA) and enables a better health promoting index while reducing the atherogenic and thrombogenic indices of meat. The cuts of meat had a smaller impact on changing the fatty acid profile than inulin or flavomycin addition. Flavomycin reduced the score of the preferences among panelists in terms of taste, color, and aroma. Rheological properties of meat should be take into account to determine their influence on consumers' preference. Inulin is a good alternative to avoid the utilization of antibiotics in the rabbits feeding. Complementary research on animal performance and economic benefits should be done to support the use of inulin as prebiotic.

Acknowledgements

Special thanks to Irene Torres Acosta for the assistance during the experimental phase with the animals and to Silvia Carrillo Dominguez for the statistical analysis recommendations. All authors read and approved the final version of the manuscript.

Compliance with ethical standards Protocols for animal housing, management, slaughter and sampling were approved by the Institutional Animal Care and Research Advisory Committee (Comité de Investigación en Animales-CINVA) at the INCMNSZ under the registration number NAN-059-09-10-1. All procedures were in accordance with the Mexican Official Norm on Principles of Laboratory Animal Care (NOM 062-ZOO-1999).

Conflict of interest

The authors declare that they have no conflict of interest.

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