Pakistan J. Zool., vol. 48(3), pp. 689-695, 2016.

Dietary Effect of Fermented Wheat Powder (Lisosan G®) on Productive Performance and Meat Quality in Intensively-Reared Rabbit

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ABSTRACT

Cereals are basic food for human consumption and for animal feeding containing a wide variety of components with nutraceutical properties. Several dietary nutraceutical supplements were used in rabbit diet to improve the meat quality. The effect of the Lisosan G® enrich-feed on the productive performance and meat quality in growing rabbits was evaluated. The trial lasted 63 days and was conducted in an experimental rabbitry on 40-weaned and clinically healthy male rabbits. Animals were fed a commercial diet and randomly assigned to four treatments: control diet (CON group) and diet supplemented with 10 g/kg (LIS_1 group) or 20 g/kg (LIS_2 group) or 30 g/kg (LIS_3 group). Performance parameters, such as body weight, average daily gain, feed intake, feed conversion indices and results relating to slaughter reliefs were not statistically affected by the experimental treatments. The Lisosan G® supplementation, at the rate of 20 g/kg feed, produced in rabbits a significant improvement of qualitative characteristics of the meat, characterized by higher oxidative stability, with a lower level of TBARS providing also a positive effect on meat shelf-life. Dietary treatments including fermented wheat powder improved meat the fatty acid profile, highlighted by a significant reduction of saturated fatty acid (SFA) and a marked increase of polyunsaturated fatty acids (PUFA), leading to an improvement of the nutritional value of the meat and with positive effects on consumer's health.

Article information

Received 4 April 2015
Revised 9 September 2015
Accepted 1 October 2015
Available online 14 March 2016

Authors' Contributions

All coauthors carried out experiments in the field and wrote the article. MN, MP, VL, LP and FV performed all laboratory analyses. MP statistically analyzed the data.

Key words

Lisosan G®, quality traits, oxidative status.

INTRODUCTION

The close relationship between food and health is increasingly addressing the consumer to change their eating habits, with a consequent increase in demand for nutraceutical foods with bioactive molecules and functional activities, able to prevent pathological events. For the consumer, food safety and animal welfare, linked to the breeding system, are taking increasingly greater importance in the supply of animal origin products, since they represent the basic assumption to get quality, healthy and safe foods (Hernández, 2008; Preziuso *et al.*, 2009; Gerencsér *et al.*, 2013; Matics *et al.*, 2014). This situation is pushing researchers and technicians to identify alternative feeding strategies involving rich-bioactive compounds dietary supplements capable of ensuring animal health and their productive performance (Gidenne and García, 2006; Maertens *et al.*, 2006).

Several dietary nutraceutical supplements were used in rabbit diet to improve the meat quality, such as olive and sunflower oil integrated with vitamin E (Lopez-Bote *et al.*, 1997), oats (Lopez-Bote *et al.*, 1998a) and essential oils of oregano (Botsoglou *et al.*, 2004). Furthermore, feeding dietary supplements, such as polysaccharides of alfalfa (Liu *et al.*, 2010) and green tea with the addition of flax oil (Eid *et al.*, 2010) improved the productive performance and meat quality in growing rabbits.

Cereals are basic food for human consumption and for animal feeding (Spiller, 2002), containing a wide variety of components with nutraceutical activities (such as fibre, vitamins, minerals, phenols, carotenoids, lignans, β-glucans and inulin Slavin, 2003). Among cereals, wheat is a viable source of natural antioxidants (Zhou *et al.*, 2004); however these bioactive compounds did not receive the same scientific attention compared to those present in fruits and/or other vegetables, even if the increased human consumption of grain and its products has been associated to reducing the risk of chronic diseases, such as cardiovascular (Okarter and Liu, 2010).

In previous research, using a dietary supplement based on powder of grain (Lisosan G®) in CCl_4-intoxicated rats, an hepatoprotective effect were observed (Longo *et al.*, 2007) and a good free radical scavenger activity due to the presence of antioxidant substances was reported (Laus *et al.*, 2013; Longo *et al.*, 2007).

There is very little information available on Lisosan G® as a dietary supplement in farming animal. In the present study, therefore, the effect of Lisosan G® enrich-feed has been evaluated on the productive performance and meat quality in growing rabbits.

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0030-9923/2016/0003-0689 $ 8.00/0

MATERIALS AND METHODS

Animals, treatment and experimental design

The trial lasted 63 days and was conducted in an experimental rabbitry on 40-weaned and clinically healthy New Zealand White × Californian cross male rabbits in accordance with European Community guidelines n. 86/609/EEC. The animals were kept in individual cages equipped with feeders and automatic watering system. The temperature and humidity of rabbitry were continuously recorded with a digital thermograph positioned at the same level as the cages. Heating and forced ventilation systems allowed the building air temperature to be maintained within 18±4°C throughout the experiment. Relative humidity was about 70±5%.

Growing rabbits were randomly divided into four groups of 10 animals each homogeneous by age (42±2 d) and body weight (1.72±0.14 kg) and fed *ad libitum* with free access to water until the end of the trial. Animals were fed a commercial diet assigned to four dietary treatments: control diet (CON group) and diet supplemented with 10 g/kg (LIS_1 group) or 20 g/kg (LIS_2 group) or 30 g/kg (LIS_3 group).

The feed was provided by Agri-zoo company (Isernia, Italy) and its chemical composition according to AOAC analysis (2000) is shown in Table I. Lisosan G® is a powder obtained from wheat (*Triticum aestivum*), which is registered with the Italian Ministry of Health as a nutritional supplement and was supplied by Agrisan company (Larciano, Italy). In the production process, firstly the whole grain is ground to a rough powder and then the fine bran and germ are separated from the other parts and collected. Water is added to moisten the mix of fine bran and germ, then selected microbic starting cultures, which typically consist of lactobacilli and natural yeast strains, are inoculated in order to initiate fermentation. When the fermentation is completed, the product is dried and the resulting dry powder is Lisosan G® (Longo *et al.*, 2007). Lisosan G® is rich in several bioactive compounds, such as polyphenols and especially flavonoids and flavonols, lipoic acid, tocopherols and polyunsaturated fatty acids.

During the test, all growing rabbits were subjected to the following controls: relief of body weight and feed intake at the start of the test (0 d), at 21, at 42 d and the end of the test (63 d) and determination of average daily gain (ADG; expressed as body weight differences divided by passed days) and feed conversion ratio (FCR; expressed as grams of feed intake divided by grams of body gain); slaughter reliefs such as body weight, gross body weight, hot carcass weight, weight of the head and pluck; post-slaughter analysis on *Longissimus lumborum* (LL) muscle such as fatty acid profile, cholesterol content, thiobarbituric acid reactive substances (TBARS), vitamins A and E were determined.

Table I.- Ingredients and chemical composition of diet feed of rabbit.

	Diets[1]
Ingredients, g/kg diet	
Sunflower meal	230.0
Alfalfa hay	220.0
Wheat bran	208.8
Alfalfa meal dehydratated, 17% CP	100.0
Beet pulp	100.0
Barley	70.0
Wheat	15.0
Calcium carbonate	15.0
Cane molasses	15.0
Palm oil	6.0
Soybean oil	7.0
Sodium chloride	4.0
Dicalcium phosphate	2.0
Vitamin and mineral premix[2]	2.5
Methionine (99%)	2.3
Lysine (78.5%)	1.4
Choline (75.0%)	1.0
Chemical composition[3]	
Moisture, % as-fed	11.0
Crude protein, % DM	15.4
Ether extract, % DM	3.3
Crude fiber, % DM	19.5
NDF, % DM	38.5
ADF, % DM	24.0
ADL, % DM	6.5
Ash, % DM	8.5
Starch, % DM	10.0

[1]CON, 0% Lisosan G; LIS_1, 1% Lisosan G; LIS_2, 2% Lisosan G; LIS_3, 3% Lisosan G.
[2]Supplied per kg of feed: vitamin A, 2000 IU; vitamin D3, 320 IU; vitamin E, 4.0 mg; vitamin B2, 0.52 mg; vitamin B6, 0.40 mg; vitamin B12, 0.006 mg; vitamin K, 0.32 mg; vitamin H, 0.020 mg; vitamin PP, 3.2 mg; folic acid, 0.10 mg; D-pantothenic acid, 2.4 mg; copper, 5.6 mg; manganese, 4.0 mg; iron, 12.0 mg; zinc, 16.0 mg; iodine, 0.060 mg; selenium, 0.040 mg.
[3] n = 2; mean±sd.

At the end of the feeding trial all animals were slaughtered in an experimental slaughterhouse and gross body weight after 12 h of fasting was recorded. The rabbits were subjected to stunning and sacrificed by bleeding following the guidelines established by the European Community (n. 86/609/EEC) and approved by the Italian Ministry of Health (L. n. 116/92), in accordance with national laws on the protection of animals at the time of slaughter or killing. The carcasses

were prepared as reported by Blasco and Ouhayoun (1996) by removing the skin, the distal part of the limbs, genital organs, the bladder and gastrointestinal tract. The hot carcasses including head and pluck were weighted and the dressing percentage was calculated. After 48 h of chilling at 4°C, on a sample of LL taken between 1st and 7th lumbar vertebra of the right side, the concentration of TBARS, according to Meineri *et al.* (2010), the content of cholesterol (Du and Ahn, 2002) and the content of vitamins A and E (Oriani *et al.,* 2001) were determined. The rest of the LL muscle samples were vacuum packaged and frozen at -20°C until fatty acid profile analysis.

The fatty acid composition of meat samples was determined after chloroform-methanol extraction (Folch *et al.,* 1957), and fatty acids were determined as methyl esters (FAME) (Dal Bosco *et al.,* 2004), using a gas chromatograph ThermoQuest Trace 2000 (SACtm-5 column 300cm×0.25mm, Supelco, USA). The fatty acid percentages were calculated with Chrom-Card software (version 1.17).

Statistical analysis

After the normal evaluation of frequency distribution, ANOVA was performed on all variables using the GLM procedure of the statistical package SPSS (2010). The growing performance and meat quality data were processed using one-way ANOVA with the dietary treatment as a main effect. The single rabbit was the experimental unit and all data are expressed as mean and the standard error of mean (SEM). The differences were considered significant at $P<0.05$.

RESULTS

Performance parameters, such as body weight, ADG, feed intake and FCR (Table II) during the whole trial, and the slaughter relief's (Table III) were not statistically affected by the experimental treatments.

The Table IV shows the mean values of meat oxidative status parameters and its contents in cholesterol. Supplementing Lisosan G® in LIS_2 group resulted in a decrease values of TBARS by 41% compared to control group ($P<0.01$); the LIS_1 and LIS_3 groups, even presenting lower values than CON group, were not statistically different.

The meat content of vitamin A and vitamin E did not show significant differences due to dietary treatment, and values were similar between experimental groups and CON group.

The cholesterol content of LL showed values slightly lower in experimental groups compared to CON group ($P>0.05$).

The Table V lists the meat values of fatty acidic profile. Lisosan G® dietary treatment resulted in a significant improvement ($P<0.01$) of fatty acid profile in experimental LIS_1, LIS_2 and LIS_3 groups, showing a decrease of saturated fatty acids (SFA) and an increase of polyunsaturated fatty acids (PUFAs); even the n-3 and n-6 fatty acids increased ($P<0.05$) in experimental groups, with a positive and lowering effect on the n-6/n-3 ratio. In particular, the SFA content decreased by 8.8% in the LIS_1 group, and by 11.5% in LIS_3 and LIS_2 groups, compared to CON group. PUFAs content increased by 19.3% in LIS_1 group, and 25.6% in LIS_3 and LIS_2 groups, compared to CON group. The n-3 fatty acids increased by 33.1, 52.0 and 48.6% in LIS_1, LIS_3 and LIS_2 groups, respectively; while the n-6 fatty acids increased by 17.1% in LIS_1 group and by 22.0% in LIS_3 and LIS_2 groups, compared to CON group.

The n-6/n-3 ratio in experimental groups showed lower trend values compared to CON group ($P>0.05$).

DISCUSSION

Lisosan G® dietary supplementation, contrary to our expectations, since is rich in highly nutraceutical components, did not improved the rabbits' performance parameters considered in the study. Lopez-Bote *et al.* (1998a), in Californian × New Zealand White rabbits fed with 300 g/kg feed of oats, compared to control diet based on barley, no significant effects on feed intake, feed conversion index and dressing percentage have found, except for average daily gain that resulted statistically higher. Also Saraee *et al.* (2014), feeding broilers chickens with green tea powder and fish oil or their combination, no effect on final body weight and carcass yield have reported, compared with those fed control diet.

Dietary supplementation with Lisosan G® resulted in a significant improvement of some meat oxidative status parameters; in particular, the marked decrease of TBARS values in LIS_2 group is entirely positive, since it is an indication of greater protection of meat from oxidation and a longer commercial shelf-life. The oxygen radicals absorbance capacity (ORAC) number of Lisosan G® (6515 μm TE/100 g fresh weight) indicates a strong antioxidant and scavenger of free radicals activity, which might be related to its high content in polyphenols (Frassinetti *et al.,* 2012). Even Aly (2012), in rats fed with oats (5%) and bran (10%) an increased serum levels of superoxide dismutase (SOD) enzyme and reduced glutathione and a decreased malondialdehyde content reported, due to the strong antioxidant phenolic compounds present in these cereals.

Table II.- Productive performances in rabbits fed the experimental diets.

	Diets[1]				Pooled SEM[2]	*P*-value
	CON	LIS_1	LIS_2	LIS_3		
Rabbits (*n*)	10	10	10	10		
Body weight (g)						
0d	1756	1762	1651	1702	22.15	0.242
21d	2403	2410	2257	2362	26.34	0.133
42d	3018	3020	2862	3001	33.19	0.262
63d	3658	3646	3483	3646	42.59	0.411
Average daily gain (g/d)						
0d-21d	30.8	30.9	28.9	31.4	0.76	0.663
22-42d	29.3	29.0	28.8	30.4	0.66	0.849
43d-63d	30.5	29.8	29.6	30.7	0.76	0.955
0-63d	30.2	29.9	29.1	30.9	0.62	0.807
Feed intake (g/d)						
0-21d	112	112	113	114	0.65	0.551
22-42d	153[a]	148[b]	151[ab]	149[ab]	0.67	0.012
43-63d	176[a]	170[b]	173[ab]	175[ab]	0.88	0.020
0-63d	147	143	146	146	0.56	0.061
Feed conversion index						
0-21d	3.64	3.62	3.91	3.63	0.12	0.613
21-42d	5.22	5.10	5.24	4.90	0.11	0.653
43-63d	5.77	5.70	5.84	5.70	0.14	0.908
0-63d	4.87	4.78	5.02	4.72	0.10	0.753

[1] Lisosan G supplementation: CON, 0% Lisosan G; LIS_1, 1% Lisosan G; LIS_2, 2% Lisosan G; LIS_3, 3% Lisosan G.
[2]SEM, standard error of the mean.

Table III.- Slaughter parameters in rabbits fed the experimental diets.

	Diets[1]				Pooled SEM[2]	*P*-value
	CON	LIS_1	LIS_2	LIS_3		
Rabbits (*n*)	10	10	10	10		
Body weight (g)	3658.00	3646.00	3483.00	3646.67	42.59	0.411
Gross body weight (g)	3409.90	3411.20	3308.00	3451.33	43.49	0.704
Hot carcass weight (g)	2284.50	2296.90	2202.50	2255.89	30.11	0.700
Dressing percentage[3] (%)	67.00	67.33	66.58	65.36	0.28	0.102
Head weight (g)	219.00	225.10	236.50	218.22	3.09	0.128
Pluck weight (g)	103.70	110.30	108.40	115.89	1.80	0.122

[1]Lisosan G supplementation: CON, 0% Lisosan G; LIS_1, 1% Lisosan G; LIS_2, 2% Lisosan G; LIS_3, 3% Lisosan.
[2]SEM, standard error of the mean;
[3]Calculated as hot carcass weight on gross body weight.

According to our results, Choe and Kim (2002) showed a decrease of TBARS levels in the liver and kidney tissue, with greater control of lipid peroxidation, in hypercholesterolemic New Zealand White rabbits fed Korean wheat enriched diet. Even Lopez-Bote *et al.* (1998a,b) in rabbit *Longissimus dorsi* muscle and in broiler meat fat, an improved oxidative stability observed following a dietary integration with oats. Flis *et al.* (2010) in *Longissimus dorsi* meat sample, immediately after slaughter, a decrease of TBARS levels noted, in pigs fed with diet containing barley and wheat.

Table IV.- Meat oxidative parameters and cholesterol content in rabbits fed the experimental diets.

	Diets[1]				Pooled SEM[2]	*P*-value
	CON	LIS_1	LIS_2	LIS_3		
Rabbits (*n*)	10	10	10	10		
TBARS (mg/100g)	0.151[a]	0.136[a]	0.089[b]	0.133[a]	0.006	0.001
Vitamin A (mg/100g)	0.142	0.150	0.166	0.154	0.008	0.810
Vitamin E (mg/100g)	0.713	0.724	0.786	0.740	0.018	0.517
Cholesterol (mg/100g)	42.65	39.73	38.56	38.61	0.983	0.440

[1] Lisosan G supplementation: CON, 0% Lisosan G; LIS_1, 1% Lisosan G; LIS_2, 2% Lisosan G; LIS_3, 3% Lisosan G.
[2]SEM, standard error of the mean;
Within a row, means without a common superscript ([a,b]) differ (P<0.05).

Table V.- Meat fatty acid profile in rabbits fed the experimental diets.

	Diets[1]				Pooled SEM[2]	*P*-value
	CON	LIS_1	LIS_2	LIS_3		
Rabbits (*n*)	10	10	10	10		
Fatty acids (g/100g)						
SFA[3]						
C14:0	2.38	2.26	2.26	2.18	0.06	0.700
C16:0	33.50[a]	30.44[ab]	29.82[b]	30.01[ab]	0.51	0.025
C18:0	9.65	9.35	8.66	8.64	0.22	0.265
C20:0	0.29	0.29	0.37	0.37	0.02	0.206
C22:0	0.71	0.66	0.67	0.67	0.02	0.842
Other	1.41a	0.71b	0.55b	0.55b	0.07	0.001
Σ SFA	47.93[a]	43.72[b]	42.34[b]	42.43[b]	0.61	0.001
MUFA[4]						
C14:1	0.26	0.39	0.27	0.27	0.03	0.263
C16:1	4.32	4.66	4.57	4.69	0.16	0.835
C18:1	24.70	23.83	24.11	23.91	0.29	0.722
C20:1	0.15	0.45	0.29	0.29	0.07	0.580
Other	0.51	0.55	0.61	0.61	0.04	0.813
Σ MUFA	29.94	29.88	29.86	29.77	0.35	0.999
PUFA[5]						
C18:2 n-6	17.73[a]	20.55[ab]	20.87[ab]	20.98[b]	0.49	0.044
C18:3 n-3	1.73	2.12	2.22	2.12	0.11	0.360
C20:3 n-3	0.13	0.22	0.20	0.20	0.02	0.260
C20:3 n-6	0.15	0.45	0.29	0.29	0.07	0.578
C20:4 n-6	1.02[a]	1.13[ab]	1.90[b]	1.90[b]	0.14	0.018
C20:5 n-3	0.22	0.36	0.34	0.34	0.04	0.636
C21:5 n-3	0.36	0.41	0.42	0.42	0.03	0.829
C22:5 n-3	0.13[a]	0.16[ab]	0.18[b]	0.18[b]	0.01	0.016
C22:6 n-3	0.40[a]	0.66[ab]	1.14[b]	1.14[b]	0.10	0.017
Other	0.27	0.33	0.23	0.23	0.02	0.108
Σ PUFA	22.13[a]	26.40[ab]	27.80[b]	27.80[b]	0.68	0.003
n-3	2.96[a]	3.94[ab]	4.50[b]	4.40[b]	0.19	0.008
n-6	18.90[a]	22.13[ab]	23.06[b]	23.17[b]	0.56	0.012
n-6/n-3 ratio	6.46	5.99	5.44	5.64	0.26	0.532

[1]Lisosan G supplementation: CON, 0% Lisosan G; LIS_1, 1% Lisosan G; LIS_2, 2% Lisosan G; LIS_3, 3% Lisosan G.
[2]SEM, standard error of the mean;
Within a row, means without a common superscript ([a,b]) differ (P<0,05);
[3]SFA, saturated fatty acid
[4]MUFA, monounsaturated fatty acid
[5]PUFA, polyunsaturated fatty acid.

Considering that high doses of polyphenols may have a pro-oxidant effect (Watjen *et al.*, 2005), it can assume that the lack of effect on TBARS meat values in LIS_3 group, could be due to the reaching of threshold dose (30 g/kg feed); while non-antioxidant activity in LIS_1 group, may be due to the minimum supplement dose used (10 g/kg feed).

The positive effect of dietary supplementation on the increase in PUFA content of meat, in the all experimental groups, may have attributed to essential fatty acids contained in Lisosan G® supplement, such as linoleic acid (C18: 2 n-6) and linolenic acid (C18: 3 n-3), which, in the rabbit as in other monogastric species, should be introduced into the animal by diet, since there is no endogenous production (Dalle Zotte, 2002). In particular linoleic acid is the precursor of PUFA n-6 family, and linolenic acid is the precursor of PUFA n-3 family, including eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids which in a significant increase resulted, with beneficial effects on the health of consumers and particularly on cardiovascular apparatus (ISSFAL, 2004).

CONCLUSION

The Lisosan G® supplementation, at the level of 20 g/kg feed, produced in rabbits an improvement of qualitative characteristics of the meat, which is characterized by reduced oxidative status, with a lower level of TBARS and with positive effects on the meat shelf-life. Slightly improvements in vitamins levels and cholesterol content were also noticed in the LIS_2, which could be developed in further research in order to determine the right dose of the feed supplement.

Moreover, dietary treatments also improved the rabbit meat fatty acid profile, highlighted by a significant reduction in SFA and a marked increase in PUFAs, leading to an improvement of the nutritional value of the meat and with positive effects on consumer's health.

Conflict of interests statement

The authors declare that they do not have conflict of interests (political, personal, religious, ideological, academic, intellectual, commercial, or otherwise) regarding the publication of the paper.

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