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PRODUCTION OF RABBIT MEAT WITH FUNCTIONAL PROPERTIES

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Abstract

The present work is based on the assumption that by supplementation thyme to defined concentration to feed of rabbits will improve the nutritional qualities of the meat and create opportunities for the development of safe products with high antioxidant activity, preserved nutritional value and easy digestion. This combination could protect against oxidative stress and increase the functional properties of the meat. To the feed of one experimental group was added 3% thyme (group T3) and to the other 5% thyme (group T5) at the expense of alfalfa hay (31.95% in control fodder). In the dispersion analysis we found that statistically significant differences were found only with respect to the weight of the liver which was lower in the groups fed with supplements of thyme compared to the control group, no significant differences were found with respect to the weight indicator. The incorporation of thyme into the fodder for fattening rabbits results in improved fatty acid composition in feed and meat, with the best results being achieved in rabbit breeding with the addition of 5% thyme leaves.

Key words*: fodder, thyme, rabbit meat, fatty acids.*

INTRODUCTION

The increasing consumer demand for conducive to good health food has pushed the meat industry to develop new strategies to optimize the nutritional composition to improve the image of meat and meat products (Toldrá and Reig, 2011). A major goal of these strategies is the presence of a biologically active substance (BAS) in the meat or meat product to provide the desired effect over a reasonable period, depending on whether or not the BAS is assimilated by the organism or is present in a significant amount or not in the final product as well as give the desired effect with its absorption for a reasonable period. The achievement of this effect is scientifically established, nutrition claims and claims for health properties are detailed and regulated by the EU (Reg. EU 1924/2006; Reg. EU 432/2012).

Consumer's preferences for natural foods direct the scientific research into the potential of using natural antioxidants. It has been found that rosemary, green tea, ginger, marjoranam and thyme show a strong inhibitory effect on the lipid oxidation of meat products (Collignan and Montet, 1998; Kanner, 2007). Thyme and rosemary are widely used spices in the culinary treatment of various types of meat and in recipes for preparing sausages, pizza, spaghetti, etc. The composition of the extracts is well known and studied and they are classified as safe for use in foods. The essential components of essential thyme oil are the phenolic compounds thymol, carvacrol, and p- cimol, which have been found to inhibit most of the pathogenic microorganisms encountered in food of animal origin. In summary the problem of providing quality and safe meat products is current in Bulgaria and internationally scale. While many scientific teams are involved in the subject abroad, there is insufficient scientific research into the use of substances of natural origin as antioxidant stabilizers in meat products.

The possibilities for the introduction of biologically active substances from plant origin into the food technology as antioxidant stabilizers, especially in the preparation of raw meat products, have not been fully investigated. Required further research and an accurate assessment of the quantity and type of extracts which may be added to the foodstuff so as to achieve the desired concentration without deteriorating the organoleptic characteristics of

the product (stronger or different odor and taste, color changes, etc., which would be unacceptable to the consumer). To achieve a proper balance between the antioxidant, flavoring and aromatizing action of plant extracts when used as additives in meat products, in-depth scientific and technological research is required. This analysis gives reason the efforts of the team to focus on searching for similar methods to improve the quality characteristics of meats in the composition of lyophilized foods with special uses.

The European Union initially restricted the use and then finally prohibited the use of antibiotics and other synthetic BAS as growth promoters in animal nutrition (Resurreccion, 2003) and the negative public opinion on the use of antibiotics, more and more scientific studies are devoted to natural alternatives (Laguerre et al., 2007; McAfee et al., 2010). The EU's decision stems from the fear that, despite the low dosing of antibiotics given for animal productivity or prophylactic purposes, may result in the formation of resistant strains of human pathogens that represent a real health risk to the population. In this regard, our main objective will be to evaluate the effect of thyme nutritional supplements (*Thymus serpyllum*) and the digestibility of these nutrients in rearing rabbits. Thyme is confirmed as a rich source of natural antioxidants and is an effective natural additive in the production of foods enriched with natural biologically active substances that are successfully applied in the meat (Stabler & Allen, 2004). Further research is needed to clarify the effects of thyme in fat deposition, bone development and mineralization. Changes in carcass fat are observed with thyme dietary supplements, but further studies are required to demonstrate whether and how these supplements can affect lipid metabolism. Therefore, our study is focused on the use of these additives to retention and protection against oxidative stress in the meat. According to the literature, a 5% of thyme supplement (*Thymus serpyllum*) is effective for increasing gamma linolenic acid (GLA) (FAO, 1997; FAO/WHO, 1991). Thyme, on the other hand, improves the oxidative stability of raw and lyophilized meat, but not of boiled meat (Peiretti and Meineri, 2011; Sikorski and Kolodziejska, 1986).

The present work is based on the assumption that by supplementation thyme to defined concentration to feed of rabbits will improve the nutritional qualities of the meat and create opportunities for the development of safe products with high antioxidant activity, preserved nutritional value and easy digestion.

MATERIALS AND METHODS

Experimental Animals

The experiment was conducted in the experimental rabbit farm of the Institute of Animal Science - Kostinbrod. Participation took 30 rabbits. The experiment started with 55 days old rabbits. The experience is of long duration from 7 weeks, to an average live weight above 2100 g. The rabbits were divided into three groups of 10 animals: one control and two experimentals. The animals are fed with whole-grained granular mixtures.

Feeding of experimental animals

The feed used in the experiment was prepared at the Agricultural Institute in Stara Zagora by recipe for combined fodder a fattening of rabbits 53- 3- 6 on August 02, 2017 (Protein-14.26%, Fibber-13.43%, Fat-1.83%, Energy-1979.62 Kcal / kg). Three percent thyme (group T3) and 5% thyme (group T5) were added to the feed of one experimental group at the expense of alfalfa hay (31.95% in control fodder). In the first two weeks of the experiment, rabbits were fed restrictively. They received 100 grams fodder of rabbit per day. After this period, the animals of the three groups were fed freely. They took water through nipple drinkers.

Weight development

During the experiment, rabbit weight data were collected weekly after approximately 18 hours of starvation.

Slaughter analysis

A total of 6 rabbits were slaughtered in each group (18 in total). The following data were taken during the slaughter: live weight before slaughter, body weight after slaughter, and weights of the individual organs.

Statistical processing and analysis of the results obtained

All results are presented as mean ± SD (standard deviation) at 6 replicates for each sample. The results are considered reliable at p

< 0.05. All statistical analyzes are performed with Excel 2013.

Biochemical Studies

Fatty acid analysis of meat performed - the total lipid extraction was performed by Bligh and Dyer (1959) with chloroform and methanol in a ratio of 1: 2. The methyl esters of fatty acids (FAME) were analyzed using a Shimadzu-2010 gas chromatograph (Kyoto, Japan). The assay is performed with a CP7420 capillary column (100 m x 0.25 mm i.d., 0.2 m, Varian Inc., Palo Alto, CA), with carrier gas-hydrogen and make-up gas-nitrogen. A five-step gas chromatographic oven program has been used.

RESULTS AND DISCUSSIONS

Six rabbits from each group were slaughtered (18 in total). On the basis of the slaughter analysis of the animals, it was established weight change of the rabbits in the different groups relative to the start of the measurements. At the end of the period, we reported an increase of about 100% in the control groups fed with 3% thyme fodder in one and 5% thyme in the other. The animals in the third group had the highest weight gain compared to the beginning of the measurements and the other two groups (Table 1).

Table 1. Relatively variation on rabbit weight by different regime on nutrition toward beginning of measurement, (%)

Regime of nutrition	Beginning	1 week	2 week	3 week	4 week	5 week	6 week	7 week
Control - usual feed - average value	100	102.92	112.34	125.52	140.18	151.18	166.31	177.27
Feed with 3% *Thymus serpyllum* - average value	100	103.62	119.78	138.75	154.46	163.15	177.14	191.13
Feed with 5% *Thymus serpyllum* - average value	100	107.65	122.97	141.52	159.86	176.98	189.95	202.22

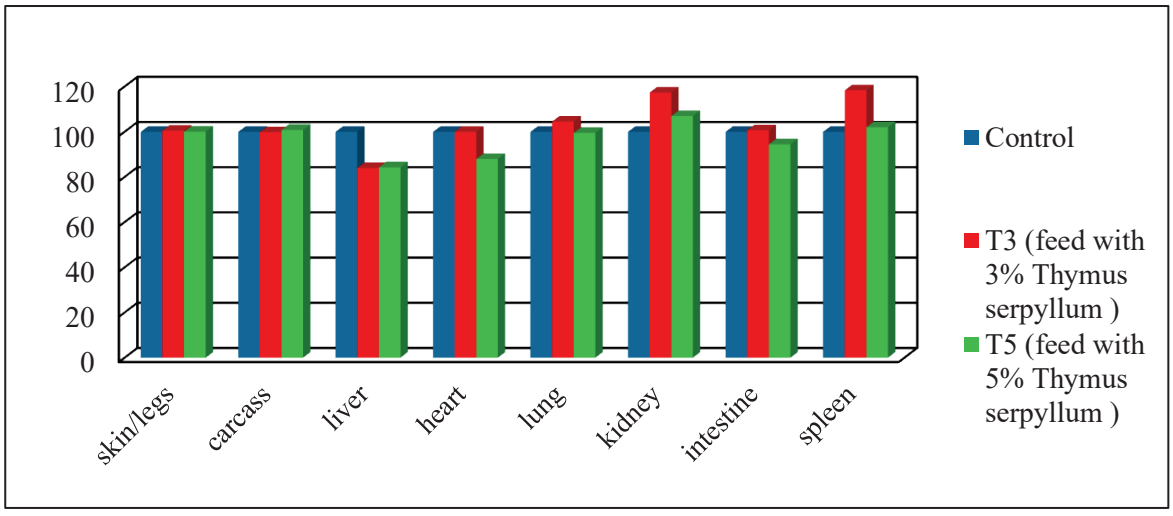

Figure 1. Weight of anatomic organs proportion on rabbits feeded with suplement *Thymus serpullum* (3 and 5%) relation to control group

It can be seen from Figure 1 that with respect to the weight of carcass meat, skin, legs, lung and intestines we have a small differences in the control group. We found lower liver weight in both variants, less heart in group T5 and increased spleen and kidney in group T3. Subsequent dispersal analysis will answer the question of how far these differences are substantial and statistically significant.

Deviation from normal organ weights in rabbits is usually a symptom of some common diseases such as coccidiosis (enlarged liver), pasteurellosis (enlarged spleen) etc.

When done dispersion analysis (Table 2) we have established that statistically significant differences were found only with respect to the weight of the liver, which is lower in the groups fed with addition of thyme compared to the control group.

Fatty acids in feed for fattening rabbits

The fatty acid composition of the feed for fattening rabbits has a saturated fatty acid (SFA) content of 40.39 g/100 g fat, monounsaturated fatty acids (MUFA)-33.41 g/100 g fat, polyunsaturated fatty acids (PUFA)-10.91 g/100 g fat, trans isomers of linoleic acid-0.67 g/100 g fat, cis isomers-3.81 g/100 g fat, omega-3 fatty acids-0.68 g/100 g fat and omega-6 fatty acids-9.65 g/100 g fat. The using of supplement from 3 and 5% thyme leaves in the fodder leads to an improvement in its fatty acid composition with respect to the content of the cis isomers of linoleic acid, respectively to 7.43 and 9.90 g/100 g fat (2 and 2.6 times the output feed), saturated and polyunsaturated fatty acids increase as follows from 12 to 13 g/100 g fat for SFA and from 3 to 7 g/100 g fat for PUFA, at the expense for MUFAs, which decrease by 8 and 9 g/100 g fat. The trans isomers of linoleic acid vary in a narrow range. The total omega-3 fatty acid content increases threefold with supplement with 3% thyme and four times with 5%. The omega-6 fatty acid content is increased to 10.82 g/100 g fat with 3% thyme added and up to 13.98% with 5% thyme supplement. The ratio between the biologically active omega-6 and omega-3 fatty acids decreases by 2.5 times as a result of their change due to the addition of thyme and the optimal ratio of 5.55 to 5% of the additive. Application of thyme as a feed additive improves the content of branched fatty acids resulting from microbiological activity and decreases by 1.6 times at 3% and 3 times at 5%. This is due to its antimicrobial and antioxidant properties (Table 3).

Table 2. Dispersion analysis on weight difference on anatomic organs in rabbit groups in dependence of regime on nutrition

Organ	Factor on investigation F_{kp} 0.05=4.96; F_{kp} 0.01=10.04; F_{kp} 0.001= 21.04					
	Between control and feed with 3% *Thymus serpyllum*		Between control and fee with 5% *Thymus serpyllum*		Between feed with 3% and 5% *Thymus serpyllum*	
	$F_{оп}$	Rank of proof	$F_{оп}$	Rank of proof	$F_{оп}$	Rank of proof
Skin/legs	0.014	-	0.001	-	0.008	-
Carcass	0.009	-	0.599	-	0.514	-
Liver	10.612	**	5.520	*	0.002	-
Heart	0.000	-	1.206	-	2.830	-
Lung	0.238	-	0.002	-	0.123	-
Kidney	2.332	-	1.053	-	0.747	-
Intestine	0.012	-	0.607	-	0.931	-
Spleen	2.022	-	0.057	-	1.257	-

P< 0.001 - ***; P< 0.01 - ** ; P< 0.05 - *

The major constituents of saturated fatty acids are lauric, palmitic and stearic acid. Lauric acid (C12:0) increased from 9.95 to 13.51 g/100 g fat with a 3% addition and up to 12.14 g/100 g fat in the addition of 5%. The palmitic acid (C-16:0) was found to be twice reduced by the addition of 5% thyme. The stearic acid (C-18:0) increases twice with addition of 3% thyme and 3 times with 5% thyme. Palmitelaidic acid (C-16:19tr) in the analyzed fodder is 19.24 g/100g fat, but the edition of 3% of thyme were reduced her by 2.5 times and with 5% of thyme by 4 times. Palmitoleic acid (C-16:1n7) has a concentration of 1.44 g/100 g fat, but due to the incorporation of thyme, its content decreases to traces in the analyzed feed. Oleic acid (C-18:1c9) in fodder for fattening of rabbits is 3.45 g/100 g fat and increases twice with 3% thyme and 2.5 times with 5% thyme addition. The linoleic acid increases from 1.55 g/100 g fat in feed to 2.20 g/100 g fat in the addition of 3% thyme and 2.27 g/100 g fat in 5% thyme. In the linoleic acid were increased with 5% thyme introduced from 3.15 in the raw material to 4.46 g/100 g fat in the additive used. As a result of the incorporation of thyme in the feed for fattening, it is found that CLA9c,11c and CLA9t,11t increase from 0.60 to 1.04 and 0.08 to 0.37 g/100 g fat. These isomers are characteristic of plant species and the probable

variation is due to a higher concentration in the thyme leaves. Changes occur and in content of omega-3 and omega-6 fatty acids. An increase in the content of dihomo gamma linolenic acid (C-20:3n6) was found as follows in 3% thyme-6 times and 5%-7 times. Arachidonic acid (C-20:4n6) increased significantly with the addition of thyme and the highest content from 1.21 g/100 g fat was found at 5%. Eicosapentaenoic acid (C-20:5n3), followed by the change in arachidonic fatty acid (Table 3).

Table 3. Fatty acids (g/100 g fat) in fodder for fattening of rabbits

Fatty acids	Control	3% *Thymus serpyllum*	5% *Thymus serpyllum*
C-12:0	9.95	13.51	12.14
C-16:0	10.49	11.06	6.95
C-18.0	6.69	16.93	20.88
C-16:19tr	19.24	7.84	5.05
C-16:1n7	1.44	0.01	0.00
C-18:1c9	3.45	6.63	8.60
C-18:2c9,12/19:0	1.55	2.20	2.27
gC-18:3n6	3.15	2.04	4.46
aC-18:3n3	0.01	0.45	0.54
CLA9c,11c	0.60	0.84	1.04
CLA9t,11t	0.08	0.23	0.37
C-20:3n6	0.04	0.25	0.28
C-20:4n6	0.03	0.70	1.21
C-20:5n3	0.07	0.16	0.39
∑CLA	0.67	1.06	1.41
∑C-18:1Trans-FA	0.64	0.50	0.39
∑ C-18:1Cis-FA	3.81	7.43	9.90
SFA	40.39	52.22	53.18
MUFA	33.41	25.27	24.52
PUFA	10.91	13.51	17.48
∑n-3	0.68	1.88	2.52
∑n-6	9.65	10.82	13.98
∑n-6/Σn-3	14.16	5.76	5.55
Branched FA	15.29	9.00	4.83

Fatty acids in rabbit leg meat rearing with fodder supplement by thyme

The fatty acid composition of analyses rabbit meat has a 75.05 g/100 g fat saturated fatty acid content (SFA) in the control and reduced to 70.56 g/100 g fat in meat obtained with 5% thyme feed. Monounsaturated fatty acids (MUFA) - in control group is 12.88 and do not change when used supplement of 3% thyme in the diet, while in 5% thyme addition increases to 18.08 g/100 g fat. Polyunsaturated fatty acids (PUFA) in the control group were the lowest - 5.24 g/100 g fat and increased to 6.63 g/100 g fat in 3% thyme and up to 7.08 g/100 g fat in 5% thyme. Trans isomers of linoleic acid in the control group meat are 2.83 g/100g fat and increase to 3.92 g/100 g fat in 3% thyme and 5.63 g/100 g fat in 5% thyme in the diet. The cis isomers in the analyzed meats are 3.28 g/100 g fat, reduced by 3% of thyme to 1.87 g/100 g fat and increased to 5.18 g/100 g fat in 5% thyme supplement of fodder. Omega-3 fatty acids increased relative to the control group as follows from 2.63 g/100 g fat in control group to 3.46 g/100 g fat in 3% thyme and up to 4.24 g/100 g fat in 5% thyme rearing group. Omega-6 fatty acids do not undergo substantial changes in the overall content. The ratio between the biologically active omega-6 and omega-3 fatty acids decreases from 1.16 g/100 g fat in the control group to 0.83 g/100 g fat when 5% thyme is added in feed. Application of the thyme as a feed additive results in a reduction in the branched fatty acid content from 6.84 g/100 g fat in the control group to 4.28 g/100 g fat in 5% thyme supplement in fodder (Table 4).

Caprylic acid (C-10:0) in the analyzed meats increased from 0.46 g/100 g fat in control group to 0.51 g/100 g fat in 3% thyme and 0.74 g/100 g fat in 5% thyme addition. The lauric

fatty acid (C-12:0) increases at 3% thyme relative to the control group from 2.74 to 5.00 g/100 g fat and in 5% of thyme group to 3.94 g/100 g fat.

Table 4. Fatty acid (g/100 g fat) in rabbit leg meat rearing with fodder supplement by *Thymus serpyllum*

Fatty acids	Control	3% *Thymus serpyllum*	5% *Thymus serpyllum*
C-12:0	2.74	5.00	3.94
C-14:0	9.54	8.69	5.91
C-16:0	17.11	24.25	20.82
C-18.0	41.01	34.16	34.90
C-16:19tr	1.51	0.77	0.88
C-18:1c9/C-18:1t12/13/	2.62	1.41	4.50
C-18:2c9,12/19:0	0.55	0.30	0.39
gC-18:3n6	0.63	0.37	0.28
aC-18:3n3	0.41	0.27	0.43
C-20:3n6	0.58	0.76	0.71
C-20:4n6	0.10	0.30	0.29
C-20:5n3	0.26	0.38	0.41
C-22:5n3	0.69	0.85	1.12
∑C-18:1Trans-FA	2.83	3.92	5.63
∑ C-18:1Cis-FA	3.28	1.87	5.18
SFA	75.05	77.21	70.56
MUFA	12.88	12.60	18.08
PUFA	5.24	6.63	7.08
∑n-3	2.63	3.46	4.24
∑n-6	3.05	3.60	3.51
∑n-6/Σn-3	1.16	1.04	0.83
Branched FA	6.84	3.56	4.28

Myristic acid (C14:0) decreases from 9.54 g/100g fat in control group to 8.69 g/100 g fat in 3% thyme group and to 5.91 g/100 g fat when 5% thyme is added to the diet. The palmitic acid (C-16:0) have a higher content between control group and group with supplement of 3% thyme in the diet from 17.11 to 24.25 g/100 g fat were established. Stearic acid (C-18:0) decreases from 41.01 g/100 g fat in control group to 34.16 g/100 g fat in T3 group and is retained in T5 group-34.90 g/100 g fat (Table 4). Palmitelaidic acid (C-16:19tr) in the analyzed meat decreased twice in the case of supplement with thyme of fodder. Oleic acid (C-18:1c9) increased in the meat of rabbits fed 5% thyme twice relative to the control group. Linoleic acid decreases from 0.55 g/100 g fat in the control group meat to 0.30 g/100 g fat with 3% thyme and 0.39 g/100 g fat with 5% thyme. Gamma linolenic acid decreases twice when 3 and 5% of thyme is added to the fodder relative to control. Alpha linolenic is lower in content using 3% thyme while 5% did not undergo changes compared to the control group of meats. Dihomo gamma linolenic acid (C-20:3n6) increases with 3 and 5% thyme incorporation by 1.3 times the control group. Arachidonic acid (C-20:4n6) increased threefold in the meat by the supplementation with 3 and 5% thyme in the rabbit diet versus the control group fed with fodder. Eicosapentaenoic acid (C-20:5n3) increased from 0.26 g/100 g fat in the meat of control group to 0.38 g/100 g fat in the 3% thyme fed group and to 0.41 g/100 g fat in 5% thyme fed group. Docosahexaenoic acid (C-22:5n3) increases from 0.65 g/100 g fat in the meat control group to 0.85 g/100 g fat in the 3% thyme fed group and up to 1.12 g/100 g fat in 5% thyme fed group.

De Andrade et al. (2018), applying a variety of rabbit rearing diets, found a lauric acid content from 3.84 to 10.03%, myristic acid from 2.55 to 2.88%, palmitic acid from 16.48 to 26.56%, stearic from 6.12 to 8.79%, C-16:1n7 from 2.30 to 4.72%, oleic from 22.93 to 28.29%, linoleic from 17.54 to 30.22%, linolenic from 1.1 to 2.4%, saturated fatty acids from 32.4 to 49.2%, MUFA from 25.1 to 35.45 and PUFA from 25.6 to 32.1%. Mattioli et al. (2017) in a study of oregano supplement, vitamin E and probiotic in rabbit nutrition and rabbit loan and leg

analysis, found higher values than ours for stearic fatty acid and polyunsaturated fatty acids and lower for myristic, palmitic, oleic, SFA and MUFA. Yonkova et al. (2017) were found a higher content of saturated and monounsaturated fatty acids and a lower content of polyunsaturated fatty acids compared to those obtained in our study and an identical ratio of omega-6 to omega-3 fatty acids. Identical results for the fatty acid composition of rabbit meat were obtained by Rasinska et al. (2018).

CONCLUSIONS

In regard to the weight of the carcass meat, skin, legs, lungs and intestines, get small differences across the control group. We found lower liver weight in both variants, less heart in group T5 and increased spleen and kidney in group T3. In the dispersion analysis we found that statistically significant differences were obtained only with respect to the weight of the liver, which was lower in the groups fed with added thyme compared to the control group. The results of the dispersion analysis show the existence of statistically significant differences in the weight of the test specimens between the thyme-supplemented and control variants, while in the feed diet supplemented with 5% thyme, the differences were the highest confidence level for almost the entire study period. Between the two variants fed with 3 and 5% thyme almost no significant differences were found with respect to the weight indicator. The incorporation of thyme into the fodder for fattening of rabbits results in an improvement of the fatty acid composition in feed and meat, with the best results being achieved by rearing rabbits fed with fodder enriched with 5% thyme leaves. The supplementation by thyme in the rabbit feed leads to a decrease in branched fatty acids, which is an indicator of improving the quality of meat and increasing the biologically active omega-3 fatty acids. Best results are achieved using 5% thyme leaves. This leads to improving fatty acid content of meat, due to the increase in biologically active fatty acids-trans and cis isomers of oleic acid, omega-3 and omega-6 fatty acids. Best results have been achieved in rearing rabbits fed with 5% fortified fodder with thyme leaves.

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