International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:11, No:1, 2017

Influence of Dietary Inclusion of Butyric Acids, Calcium Formate, Organic Acids and Its Salts on Rabbits Productive Performance, Carcass Traits and Meat Quality

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Abstract**—**Animal nutritionists and scientists have searched for alternative measures to improve the production. One of such alternative is use of organic acids as feed additive in animal nutrition. The study was conducted to investigate the impact of butyric acids, calcium formate, organic acids, and its salts (BCOS) additives on rabbit's productive performance, carcass traits and meat quality. The study was conducted with 14 Californian breed rabbits. The rabbits were assigned to two treatment groups (seven rabbits per each treatment group). The dietary treatments were 1) control diet, 2) diet supplemented with a mixture BCOS - 2 kg/t of feed. Growth performance characteristics (body weight, daily weight gain, daily feed intake, feed conversion ratio, mortality) were evaluated. Rabbits were slaughtered; carcass characteristics and meat quality were evaluated. Samples loin and hind leg meat were analysed to determine carcass characteristics, pH and colour measurements, cholesterol, and malonyldialdehyde (MDA) content in loin and hind leg meat. Differences between treatments were significant for body weight (1.30 vs. 1.36 kg; $P<0.05$), daily weight gain (16.60 vs. 17.85 g; $P<0.05$), and daily feed intake (78.25 vs. 80.58 g; $P<0.05$) for control and experimental group respectively for the entire experimental period (from 28–77 days old). No significant differences were found in feed conversion ratio and mortality. The feed additives insertion in the diets did not significantly influence the carcass yield or the proportions of the various carcass parts and organs. Differences between treatments were significant for pH value after 48h in loin (5.86 vs. 5.74; $P<0.05$), hind leg meat (6.62 vs. 6.65; $P<0.05$), more intense colour b* of loin (5.57 vs. 6.06; $P<0.05$), less intense colour a* (14.99 vs. 13.15; $P<0.05$) in hind leg meat. Cholesterol content in hind leg meat decreased by 17.67 mg/100g compared to control group ($P<0.05$). After storage for three months, MDA concentration decreased in loin and hind leg meat by 0.3 μmol/kg and 0.26 μmol/kg respectively compared to that of the control group ($P<0.05$). The results of this study suggest that BCOS could potentially be used in rabbit nutrition with consequent benefits on the rabbits' productivity and nutritional quality of rabbit meat for consumers.

Keywords**—**Butyric acids, calcium formate, meat quality, organic acids salts, rabbits, productivity.

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I. INTRODUCTION

RABBIT meat for its dietetic and nutritional characteristics is accepted by the consumer as a product of high quality meat [1], [2]. Rabbit meat is commonly consumed in many European countries, and its production plays an important role in national economies; it is lean, and its lipids are highly unsaturated (60% of the total fatty acids), rich in protein (20-21%) of high biological value [3], [4], low cholesterol content [5], and noticeable quantities of linolenic fatty acid (C18:3n3). Also, it displays a low sodium content and a high phosphorus content and can be a good source of B vitamins [1]. Therefore, rabbit meat is characterized by a high digestibility and may be fortified with bioactive compounds to obtain a "functional" product [3], [6].

For the investigation of rabbit meat quality, the primary attention is paid to the pH, color, water-holding ability of the meat [7], [8].

Antibiotic as growth promoters in feed for farm animals have been banned in the European Union from 2006, and this results in the pressures on meat exporters around the world, therefore it has increased interest in usage of organic acids. The organic acids are used as a growth promoter and for improvement of the feed conversion efficiency in farm animals [9]. Therefore, organic acids are among the prospective replacements for antibiotics in rabbit feeding [10]. Organic acids and their salts are generally considered as safe (GRAS) and have been approved by most member states of EU to be used as the feed additives in animal production.

Acidifiers have also been investigated for intensive rabbit production diets, either as organic acids or their salts with research being absorbedly on both health and productive performances [11]. However, scientific literature provides non-sufficient data on butyric acids, calcium formate, different organic acids, and its salts to the rabbit's productive performance, carcass traits, and meat quality. The aim of this study was to investigate the influence of butyric acids, calcium formate, organic acids, and its salts in rabbit's diets on rabbit's productivity, carcass characteristics, and meat quality.

II. Materials and Methods

A. Management of Experimental Rabbits

The feeding study was conducted in 2014–2015 in the individual rabbit breeding farm and the determination of rabbits' carcass traits and meat quality were performed in the animal productivity laboratory under the Institute of Animal Rearing Technologies of Veterinary Academy of Lithuanian University of Health Sciences.

The experiment was carried out with Californian breed rabbits of 28–77 days old which were sampled by weight. The rabbit's weight was 452±34 g at start of the study. During the study, 14 rabbits were divided into two groups, including seven rabbits in each. The rabbits were stored in individual wire cages with grid floors and an individual vessel for watering and feeding. Storage conditions were the same for all groups.

The rabbits were fed a compound feed diets (control group), experimental group was supplemented with a mixture (*Novibac*® and *Novyrate*® - from INNOV AD nv/sa, Belgium – a commercially available product that contains butyric acid, citric acid, calcium formate and propionate, silicic acid, zeolite) dosed at *Novibac*® 1 kg/t + *Novyrate*® 1 kg/t of feed.

The main components of the compound feed were the following: hay, corn, oats, wheat, sunflower, vegetable oils and minerals. Rabbits were fed twice a day (ad libitum).

TABLE I
Nutrition Indicators of Compound Feed

Indicators	Value
Digestive energy, kcal	2370.70
Metabolized energy, kcal	2257.20
Crude protein*, %	16.40
Crude fibre*, %	16.39
Moisture*, %	10.65
Starch*, %	9.56
Sugar, %	4.38
Total lysine, %	0.65
Methionine + cysteine, %	0.65
Tryptophan, %	0.20
Linolenic acid, %	1.04
Threonine, %	0.61
Total methionine, %	0.39
Available phosphorus, %	0.37
Calcium*, %	1.29
Phosphorus*, %	0.59
Sodium, %	0.25
Chlorine, %	0.54

*analyzed values

Contents of the premix: vit. A – 10.08 TV, vit. D3 – 1.14 TV, vit. E – 50.30 mg/kg, vit. K3 – 0.99 mg/kg, vit. B1 – 3.71 mg/kg, vit. B2 – 2.80 mg/kg, vit. B5 – 9.80 mg/kg, vit. B12 – 0.01 mg/kg, nicotinic acid – 20.40 mg/kg, folic acid – 0.22 mg/kg, choline chloride – 170.00 mg/kg, magnesium – 76.28 mg/kg, iron – 317.00 mg/kg, zinc – 110.89 mg/kg, copper – 19.16 mg/kg, cobalt – 0.29 mg/kg, iodine – 0.67 mg/kg, selenium – 0.31 mg/kg.

B. Characteristics of Preparations Used in the Study

Novyrate® – consists of salts of fatty acids (butyric acid of 98%) (100%).

Novibac® – calcium formate (55.0%), calcium propionate (10.0%), citric acid (10.0%), medium-chain fatty acids (0.50%), plant extracts (0.30%) silicic acid precipitated and joined together (2.0%), zeolite (22.20%).

C. Zootechnical Methods

During the feeding study, individual rabbit weight, daily feed intake, and mortality were recorded, and then, rabbit's daily weight gain and feed conversion ratio were calculated.

D. Physiological Methods

At 77 days of age, five rabbits per group were slaughtered. The carcasses were prepared as reported by [12]. The slaughtered rabbits were bled, and the skin, genitals, urinary bladder, gastrointestinal tract and the distal part of the legs were removed. The carcasses were chilled at 4 °C for 24h in a ventilated room. From the remaining carcass head, liver, heart, lungs, esophagus, trachea, thymus gland, and kidney free of perirenal fascia were dissected and weighed. From the reference carcass, the hind legs, and loin muscle were separated. Body weight at slaughter and hot carcasses weight were recorded. The dressing percentage was calculated as kilograms of hot carcass weight per kilograms of body weight.

The pH at 1h (pH1h), at 24h (pH24h), at 48h (pH48h) and at 72h (pH72h) post-mortem were determined on loin and hind leg muscle by using a InoLab WTW 720 pH-meter model with a driven electrode.

Colour measurements were performed according to the lab system (L*: lightness; a*: redness; and b*: yellowness) using a Minolta colorimeter CR-300® (Minolta Camera Co., Osaka, Japan, illuminant D65 and 0° observer) on a freshly cut loin and hind leg slice, left to oxygenate at 4 °C for 1h after slaughter.

The cholesterol content in rabbits' loin and hind leg muscle was determined by high performance liquid chromatography method described by [13].

Degree of lipids oxidation in rabbits' loin and hind leg muscle was measured 24h postmortem and after three months of keeping at the -18 °C temperature in refrigerator according the methods [14], using HPLC (Varian system).

E. Statistical Analysis

The results of the experiment were analyzed using the 1-way ANOVA test, and significant differences between groups were determined by Duncan's multiple range test. STATISTICA 8.0. for Windows TM software was used. Differences were considered significant at $P<0.05$.

III. Results and Discussion

Performance data of growing rabbits are reported in Table II. Addition of butyric acids, calcium formate, organic acids, and its salts significantly ($P<0.05$) increased slaughter weight, daily weight gain, and daily feed intake of the experimental group rabbits. The analysis of the effect of feed additives had a positive impact on livability of rabbits. This result agreed with [15] who found that addition of organic acid (formic and lactic acid) increased the live weight gain of rabbit. The positive effect of organic acids on rabbit growth indicators was found by [16] using citric acid on 30-86 days, by [17] using the mixture of microencapsulated calcium formate and citric acids on 28-77 days and others [18], [19]. However, the impact of organic acids in the feed on rabbits' productivity remains unclear.

TABLE II
EFFECT OF BUTYRIC ACIDS, CALCIUM FORMATE, ORGANIC ACIDS, AND ITS SALTS ON PRODUCTIVE PERFORMANCE (N=7) AND CARCASS TRAITS (N=5) OF RABBITS

Indices	Groups	
	Control	Experimental
Body weight at 28 d of age, g	483.33±22.10	481.33±18.90
Slaughter weight at 77 d of age, kg	1.30±0.47[a]	1.36 ±0.46[b]
Feed conversion ratio (28–77 d), kg/kg	4.71±0.60	4.51±0.49
Daily weight gain (28–77 d), g	16.60±0.75[a]	17.85±0.94[b]
Daily feed intake (28–77 d), g	78.25±10.54[a]	80.58±9.47[b]
Hot carcass weight, g	796±17.98[a]	873±25.61[b]
Internal organs weight, g*	85.10±4.25	83.90±3.40
Skin weight, g	341.23±10.45	352.69±12.69
Dressing out percentage, %	61.15±3.20[a]	64.19±5.87[b]

[a, b] – means within each row with different superscripts are significantly different at P < 0.05;
*- heart, liver, spleen, lung, kidneys.

Rabbit carcass and meat quality is influenced by breed, age of animals, their diet, ante and post mortem factors, etc. [20]. Rabbit meat does not have a very strong flavour, being comparable but not identical to chicken [21]. Tenderness varies with age and is tenderer in the younger rabbits [22].

Table II shows the effect of the analysed additives on carcass traits of rabbits. There were insignificant differences in the slaughter and hot carcass weight among the group. Supplementation of butyric acids, calcium formate, organic acids, and its salts in the rabbits feed had no effects neither on final live body slaughter nor on the carcass weights compared with control group. It could be noticed that the butyric acids, calcium formate, organic acids, and its salts group showed the highest final live body slaughter and hot carcass weights (1.36 kg and 873 g, respectively), in the other side, the control group showed the lowest final live body slaughter and carcass weights (1.30 kg and 796 g, respectively). The carcass traits of fore, loin and hind parts, internal organs, and heads were trend of slaughter and hot carcass weight, when showed higher dressing out percentage of experimental group compared with the control group (64.19 ($P<0.05$) and 61.15 ($P>0.05$); respectively).

pH is the most important factor of meat quality. The effect of dietary supplementation with butyric acids, calcium formate, organic acids, and its salts (Table III) pH in loin meat after 48h (5.86 vs. 5.74) and in hind leg meat after 48h (6.62 vs. 6.65) and 72h (6.65 vs. 6.56) were significantly differed ($P<0.05$) in comparison to control rabbit meat fed on basal diet. 72h after post-mortem, rabbits' meat has been completely mature.

Our results are in agreement with those reported by [23]-[26] with different raw material supplementation in rabbit feed. Similar results were found by [27] being from 5.82 for New Zealand rabbits to 5.89 for Californian rabbits 24h after slaughtering. However, these authors asserted that for meat of good quality, pH should be in the range of 5.4 to 5.8 after 24h of meat storage. Additionally, [28] assessed differentiation in the pH value which changes depending on the part of carcass. The lowest pH was measured in the loin (5.56), the highest in the thigh (5.71) after 24h of meat storage.

TABLE III
EFFECT OF BUTYRIC ACIDS, CALCIUM FORMATE, ORGANIC ACIDS, AND ITS SALTS ON PH VALUES IN MEAT OF RABBIT'S (N=5)

Hours	Control group	Experimental group
	Loin	
1	5.85±0.16	5.83±0.10
24	5.82±0.08	5.79±0.07
48	5.86±0.06[a]	5.74±0.13[b]
72	5.76±0.10	5.72±0.10
	Hind leg	
1	6.85±0.11	6.60±0.23
24	6.79±0.06	6.57±0.27
48	6.62±0.04[a]	6.65±0.19[b]
72	6.65±0.07[a]	6.56±0.09[b]

[a, b] – means within each row with different superscripts are significantly different at P < 0.05.

Reference [29] reported that the lowering of pH in muscles is due to the accumulation of lactic acid after stunning, and that both the rate and the extent of the post-mortem pH fall are influenced by intrinsic factors such as species, the type of muscles, and variability between animals. Also, [30] indicated that it is one of the most significant post-mortem changes that occur during the conversion of muscles to meat. This parameter affects the structure of proteins as well as water retention capacity of the tissue, and may modify the sensorial quality of the meat – mainly color and tenderness [27], [31].

TABLE IV
EFFECT OF BUTYRIC ACIDS, CALCIUM FORMAT, ORGANIC ACIDS AND ITS SALTS ON MEAT COLOR INTENSITY OF RABBIT'S (N=5)

Indices	Control group	Experimental group
	Loin	
L*	53.06±0.37	53.17±0.70
a*	13.68±0.36	13.69±0.61
b*	5.57±0.24[a]	6.06±0.18[b]
	Hind leg	
L*	52.97±1.41[a]	55.53±0.90[b]
a*	14.99±1.19[a]	13.15±0.67[b]
b*	4.74±0.21	4.77±0.31

[a, b] – means within each row with different superscripts are significantly different at P < 0.05;
L – lightness, a – redness, and b* - yellowness

The analysis of meat colour intensity of rabbits (Table IV) showed that butyric acids, calcium formate, organic acids, and its salts increased b* indices in loin meat (5.57 vs 6.06; $P<0.05$), L* indices in hind leg meat (52.97 vs. 55.53; $P<0.05$) and decreased a* indices in hind leg meat (14.99 vs. 13.15; $P<0.05$) in the experimental group as compared to the control group. Generally, the increase in lightness (L*) can be due, in part, to some modifications occurring in the muscular fibres during storage, such as the reduction in the cellular membrane integrity, also caused by the shrinkage of myofibrils. This, in turn, can cause a passage of liquids from the cells to extracellular spaces, which leads to an increase in light reflection.

From a commercial point of view, an increase in the lightness in rabbit meat during storage could be considered as a positive factor, since consumers associate a light colour in "white meats" with better dietetic and nutritional characteristics [32].

TABLE V
EFFECT OF BUTYRIC ACIDS, CALCIUM FORMATE, ORGANIC ACIDS, AND ITS SALTS ON CHOLESTEROL CONTENT IN MEAT OF RABBIT'S, MG/100G (N=5)

Groups	Loin	Hind leg
Control	48.41±2.34	94.25±6.39^{a}
Experimental	46.81±4.25	76.58±5.98^{b}

$^{a, b}$ – means within each column with different superscripts are significantly different at P < 0.05.

The cholesterol content of the body is determined by genetic and environmental factors, with nutrition playing an important role [33]. Results of the total cholesterol content in rabbit muscles are presented in Table V. A positive tendency to lower cholesterol content in the hind leg was observed in the experimental group (94.25 mg/100 g vs. 76.58 mg/100 g; P<0.05).

Our results are in agreement with data reported by [34]. They showed that the meat cholesterol level is higher and ranged between 120 mg/100g meat for Californian rabbit to 145 mg/100g for New Zealand White rabbits. Additionally, the highest total cholesterol content (163.3 mg/100g) for rabbit meat was observed by [35]. Reference [36] found that amount of cholesterol in rabbit meat was about 59 mg/100 g of muscle.

TABLE VI
EFFECT OF BUTYRIC ACIDS, CALCIUM FORMATE, ORGANIC ACIDS AND ITS SALTS ON THE STORAGE TIME ON THE LIPID OXIDATION VALUES (MDA) IN THE RAW RABBITS MEAT, MMOL/KG (N=5)

Groups	Loin		Hind leg	
	24h	3 months	24h	3 months
Control	0.45±0.26	0.95±0.50^{a}	0.51±0.37	1.10±0.71^{a}
Experimental	0.37±0.23	0.65±0.35^{b}	0.46±0.40	0.84±0.46^{b}

$^{a, b}$ – means within each column with different superscripts are significantly different at P < 0.05.

Aging is one of the most critical factors that influence meat quality. During aging, the process of muscle conversion into meat is accompanied by quantitative changes in several metabolites. Consequently, meat becomes unfit for human consumption as it is considered to be spoiled. Spoilage of raw meat accounts greatly for major annual losses to meat processors and retailers [37].

Lipid oxidation reason disadvantage of nutrition. MDA has long been regarded as an index of oxidative rancidity [38].

The effects of dietary treatments on the oxidative stability of meat measured at different time periods are shown in Table VI. Supplementation of butyric acids, calcium formate, organic acids, and its salts had no effect on MDA concentration of fresh meat muscles (24h). MDA concentration in the loin and hind leg meat after refrigerated storage for three months in experimental group decreased by 0.3 µmol/kg (P<0.05) and 0.26 µmol/kg (P<0.05) respectively, compared with the control group.

IV. CONCLUSION

The results of this study suggest that butyric acids, calcium formate, organic acids, and its salts significant increased daily weight gain if rabbits, the slaughter and hot carcass weights, dressing out percentage, pH in hind leg, yellowness color intensity in loin and lightness color intensity in hind leg compared with the control group. The analyzed feed additives significantly reduced pH in loin meat, redness color intensity in hind leg, cholesterol content in hind leg, and in oxidative stability of rabbits' meat after three months of storage. Consequently, these feed additives can be incorporated into feeding and to be the alternative for antibiotic growth promoters in rabbits' nutrition.

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