NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 56, 2023 (4): 3–15
ISSN 1337-9984, E-ISSN 1338-0095
SCI CELL

HEMPSEED CAKE IN RABBIT NUTRITION: LIVESTOCK PERFORMANCES, QUALITY OF MEAT, DIGESTIBILITY OF NUTRIENTS AND ANIMAL HEALTH STATUS

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ABSTRACT

The objective of this study was to evaluate the effect of dried hempseed cake (by-product of oil production) supplementation (5 % – EG1 and 10 % – EG2) to rabbit feed mixture on livestock performances, quality of meat, digestibility of nutrients and animal health status. The chemical composition of the *Longissimus thoracis* and *lumborum* muscle including the content of fatty acids and amino acids, and the biochemical parameters in caecum and blood of growing rabbits were investigated. No significant differences among the experimental groups in feed intake, body weight and carcass parameters were found. The digestibility trial was performed using the balance method. Feed mixtures differed in digestible energy content i.e., crude protein, crude fibre and fat. The resulting digestibility coefficients for protein fell within the range of 66.72 – 74.18 %, fat digestibility was in the interval from 88.73 to 89.34 % and crude fibre digestibility was in the range from 27.56 to 34.59 %. Fatty acid profile in intramuscular lipids represents the highest content of monounsaturated fatty acids (MUFA; 47.72 – 48.51 %), followed by the content of saturated acids (SFA; 33.75 – 33.87 %) and a low content of polyunsaturated fatty acids (PUFA; 11.23 – 12.08 %). Hempseed oil cake could be included in rabbits' diet with beneficial effect on carcass quality and can enhance the nutritional quality of rabbit meat with the focus on essential amino acids. The tested blood serum parameters were within the range of the physiological reference values with no statistical differences between experimental groups, except for glucose and cholesterol content. The data on volatile fatty acids (VFA) show that most intensive process was in the caecum of rabbits in the experimental groups EG1 and EG2. All obtained data let us to recommend the inclusion of hempseed cake up to 10 % in rabbit diet without any negative effect on animal welfare, livestock performance and quality of meat.

Key words: hempseed cake; rabbits; meat

INTRODUCTION

Hemp (*Cannabis sativa* L.) is an environmentally sustainable plant widespread worldwide. Following the reintroduction of its cultivation, hemp is attracting interest, especially in the food/feed industry. However, it was only with Regulation (EU) No 1307/2013, that hemp cultivation was also included among those qualifying for Common Agricultural Policy (CAP) payments, with the condition to cultivate only seeds of varieties registered in the European catalogue with a Δ9-tetrahydrocannabinol (THC) content (i.e., the plant's recognised psychoactive compound) of less than 0.2 % (Sorrentino, 2021; EFSA, 2011). Teleszko *et al*. (2022) reported the nutritional value of the Polish hemp seeds of the Bialobrzeskie and Henola

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Received: November 8, 2023
Accepted: December 14, 2023

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https://doi.org/10.36547/sjas.841

varieties, including the profile/content of fatty acids and amino acids. Hempseed cakes were found to be rich in protein, fat and dietary fibre. Polyunsaturated fatty acids (PUFA) dominated the unsaturated fatty acid (UFA) profile. Their average proportion from the total fatty acids (FA) was as high as 75 %. Linoleic acid belonging to this group accounted for 55 % of the total FA. By pressure extraction of hemp seed, it is possible to obtain a high siccative index industrial oil and an oil cake (12 – 14 % residual oil) with high protein, fibre and energy level and other components, the consumption of which can be beneficial for health (House *et al*., 2010; Siano *et al*., 2018; Razmaité *et al*., 2021). Hemp seed oils are also a source of vitamins and other compounds with antioxidant activity, especially tocopherols and other bioactive compounds, such as phenolics and phytosterols, including β-sitosterol and campesterol. This study explored the potential for using seed cake from hemp (*Cannabis sativa* L., variety Finola) as a protein feed for rabbits. The objective of this study was to determine the effects of hempseed cake (HC) supplementation of rabbit diets, at 5 % or 10 % proportion, on the growth performance, nutrient digestibility, biochemical parameters in the digestive tract, the meat composition and the haemato-biochemical parameters of growing rabbits.

MATERIAL AND METHODS

Experimental design

All care and experimental procedures were approved by the Slovak Veterinary and Food Administration and by the Ethic Commission of both institutes (permission code: SK CH 17 021). The study was carried out in April and May 2022 at the National Agricultural and Food Centre, Research Institute for Animal Production Nitra / Institute of nutrition and small farm animals, Lužianky, Slovak Republic.

A total number of 66 post-weaned rabbits (aged 35 days, meat line M91 and P91 hybrid rabbits) were randomly divided into 3 groups; 22 animals in each group (control-CG, experimental group EG1 and EG2). They were housed in standard metal cages, two animals per cage. The cages allowed faeces separation. A cycle of 16 h of light and 8 h of dark was used throughout the experiment. Temperature and humidity were recorded continuously by a digital thermograph positioned at the same level as the cages. Heating and forced ventilation systems allowed air temperature in the building to be maintained within 14 ± 4 °C during the experiment. Relative humidity was about 60 ± 5 %. The rabbits in the control group were fed a commercial mixture (3.5 mm in diameter) for growing rabbits (KV; SIGI TRADE, Ltd.,

Table 1. Composition of the experimental diets

Feed ingredients in g.kg^{-1} in original feed	Control group	EG1 with 5 % hempseed cake	EG2 with 10 % hempseed cake
Alfalfa meal	360	342	342
Sunflower meal	55	52	52
Rape-seed meal	55	52	52
Hempseed cake	0	50	100
Wheat bran	90	85	81
Oats	130	124	117
Dry malting sprouts	150	143	134
Maize	50	47	43
Sodium chloride	3	3	3
Minerals and Vitamins mixture*	17	17	15
Barley	80	75	52
Limestone	10	10	9

*Commercial premix (KV; SIGI TRADE, Ltd. Slovak Republic), provided per kg diet: Vit. A 6000 IU; Vit. D_3 1000 IU; Vit. E 50 mg; Vit. B_1 1.7 mg; Vit. B_2 8.0 mg; Vit. B_6 3.0 mg; Vit. B_{12} 0.01 mg; Vit. K_3 0.5 mg; biotin 0.2 mg; folic acid 0.5 mg; nicotic acid 45 mg; Se 0.2 mg; choline chloride 450 mg.

Dvory nad Žitavou, Slovak Republic). The rabbits in the 1st experimental group were fed granulated mixture including 5 % hempseed cake. The rabbits in the 2nd experimental group were fed granulated mixture including 10 % hempseed cake. Rabbits were fed *ad libitum* and water was also provided using nipple drinkers. The samples of individual feeds were analysed for content of nutrients (Tables 1 and 2), according to the procedures of the AOAC (2005), and starch, according to the alpha-amyloglucosidase method. The content of metabolizable energy of sample mixture was calculated by the equation of Wiseman *et al*. (1992).

Evaluation of growth performance and carcass traits

The body weight of each animal was recorded weekly during the whole study. Weight of the feed mixture was checked daily and average daily weight gain (ADWG) and feed conversion ratio as well as mortality were calculated at the end of the experiment (Table 3). Between 65 and 70 days of age, 5 rabbits from each group were selected for digestibility tests using the balance method. The digestibility test was performed in accordance with the recommended methodology E.G.R.A.N. (2001). The faeces were collected individually during 4 consecutive days according to the European reference method for rabbit digestion trials. Faecal sampling was executed every 2 hours. Faeces were collected in bags during the daytime. Every day in the morning, faeces were mixed with a handy mixer, the average samples were pre-dried (at 60 °C for 36 h in a dryer) and grinded (1 mm screen) with a laboratory grinder for chemical analysis. The chemical composition was analysed in accordance with the methods of the Association of Analytical Chemists (AOAC, 2005) for dry mater (DM), crude protein (CP), crude fibre (CF) by the standard Weende method in nitrogen free extract, ash and organic matter. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were pre-treatments. Hemicellulose was calculated as NDF-ADF, cellulose - as ADF-ADL, crude fat of feeds and acid analysed sequentially (Van Soest *et al*., 1991; Gidenne *et al*., 2001; Gidenne *et al*., 2002) with a thermo-stabile amylase detergent, fibre faeces were determined in a Soxlet extractor system. Starch was determined by polarimetric method on Polarimeter ADP 220 (Bellingham and Stanley Ltd., United Kingdom) following the regulations of the Ministry of Agriculture of the Slovak Republic No. 2145/2004-100.

Biochemical parameters in the blood of rabbits (*in vivo*)

Blood was sampled from five rabbits, individually from each of them, at day 35 (day zero, without additives)

Table 2. Chemical composition of the experimental diets

Chemical analysis in g.kg^{-1}; *mg.kg^{-1}	Hempseed cake	Control group	EG1 with 5 % hempseed cake	EG2 with 10 % hempseed cake
Crude proteins	331.12	152.54	165.23	162.79
Crude fibre	286.17	156.63	151.19	153.99
Fat	113.87	28.13	33.97	33.05
Ash	58.97	77.31	80.14	84.16
Starch	10.85	188.29	189.18	181.62
Organic matter	841.78	810.21	817.41	821.71
ADF	325.33	145.53	182.19	185.43
NDF	382.52	334.36	334.08	328.44
Calcium (Ca)	1.91	11.74	9.77	12.06
Phosphorus (P)	7.57	5.89	6.34	6.82
Magnesium (Mg)	4.95	2.86	2.18	2.55
Sodium (Na)	0.23	1.31	1.61	1.23
Potassium (K)	8.60	10.17	10.37	9.82
Copper (Cu) *	23.03	22.32	23.14	21.18
Iron (Fe)*	150.16	503.63	624.49	577.00
ME MJ.kg^{-1}	11.16	10.74	11.17	10.99

ADF – Acid detergent fibre; NDF – Neutral detergent fibre; ME Metabolizable energy; data expressed on raw feed.

and day 77 (n = 5/group) from the marginal ear vein (*Vena auricularis*) into dry non-heparinized Eppendorf tubes for biochemical analyses. Blood serum was obtained by centrifugation at 3000 × g for 10 min and stored frozen (at -18 °C) in plastic vials until analysis. To test the biochemical parameters – total proteins (TP; g/L), albumins (g/L), urea (mmol/L), glucose (mmol/L), triglycerides (mmol/L), total cholesterol (mmol/L), alanine aminotransferase (ALT; µkat/L), calcium (mmol/L) and phosphorus (mmol/L), a commercial Dialab kit (Czech Republic) and an automated biochemical analyser ELLIPSE (AMS, Italy), based on turbidimetric clinical assay, were used according to the manufacturer's instructions and the methods of serum parameter determination. The activity of blood aspartate aminotransferase (AST; µkat/L), gamma-glutamyl transferase (GGT; µkat/L) and alkaline phosphatase (ALP; µkat/L) were measured using commercial DiaSys sets (Diagnostic Systems GmbH, Holzheim, Germany) on the Rx Monza device (Randox Laboratories Ltd., London, United Kingdom).

Slaughter procedure and meat quality parameters

Five animals at 70 days of age from each group were slaughtered and samples were taken. After electro-stunning (90 V for 5 sec), the rabbits were slaughtered at an experimental slaughterhouse by cutting the *carotid* and *jugular veins* and bleeding out. The *Longissimus thoracis* and *lumborum* muscles (LTLM) were separated by removing the skin and connective tissue, chilled and stored at 4 °C for 24 h until physico-chemical analysis. The physico-chemical characteristics and chemical composition were determined by standard methods (STN 570185). The contents of water, protein and fat were estimated using a FoodScaneTM – Meat Analyser (FOSS, Denmark) by the FT IR method (Fourier Transform infrared Spectroscopy) and expressed in g.100 g^{-1} (Burns and Ciurczak, 2007). From these data, energy value was calculated according to the equation of Strmiska *et al*. (1988): Energy value (kJ.100 g^{-1}) = 16.75 x protein content +37.65 x fat content. The ultimate pH was determined 24 h *post-mortem* with a Radelkis OP-109 (Jenway, England) with a combined electrode penetrating 3 mm into the LTLM. The electrical conductivity (µS.cm^{-1}), defined as locations of muscles, was evaluated using PMV 51 (Tecpro Metall GmbH, Neuss, Germany). Colour measurements were taken on LTLM surface of the carcass at 24 h after bleeding. Colour characteristics were expressed using the CIE L*a*b system (lightness-L*, 0: black and 100: white; redness and greenness-a*; yellowness and blueness-b*) using a Lab Miniscan (HunterLab, Reston, VA, USA). Lightness measurements at room temperature were also taken. The water holding capacity was determined by compression method at constant pressure (Hašek and Palanská, 1976). The analysed samples (0.3 g in weight) were placed on filter papers (Schleicher and Schuell No. 2040B, Dassel, Germany) with tweezers previously weighed. Together with the papers, samples were wedged between Plexiglass plates and then subjected to a pressure of 5 kg for 5 min. The results were calculated from the difference in weight between the slips with aspirating spot and the pure filter paper.

For macro- and micro-element analysis, samples were ashed at 550 °C, the ash was dissolved in 10 ml of HCl (1:3) and minerals were determined by AAS iCE 3000 (Termo, United Kingdom). The phosphorus content was determined by molybdovanadate reagent Camspec M501 (Spetronic Camspes Ltd, United Kingdom). Mineralized samples were analysed for Ca, P, Mg, Na, K, Fe, Zn, Cu and Mn content. For mineral content determination the spectrometer AAS iCE 3000 (Thermo, UK) was used. Contents of mineral nutrients in feeds and faeces were estimated in graphite cuvette through electrothermal atomization. Content of Ca was estimated at the wavelength of 422.7 nm, Fe – at 248.3 nm, Zn – at 213.9 nm, Cu – at 324.8 nm and content of P – at 410.0 nm, as phosphomolybdic yellow (Official Journal L 206, 29/07/1978, p.0043-0055). All the analyses were performed in triplicate.

The diets' amino acid composition was analysed by ion-exchange chromatography on AAA (Ingos Prague, Czech Republic) after acid hydrolysis with 6M HCl, while methionine and cystine – after oxidation hydrolysis. The fatty acid (FA) composition of the LTLM samples was determined by the method of Ouhayoun (1992) and Bannon *et al*. (1982) using gas chromatography of fatty acid methyl ester (FAME) on GC 6890N (Agilent Technologies, Switzerland). The results were expressed as a percentage of total fatty acids. Fatty acid composition varied a lot, and it is expressed as a share of SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), lipid profile indices Σ PUFA / Σ SFA and Σ n-6/ Σ n-3 index.

Parameters of caecum (*post-mortem*)

The caecal samples, collected from each of the five slaughtered rabbits (n = 5/group), were analyzed for pH and lactic acid (g.100 g^{-1}). Volatile fatty acid (VFA) concentrations (acetic, propionic, butyric, iso-butyric, valeric, iso-valeric and caproic acids) were determined (mmol/100 g) using Gas Chromatograph (GC Carlo Erba 8000 Top) from samples of caecal content (15 g) on the day 70. A glass column (average diameter 3 mm, length 180 cm) was filled with N_2 (30 mL), H_2 (20 mL) and air (240 mL) and a sample (1 µL) for diffusion. As the standard column was used, isocaproic acid (SP 1200 H_3PO_4) on Chromosorbe WAW; was separated at 130 °C. Lactic acid was separated at 125 °C on Chromatone N-AW-DMCS. The pH value was measured with a Jenwey 3310 pH-meter (Essex, England). Ammonia-N concentration was measured by the micro-diffusion according to Conway (Voight and Steger, 1967).

Statistical analysis

All measurements were made in duplicate and results in tables are reported as means ± standard deviation (SD). The data were analysed by a one-way analysis of variance (ANOVA) with the post-hoc Tukey's multiple comparison test. Mean values within the same row having different superscripts indicate significant difference for ($P \leq 0.05$).

RESULTS AND DISCUSSION

The experiment lasted for 42 days, until the animals attained the slaughtering weight 2.5 kg. Rabbits remained healthy throughout the study period. Health state was considered on the absence of clinical signs. The purpose of the present study was to establish the real possibilities of utilization of dried hempseed cake, a by-product from pressure oil extraction of hempseed.

Zootechnical parameters of rabbits are shown in Table 3. No differences were observed in feed intake and feed conversion ratio ($P > 0.05$) among the groups. The average dressing out percentage was calculated as the ratio of dressed weight to the live weight. Beneficial effect of hempseed cake on the carcass yields of rabbits was observed in the experimental groups compared to the control group, but the difference was not statistically significant. The overall mortality at the end of growing phase was 1 (EG1) vs. 3 rabbits (EG2) and was not related to the type of the diet. The digestibility coefficients of nutrients (Table 4) showed significant effect of a diet. The resulting coefficients of crude fibre digestibility and its fractions were lowest for the rabbits in the control group. For other nutrients (crude protein, crude fat, crude fibre and organic matter, sodium and potassium) we recorded higher coefficients of digestibility in the experimental groups. Coefficients of digestibility of crude protein and crude fibre in our experiment in the EG1 group were higher than those published by Lebas *et al*. (1988) and Ondruška *et al*. (2010). This was associated with a reduction of fibre digestibility (NDF fraction) in the EG2 group.

Results of selected meat quality parameters are presented in Table 5. Generally, rabbit meat is characterized by low-fat content, when compared with other meat species (Dalle Zotte and Szendro, 2011). In our experiment, content of fat in the experimental

Table 3. Effect of treatment on rabbit livestock parameters

Tested of parameters (n = 22 / group)	CG Control group	EG1 with 5 % hempseed cake	EG2 with 10 % hempseed cake	*P*-value
Initial body weight (1 d), g	1010 ± 98	1085 ± 130	1115 ± 146	0.185
Intermediate live weight (21 d), g	1800 ± 162	2119 ± 188	2080 ± 173	0.275
Weight at slaughter (42 d), g	2615 ± 206	2635 ± 251	2651 ± 242	0.301
Average daily gain (1 - 42 d), g.d^{-1}	38.10	36.90	36.57	0.243
Feed conversion ratio (1 - 42 d)	3.423 ± 0.131	3.447 ± 0.271	3.453 ± 0.343	0.157
Average daily feed intake, g	153	150	154	0.166
Carcass yield (%)	52.65 ± 0.57	56.11± 1.59	56.03 ± 0.61	0.211
Mortality, n (%)	2 (9 %)	1 (4.5 %)	3 (13.6 %)	0.118

P > 0.05 non-significant effect from control; data expressed as mean ± SD.

Table 4. Apparent digestibility coefficients of the diets (%)

Parameter n = 5 / group	Treatment groups			*P*-value
	CG1 Control group	EG1 with 5 % hempseed cake	EG2 with 10 % hempseed cake	
Dry matter	63.20 ± 0.31^{A}	62.80 ± 0.40^{A}	58.51 ± 2.39^{B}	0.0066
Crude protein	66.72 ± 1.09^{a}	74.18 ± 5.51^{b}	67.88 ± 2.98^{a}	0.0236
Fat	80.89 ± 2.37^{A}	88.73 ± 1.22^{B}	89.34 ± 2.11^{B}	0.0014
Crude fibre	21.89 ± 2.36^{A}	27.56 ± 2.01^{b}	34.59 ± 3.01^{B}	0.0020
ADF	14.40 ± 1.03^{A}	27.50 ± 2.01^{B}	19.24 ± 4.70^{b}	0.0003
NDF	36.71 ± 2.30^{c}	38.75 ± 1.27^{ac}	31.02 ± 4.43	0.0432
Starch	93.19 ± 0.37^{a}	95.75 ± 0.54^{b}	95.31 ± 0.39^{b}	0.0055
Ash	47.69 ± 1.72^{a}	39.64 ± 1.07^{b}	37.07 ± 4.30^{b}	0.0011
Organic matter	64.71 ± 0.23^{a}	64.97 ± 0.41^{a}	60.39 ± 2.38^{b}	0.0086
Calcium (Ca)	65.30 ± 0.97^{A}	43.18 ± 3.74^{b}	38.24 ± 3.18^{B}	0.0013
Phosphorus (P)	14.88 ± 2.06^{a}	21.96 ± 3.80^{b}	20.60 ± 4.88^{b}	0.0087
Magnesium (Mg)	41.07 ± 2.11^{a}	36.26 ± 5.97^{b}	33.54 ± 4.56^{b}	0.0082
Sodium (Na)	57.13 ± 6.29	78.70 ± 5.93	64.15 ± 8.22	0.1403
Potassium (K)	76.61 ± 6.25^{a}	84.57 ± 2.32^{b}	84.78 ± 1.57^{b}	0.0315
Copper (Cu)	11.55 ± 4.94^{A}	8.03 ± 3.39^{B}	7.83 ± 3.02^{B}	0.0012
Iron (Fe)	20.05 ± 3.31^{a}	17.41 ± 5.13^{ab}	13.88 ± 3.76^{b}	0.0187

^{a,b} – $P < 0.05$; ^{A,B} – $P < 0.01$ Significant difference.

groups was higher than in the control group, most probably due to the high content of fat in hempseed cake, which, in turn, resulted in the higher energy value in experimental meat samples. Only small differences were found between the individual components, which correspond with the results of other authors (Bianospino *et al*., 2006; Dalle Zotte and Szendrő, 2011; Chrastinová *et al*., 2018; Pogány Simonová *et al*., 2019; Lauková *et al*., 2016; 2017; Kalafová *et al*., 2014; 2015). It also has an optimal content of zinc, copper, phosphorus, calcium and cobalt (Zadina *et al*., 2004). The pH of rabbit meat in the EG1 group was lower when compared to the EG2, but the differences were not significant (P > 0.05). The pH value depends on the balance of muscle energy metabolism and represents a key role in the maintenance of the meat quality during storage. Meat colour is the most important factor affecting consumer acceptance and purchasing decisions. Dalle Zotte (2002) reported that mean L*a*b* colour values of the rabbit LTLM muscle are L* = 56 – 60, a* = 2.6 – 3.4 and b* = 4.0 – 5.0. Higher values of yellowness could relate to free radicals, produced by lipid oxidation during storage and/or manipulation, which can oxidise hem pigments, causing discolouration of meat and meat products (Münch, 2004). In our experiment the dietary strategy did not influence meat colour parameters. Furthermore, a positive correlation between water holding capacity and intramuscular fat content (Hernández *et al*., 2000) as well as the ultimate pH (Lambertini *et al*., 1996) was noticed.

As shown in Table 6, the intramuscular lipids were characterized by the highest percentage of monounsaturated fatty acids (MUFA). In this study, the intramuscular lipids in the LTLM muscles were also characterized by a higher percentage of saturated (SFA) and lower percentage of polyunsaturated fatty acids (PUFA) with no statistical differences among experimental groups. Selection of different feeding and breeding strategies could increase functional ingredients in rabbit meat, particularly essential fatty acids – EPA (Eicosapentaenoic acid), DHA (Docosahexaenic acid), CLA (Conjugated linoleic acid) and BCFA (branched chain fatty acids. Moreover, natural trans-vaccenic acid could be metabolized into conjugated linoleic acid (CLA) and, besides vaccenic acid did not inhibit the metabolic conversion of linoleic to arachidonic acid (Kummerow, 2009). Food and Drug Administration (FDA) assumed that some of trans acids might be from the natural vaccenic acid that had

Table 5. The effect of dietary supplementation with hempseed oil cake on selected processing technology parameters and chemical characteristic of *MLTL* muscles 24 h *post-mortem*

Parameter n = 5 / group		CG Control group		EG1 with 5 % hempseed cake		EG2 with 10 % hempseed cake		*P*-value
		means	SD	means	SD	means	SD	
Content of water	g.100 g^{-1}	75.06	0.48	74.32	0.44	74.38	0.39	0.0611
Total proteins	g.100 g^{-1}	22.68	0.27	23.38	0.32	23.11	0.18	0.0745
Content of fat	g.100 g^{-1}	1.03^{a}	0.15	1.52^{b}	0.28	1.57^{b}	0.33	0.0218
Collagen	g.100 g^{-1}	0.78	0.12	0.77	0.08	0.75	0.13	0.4208
Energy value	kJ.100 g^{-1}	421.32^{a}	7.60	448.99^{b}	8.28	446.20^{b}	14.79	0.0167
Ash	g.100 g^{-1}	0.56	0.15	0.43	0.12	0.53	0.14	0.4848
Cholesterol	g.100 g^{-1}	0.37	0.02	0.37	0.02	0.39	0.05	0.4506
Content of minerals element in MLTL muscles in original matter								
Calcium (Ca)	g.kg^{-1}	0.136	0.056	0.113	0.014	0.104	0.017	0.2822
Phosphorus (P)	g.kg^{-1}	2.207	0.023	2.172	0.114	2.235	0.094	0.1691
Magnesium (Mg)	g.kg^{-1}	0.261	0.034	0.274	0.037	0.255	0.007	0.0287
Sodium (Na)	g.kg^{-1}	0.312^{a}	0.022	0.242^{b}	0.025	0.310^{a}	0.022	0.0461
Potassium (K)	g.kg^{-1}	4.319	1.021	3.790	0.171	3.854	0.070	0.0707
Zinc (Zn)	mg.kg^{-1}	8.063^{a}	1.029	10.706^{b}	1.282	7.478^{a}	1.286	0.0116
Copper (Cu)	mg.kg^{-1}	1.701^{a}	0.024	1.305^{ab}	0.118	1.003^{b}	0.174	0.0103
pH 24		5.94	0.03	6.00	0.06	6.06	0.04	0.1734
Water holding capacity	g.100 g^{-1}	25.86	3.44	27.23	3.99	24.62	5.23	0.1498
Electric conductivity	µS.cm^{-1}	1.20	0.51	0.91	0.470	0.81	0.19	0.2252
Colour Lightness, (0: black and 100: white)	L	54.02	1.69	52.81	2.33	53.61	0.89	0.2930
Redness (500−700 nm)	a	1.36	1.24	2.23	0.28	1.00	0.64	0.2871
Yellowness (445−578 nm)	b	8.50	1.29	9.77	2.11	8.84	1.79	0.1940

[a,b] Means in the same row followed by different letters differ significantly $P < 0.01$.

no harmful effects and suggested that approximately 2.6 % of the total daily fat intake were from trans-fat and 50 % of the trans fatty acids might be from vaccenic acid. Therefore, the presence of vaccenic acid (trans-11-18:1) in rabbit fat should not be a limiting factor for the use of meat. Small quantity CLA in meat MLTL rabbits is due to fermentation in caecum and caecotrophy gains (Marounek *et al*. 2006).

Table 7 summarizes the determined contents of 17 amino acids observed in *longissimus thoracis* and *lumborum* muscle (g.kg^{-1} of original matter). The essential amino acid composition is one of the most important nutritional qualities of a protein. Some of these amino acids can be synthesized by living organisms from other nitrogenous material. Other amino acids are essential to the diet and cannot be synthesized *in vivo*, however, must be ingested as such. Isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (with histidine for infants) are known as the essential amino acids and must all be provided via the eaten food (Henchion *et al*., 2017). Research of rabbit meat amino acid (AA) composition and its influence by natural additives treatment is still limited. The tested AA levels varied from 93.882 to 101.645 g.kg^{-1} for EAAs and from 99.587 to 103.368 g.kg^{-1} for NEAAs. These data are comparable to the most presented in before mentioned studies, but they are lower than AA levels noted during phyto-additive application to rabbits (Pogány Simonová *et al*., 2010). While hempseed cake alone significantly increased mostly isoleucine, leucine, lysine, threonine and valine amounts from EAAs; the stimulating effect was not noted on methionine, phenylalanine and cysteine level. The diet supplementation with agricultural and

Table 6. Fatty acid composition of total intramuscular fat from *Musculus longissimus thoracis et lumborum* (% from total fatty acids)

Fatty acids n = 5 / group Age at slaughter (77 d)	CG1 Control group	EG1 with 5 % hempseed cake	EG2 with 10 % hempseed cake	*P*-value
C12:0 (Lauric a.)	0.115 ± 0.003	0.110 ± 0.006	0.113 ± 0.005	0.3339
C14:0 (Myristic a.)	1.402 ± 0.026	1.343 ± 0.080	1.376 ± 0.057	0.0669
C16:0 (Palmitic a.)	24.349 ± 0.254	24.376 ± 0.221	24.583 ± 0.084	0.4076
C17:0 (Heptadecanoic a)	0.295 ± 0.012	0.290 ± 0.055	0.299 ± 0.049	0.3810
C18:0 (Stearic a.)	10.503 ± 0.302	10.530 ± 0.456	10.701 ± 0.131	0.4820
Total saturated FA	33.754 ± 0.832	34.832 ± 2.902	33.874 ± 1.836	0.1539
C18:1n-9c (Oleic a.)	26.912 ± 9.805	33.290 ± 12.697	30.148 ± 9.937	0.4842
C18:1 11c/15t (Vaccenic a.)	5.013 ± 0.142	4.952 ± 0.197	4.903 ± 0.122	0.1762
C20:1 (Eicosenoic a.)	0.674 ± 0.110	0.702 ± 0.137	0.643 ± 0.116	0.1629
Total monounsaturated FA	47.720 ± 1.623	47.774 ± 2.741	48.514 ± 2.027	0.3841
C18:2n-6 (Linolic a.)	3.832 ± 0.743	4.812 ± 1.714	4.898 ± 1.007	0.0500
C18:2 9c/11t (CLA)	0.126 ± 0.018	0.134 ± 0.018	0.128 ± 0.007	0.1210
C18:3n-3 (α-Linolenic a.)	0.193 ± 0.019	0.224 ± 0.031	0. 197 ± 0.016	0.4730
Total essential fatty acids	8.812 ± 1.194	8.794 ± 1.916	8.234 ± 0.709	0.4498
C20:4n-6 (Arachidonic a.)	1.814 ± 0.282	2.062 ± 0.259	1.969 ± 0.282	0.3428
C20:5n-3 (Eicosapentaenoic a.)	0.102 ± 0.027	0.113 ± 0.022	0.090 ± 0.017	0.1491
C22:5n-3 (Docosapentaenic a.)	0.127 ± 0.008	0.136 ± 0.007	0.137 ± 0.009	0.0580
C22:6n-3 (Docosahexaenic a.)	0.030 ± 0.002	0.035 ± 0.003	0.033 ± 0.005	0.4666
Total polyunsaturated FA	12.076 ± 1.158	11.226 ± 2.027	12.032 ± 1.398	0.4234
AI Atherogenicity index	0.555	0.506	0.499	
Σ PUFA/ Σ SFA	0.358	0.322	0.355	

P > 0.05; Not significant differences from control. SFA: saturated fatty acids, include C8:0, C10:0, C12:0, C14:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0. MUFA: monounsaturated fatty acids, include C16:1 n-7, C18:1 n-9c, C18:1 n-9t, C22:1. PUFA: polyunsaturated fatty acids, include C18:2 n-6, C18:3 n-3, C20:4 n-6, C20:5n-3, C22:5 n-6, C22:6n-3. AI: Atherogenicity index = (C12+4xC14+C16) / (Σ PUFA+C18:1+other MUFA) (Ulbricht and Southgate, 1991).

food industrial by-products, rich in fibre and protein, could be included in rabbits' diet with beneficial effect on carcass quality and can enhance the nutritional quality of rabbit meat with the focus on EAAs. These results are important, as generally only few reports are focused on rabbit meat's AA content, especially its changes and application. Therefore, it can expand the existing knowledge in this field.

Data of a blood serum analyses are shown in Table 8. The tested serum parameters were in the range of normal values defined in previous studies with rabbits, although there are physiological differences on reference ranges in rabbit serum (Burnett *et al*. 2006; Kociniewska *et al*. 2012). Blood glucose is an important source of energy for many cells, and this is a parameter of the balance between glucose source/availability and utilization. Higher glucose content was observed in feedstuffs as well as during dietary application on animal blood profile. The increase in glucose can be explained by transformation of lactic acid on pyruvate through the gluconeogenesis in the liver. On the contrary, reduced glucose level was spotted in the control group. Potentially, glucose accumulation was reduced by increased H^+ concentration due to higher organic acid values in the caecal content, which inhibited gluconeogenesis (Illes *et al*., 1977).

Indigestible fibre is important to stimulate gut motility that moves digesta and fluid into the caecum for fermentation and stimulates appetite and ingestion of caecotrophs. Fermentable fibre is important to provide a substrate for caecal microbiota, to provide optimal caecal pH and volatile fatty acid production and prevent proliferation of pathogenic bacteria in the caecum. Caecal bacteria are helpful in nutrient digestion due to the ability to produce bacterial enzyme, which hydrolyse plant cell wall components otherwise indigestible by the host's intestinal digestive enzymes. These indigestible elements, such as lignin, cellulose,

Table 7. The content of essential amino acids in *Longissimus thoracis and lumborum* muscles of rabbit tissue (g.kg^{-1})

Amino acids n = 5 / group	CG Control group	EG1 5 % hempseed cake	EG2 10 % hempseed cake	*P*-value
Arginine	11.511 ± 0.658	12.547 ± 0.344	12.220 ± 0.482	0.1680
Cystine	2.438 ± 0.119	2.396 ± 0.102	2.398 ± 0.085	0.1001
Phenylalanine	7.317 ± 0.406	8.103 ± 0.173	8.001 ± 0.290	0.8322
Histidine	9.530 ± 0.819	8.800 ± 0.272	8.634 ± 0.365	0.9422
Isoleucine	6.462 ± 1.341^{a}	8.373 ± 0.480^{b}	8.292 ± 0.570^{b}	0.0006
Leucine	15.183 ± 0.877^{a}	16.326 ± 0.337^{b}	15.933 ± 0.528^{b}	0.0005
Lysine	16.447 ± 1.054^{a}	17.974 ± 0.448^{b}	17.671 ± 0.679^{b}	0.0201
Methionine	6.359 ± 0.305	5.700 ± 0.178	5.762 ± 0.160	0.1200
Threonine	11.585 ± 0.660^{a}	12.375 ± 0.433^{b}	12.208 ± 0.426^{b}	0.0025
Valine	7.050 ± 1.403^{a}	9.051 ± 0.473^{b}	8.889 ± 0.737^{b}	0.0006
Σ EAA	93.882 ± 7.643^{a}	101.645 ± 3.240^{b}	100.009 ± 4.322^{b}	0.0282
Aspartic acid	23.335 ± 1.568^{a}	21.747 ± 0.438^{b}	21.616 ± 0.632^{b}	0.0003
Serine	9.742 ± 1.592^{a}	8.321 ± 0.285^{b}	8.086 ± 0.367^{b}	0.0002
Glutamic acid	33.998 ± 1.442	34.112 ± 0.671	34.087 ± 1.069	0.0869
Proline	8.468 ± 0.507	7.597 ± 0.307	7.774 ± 0.238	0.0849
Glycine	9.046 ± 0.358	8.967 ± 0.280	8.845 ± 0.283	0.0820
Alanine	11.695 ± 0.344	11.793 ± 0.277	11.532 ± 0.337	0.0698
Tyrozine	7.084 ± 0.374	7.788 ± 0.159	7.647 ± 0.246	0.1648
Σ NEAA	103.368 ± 6.185	100.325 ± 2.418	99.587 ± 3.172	0.1241
Sum of amino acids	198.250 ± 13.827	201.970 ± 5.658	199.596 ± 7.494	0.0931

Results are presented as mean ± SD; Mean values within the same row having different superscripts indicate significant difference for Tukey test $P > 0.05$. Σ EAA-Total essential amino acids; Σ NEAA-Total non essential amino acids; Tryptophan not determined; TAA-total amino acids.

Table 8. Effect of selected biochemical and haematological parameters in blood of rabbits

Parameter n = 5 / group (mean ± SD)	Day 0/1	CG Control group	EG1 with 5 % hempseed cake	EG2 with 10 % hempseed cake	*P*-value
Total proteins g/L	59.90 ± 1.19	62.19 ± 4.76	59.58 ± 0.59	58.95 ± 4.35	0.2962
Albumin g/L	42.84 ± 1.15	43.3 ± 2.04	39.8 ± 2.20	40.6 ± 1.43	0.3009
Urea mmol/L	4.33 ± 0.28	4.33 ± 0.39	5.18 ± 0.37	4.42 ± 0.18	0.4601
Triglyceride mmol/L	1.28 ± 0.28	0.91 ± 0.26	1.45 ± 0.43	0.92 ± 0.21	0.3729
Cholesterol mmol/L	1.86 ± 0.13^{a}	1.10 ± 0.11	1.58 ± 0.38^{b}	1.34 ± 0.09^{b}	0.0169
Glucose mmol/L	4.33 ± 0.23^{a}	8.68 ± 0.22	9.17 ± 0.57^{b}	9.56 ± 0.53^{b}	0.0051
ALT µkat/L	0.30 ± 0.20	0.64 ± 0.06	0.47 ± 0.56	0.45 ± 0.18	0.2965
AST µkat/L	0.76 ± 0.34	0.82 ± 0.47	0.92 ± 0.12	0.93 ± 0.16	0.1948
GGT µkat/L	0.16 ± 0.11	0.24 ± 0.06	0.39 ± 0.04	0.14 ± 0.02	0.3142
ALP µkat/L	1.77 ± 0.11	3.04 ± 0.86	1.50 ± 0.38	1.71 ± 0.80	0.2559
Calcium mmol/L	3.10 ± 0.12	3.60 ± 0.03	3.85 ± 0.06	3.92 ± 0.24	0.1823
Phosphorus mmol/L	1.93 ± 0.04	2.51 ± 0.04	1.99 ± 0.07	2.00 ± 0.05	0.3978

[a]Values within a row with diverse superscripts differ significantly at $P < 0.05$; Reference value: TP – total proteins: 28 – 100 g/L; albumin: 26 – 46 g/L; Urea: 2.1 – 8.4 mmol/L; GLu=glucose: 5.5 – 8.6 mmol/L; TG = triglycerides, up to 1.44 mmol/L; cholesterol: 0.28 – 2.1 mmol/L; urea: 2.1 – 8.4 mmol/L; ALT: 0.3 – 2.13 µkat/L; ALP = alkaline phosphatise: 1 – 2.7 µkat/L; AST = aspartate aminotransferase: 0.23 – 0.93 µkat/L; ALT = Alanine amino transferase: 0.25 – 0.95 µkat/L; GGT = gamma glutamyl transferase: 0.14 – 0.84 µkat/L; Ca: 3 – 4.2 mmol/L.

Table 9. The effect of hempseed oil cake on parameters in caecal content of rabbits

Parameter n = 5 / group	Unit	CG Control group	EG1 with 5 % hempseed oil cake	EG2 with 10 % hempseed oil cake	*P*-value
pH	-Log mol c	6.413 ± 0.410	5.670 ± 0.940	6.410 ± 0.288	0.1661
Acetic acid	mmol.100 ml^{-1}	5.697 ± 0.509[a]	12.143 ± 2.141[b]	17.693 ± 4.765[b]	0.00135
Propionic acid	mmol.100 ml^{-1}	0.399 ± 0.064[a]	0.486 ± 0.058[a]	0.761 ± 0.425[b]	0.04604
Isobutyric acid	mmol.100 ml^{-1}	0.014 ± 0.005[a]	0.005 ± 0.004[b]	0.003 ± 0.002[b]	0.00187
Butyric acid	mmol.100 ml^{-1}	0.732 ± 0.179[a]	2.898 ± 1.041[b]	3.614 ± 0.962[b]	0.00157
Iso valeric acid	mmol.100 ml^{-1}	0.177 ± 0.152	0.119 ± 0.047	0.272 ± 0.126	0.07452
Valeric acid	mmol.100 ml^{-1}	0.089 ± 0.013	0.110 ± 0.039	0.097 ± 0.017	0.29747
Caproic acid	mmol.100 ml^{-1}	0.011 ± 0.011[a]	0.088 ± 0.075[b]	0.104 ± 0.049[b]	0.00544
Lactic acid	g.100g^{-1}	0.024 ± 0.036[a]	0.016 ± 0.005[b]	0.013 ± 0.003[b]	0.02522
Total concentration of volatile fatty acids	mmol.100 g^{-1}	7.119 ± 0.495[a]	15.850 ± 2.930[b]	22.543 ± 5.839[b]	0.00211
N-NH_3	mmol.L^{-1}	21.243 ± 4.279[a]	15.192 ± 4.553[b]	12.693 ± 5.181[b]	0.00369

[a]Values within a row with diverse superscripts differ significantly at $P < 0.05$.

hemicellulose and pectin, are hydrolysed by bacterial enzymes into smaller compounds and fermented into end products, namely ammonia, volatile fatty acids (VFA), intermediary metabolites (succinic, formic and lactic acid) and gases (CO_2, CH_4 and H_2; Marounek *et al*., 2006; Combes et a., 2013). During fermentation processes, volatile fatty acids (VFA) are produced and continuously supply 30 – 50 % of adult rabbit's energy requirements. The data on volatile fatty acids (VFA) show that most intensive process was run in the caecum of rabbits in the experimental groups EG1 and EG2 (Table 9). The content of ammonia-N in the caecal samples had influence on pH value. These VFA are detected in a specific ratio in rabbits, with a predominance of acetate, followed by butyrate and propionate. The lactic acid and volatile fatty acid concentrations in caecum of the rabbits were significantly influenced by the treatment, without any statistical influence on pH values between groups.

CONCLUSION

According to present results, we can conclude that up to 10 % hempseed cake can be used in rabbit diet without any negative effect on livestock performance and health status of animals. Addition of hempseed oil cake into complete feed mixtures to rabbits did not negatively influence the physical parameters of meat and raised its energy value. The hempseed oil cake could be included into rabbit diet with beneficial effect on carcass quality and the nutritional quality of rabbit meat with the focus on essential amino acids. These results are important, as only a few reports focus on rabbit meat's AA content, and this study can expand the existing knowledge in this field. Fermentation processes in the caecum were stimulated by hempseed oil cake in complete feed mixtures for rabbits compared with values in the control group. Further experiments are required to confirm the promising beneficial effect of hempseed cake in rabbits.

ACKNOWLEDGEMENT

This publication was supported by the Operational Programme Integrated Infrastructure within the project: Sustainable smart farming systems taking into account the future challenges 313011W112, co-financed by the European Regional Development Fund. The authors would like to thank to Dr. Roman Mlynár, Dr. Tomáš Sládeček, Ms. Jana Zeleňáková, Mr. Ján Pecho and Mr. Peter Fridrich for their excellent technical assistance.

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All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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