https://doi.org/10.17221/82/2025-CJAS

Effect of dietary hop extracts and humic substances on the growth performance, carcass yield, blood biochemistry parameters, and meat quality of rabbits

Zuzana Lacková[1], František Zigo[1], Zuzana Farkašová[1], Jakub Ješík[2], Jana Záhumenská[3], Silvia Ondrašovičová[4], Andrej Récky[1], Henrieta Arpášová[5]*

[1]*Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic*
[2]*Clinic of Ruminants, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic*
[3]*Department of Hygiene, Technology and Health Food Safety, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic*
[4]*Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic*
[5]*Institute of Animal Husbandry, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic*

**Corresponding author: frantisek.zigo@uvlf.sk*

Citation: Lacková Z., Zigo F., Farkašová Z., Ješík J., Záhumenská J., Ondrašovičová S., Récky A., Arpášová H. (2025): Effect of dietary hop extracts and humic substances on the growth performance, carcass yield, blood biochemistry parameters, and meat quality of rabbits. Czech J. Anim. Sci., 71: 345–356.

Abstract: This study aimed to evaluate the effects of hop extract in the form of xanthohumol (XA) and humic substances (HS) supplementation in the rabbit diet on growth performance, carcass traits, blood biochemical parameters, and the qualitative and sensory properties of rabbit meat. Experimental material consisted of 60 Big Light Silver × Chinchilla Giant crossbred rabbits, randomly assigned to three dietary groups. Experimental groups, XA and HS, were fed pellets enriched with 1.0% XA or 1.0% HS, respectively, for 63 days during the fattening period. Control group (C), consisting of rabbits that received a basal diet without supplements. In the XA group, the higher final live weight and carcass weight were recorded at the end of the fattening period, along with increased protein content and collagen-free muscle protein (BEFFE) in the leg and loin muscles, compared to the control group. In contrast, the HS-supplemented group exhibited the lower final live weight and carcass weight, along with reduced fat content in the leg and loin muscles, as reflected in lower blood cholesterol levels. Additionally, elevated blood serum calcium and phosphorus levels were observed in the HS group. Higher values of colourimetric parameters L^* and h^* (lightness and hue angle) were recorded in the leg meat of the XA group compared to the control group. Moreover, the results demonstrated that supplementation with 1.0% XA was more effective in delaying lipid oxidation in meat compared to the control group on the 7th day of refrigerated storage. In conclusion, the primary benefit of supplementing rabbit diets with 1% xanthohumol is improved growth performance, positively influenced by increased protein content, reduced fat content, and enhanced oxidative stability of the meat.

Keywords: colour; muscle; organic matter; sensory; supplementation; weigh; xanthohumol

Supported by Project KEGA (No. 011UVLF-4/2024).

https://doi.org/10.17221/82/2025-CJAS

Meat is recognised as a key dietary source to meet human nutritional requirements, particularly for high-quality protein. However, protein quantity alone is not sufficient to define the nutritional value of a protein source; both the content and digestibility of essential amino acids (AA) must be considered (Dalle Zotte and Cullere 2024). From a nutritional standpoint, rabbit meat can be regarded as an ideal choice due to its unique composition. In addition to its high biological value protein content, it is an excellent source of minerals, particularly potassium, phosphorus, and selenium while being low in sodium and cholesterol and rich in B vitamins. Notably, rabbit meat is considered one of the richest dietary sources of vitamin B12 among all meat species (Dalle Zotte et al. 2020).

It is well-documented that the continuous use of antibiotics as growth promoters leads to their accumulation in animal tissues, and consumption of such products by humans may contribute to the development of antibiotic resistance. In recognition of this risk, the European Community banned the use of antibiotics as growth promoters in 2006 (Ondruska et al. 2012). Consequently, nutritionists have focused on alternative feed additives such as organic acids, antioxidants, and probiotics. Among these, humic substances (HS) are considered a promising substitute for antimicrobial agents due to their beneficial effects on production performance, immune function, and animal health. HS can bind various toxic compounds, forming insoluble complexes, which enhances their role as adsorbents and reduces the absorption of harmful endotoxins, an important factor in safeguarding both animal and human health. Furthermore, HS exhibits antibacterial, antiviral, and antimicrobial properties, thereby contributing to the sustainability and efficiency of animal production systems (Ondruska et al. 2012).

Xanthohumol (XA, 3'-[3,3-dimethylallyl]-2',4',4-trihydroxy-6'-methoxychalcone) is the most abundant prenylated flavonoid found in hops. XA has attracted significant scientific interest due to its wide range of biological effects. Laboratory studies have demonstrated the potential benefits of xanthohumol as a dietary supplement (Miranda et al. 2016). XA exhibits multiple biological activities, including antioxidant, anti-inflammatory, anticarcinogenic, antimicrobial, and chemoprotective effects. In addition, it inhibits bone calcium resorption, suppresses lipoxygenase enzymatic activity, possesses strong phytoestrogenic properties, supports metabolic homeostasis, and stimulates appetite.

However, many of the existing studies on XA and HS have primarily focused on their effects on animal health, with less emphasis on their economic implications and production-related outcomes. Due to their unique composition and beneficial properties, both XA and HS can exert complex physiological effects, enhancing animals' disease resistance and promoting improved performance (Ondruska et al. 2012; Miranda et al. 2016). Therefore, the present study aimed to evaluate the effects of XA and HS supplementation in pelleted feed on growth performance, carcass traits, and the qualitative and sensory characteristics of rabbit meat.

MATERIAL AND METHODS

The animal study protocol was approved by the Ethics Commission of the University of Veterinary Medicine and Pharmacy in Kosice (Protocol Code EKVP/2024-2 and the date of approval is 28 August 2024).

Housing of rabbits and feed supplementation

The experimental animals consisted of 60 crossbred silver and chinchilla rabbits (28 females, 32 males). Rabbits were housed under the same conditions in an air-conditioned hall. The young animals were raised with their mothers until the 35th day of age and kept in pens with deep bedding. At the weaning and during the experiment, 16 h of light and 8 h of darkness were provided. The average temperature in the hall was 22 ± 2 °C, and the relative humidity was 70 ± 5%. Before the weaning, all rabbits were provided commercial feed *ad libitum*. After weaning, the growing rabbits were housed in two-tier metal mesh cages designed for commercial rabbit rearing, with 4 animals of the same sex in each cage. The cage area in which the rabbits were kept was in accordance with the conditions for keeping fur-bearing animals (The Ministry of Agriculture 1998). The rabbits were included in a veterinary prophylaxis program for this group of animals (vaccinations against: haemorrhagic disease – Pestorin – 1 ml

https://doi.org/10.17221/82/2025-CJAS

intramuscularly, *myxomatosis* – Myxoren – 1 ml subcutaneously). After weaning, the rabbits were housed in two-tier metal mesh cages designed for commercial rabbit rearing, with four animals of the same sex in each cage.

At the beginning of the experiment, the animals were divided into three groups (n = 20/group) with five replicates in each group: C (control group, standard diet without additives), group XA (received standard feed + 1.0% INHE Xanthohumol, P.R. China), group HS (received standard feed + 1% Humac Natur AFM, Slovakia – humic acids). XA and HS were incorporated during the production of pellets when mixing the individual components.

The control group was fed a basal diet without any additives for growing rabbits, according to the recommendations given in the international feeding standards containing alfalfa flakes, barley, wheat bran, corn meal, calcium carbonate, extracted rapeseed meal, vitamin-mineral premix, NaCl and nutritional supplements (Table 1).

During the entire period of fattening (from the 35th to the 98th day of age), group XA was fed basal diet pellets with 1% of xanthohumol (INHE Xanthohumol; Shaanxi Inhealth Nature Industry Co., Ltd., P.R. China; 50% share of XA and 10% of hop flavonoids) and group XS was fed basal diet pellets with 1% addition of humic substances (Humac® Natur AFM; Košice, Slovak Republic; minimum 65% share of natural humic acids, maximum 5% fulvic acids, Ca 42 278 mg/kg; Na 7 129 mg/kg; K 903 mg/kg; Br 54.7 mg/kg; Mg 5 111 mg/kg; Fe 19 046 mg/kg; Cu 15 mg/kg; Zn 37 mg/kg; Mn 142 mg/kg; Co 1.24 mg/kg; Se 1.67 mg/kg; V 42.1 mg/kg; Mo 2.7 mg/kg in dry matter, crude fibre 24.3 g/kg, moisture maximum 21%, pH 5.8, particle size 0–200 μm).

Growth performance and carcass yield of rabbits

Throughout the study period (63 days), the animals were pelleted fed *ad libitum* and had free access to drinking water. After the experimental growing period, 10 rabbits (5 males and 5 females) were randomly selected from each group. The weight of the rabbits was determined by weighing at ages 35 (start of XA and HS supplementation) and 98 days (end of XA and HS supplementation). Carcass weight was recorded after slaughter (decapitation, removal of skin and distal parts of the limbs, and evisceration). At the end of the experiment, after 9 weeks of XA and HS administration, muscle samples were collected from the rabbits for further investigation.

Slaughter analysis was conducted during post-slaughter processing. Data included: the body weight of the rabbit after 16 h of fasting and the weight of the left leg with bone.

Based on the collected data, the dressing-out percentage (DPC) was calculated according to the method of Gugolek et al. (2008) using the following formula:

$$\text{DPC (\%)} = \frac{\text{carcass weight after slaughter without the head and offal}}{\text{body weight of the animal before slaughter}} \times 100\% \quad (1)$$

Table 1. Analytical composition of the feed mixture

Proportion of components in the feed mixture (%)		Analytical composition of mixture (g in 1 kg feed mixture)		Nutritional additives (in 1 kg feed mixture)	
Alfalfa flakes	42.0	Dry matter	893	Vitamin A	min. 9 800 I.U.
Barley	11.2	Nitrogenous substances	215	Vitamin D3	min. 1 870 I.U.
Wheat bran	30.9	Fibre	202	Iron	74 mg
Corn meal	6.5	Starch	145	Iodine	1.1 mg
Extracted rapeseed meal	7.2	Ash	71	Copper	14.5 mg
Vitamin-mineral premix	1.5	Fat	51	Manganese	108 mg
Calcium carbonate	0.4	Calcium	9	Zinc	79 mg
NaCl	0.3	Sodium	2.3	Selenium	0.28 g
–		Phosphorus	6.8	Methionine	0.38 g
–		–		Lysine	0.85 g

https://doi.org/10.17221/82/2025-CJAS

The separation of the hind limbs was performed on the sides of the *os coxae* and the posterior part of *m. iliopsoas*: *m. psoas major* and *m. iliacus* (*pars lateralis* and *pars medialis*) (Blasco and Ouhayuon 2010).

Physicochemical analysis of loin and leg meat samples

The loin and leg meat samples were frozen at –21 °C 24 h after slaughter until the meat quality analysis. For the meat quality analysis, eight rabbits from each experimental group were randomly selected. In these samples, ash, water, fat and protein content, collagen, meat protein content without connective tissue content (BEFFE), NaCl, pH, malondialdehyde (MDA) content, and sensory characteristics were evaluated.

The proportion of individual chemical components in the homogenised meat samples loin and leg was determined using a TANGO FT-NIR spectrophotometer (Bruker, Germany) with a resolution of 16 cm^{-1}, a measurement time of 64 scans, and the measurement of one sample was repeated three times.

The pH of the homogenised meat samples was analysed using a digital inoLab pH 340i meter (Wissenschaftlich-Technische Werkstatten, Germany).

Determination of the biochemical parameters

On the last day of fattening, blood samples (12 ml) were collected for evaluation of biochemical parameters, and rabbits were killed after stunning with a spring-loaded slaughter gun (Agrofortel s.r.o., Prague, Czech Republic) followed by cutting the *jugular vein* and exsanguination (European Commission 2019). To evaluate biochemical parameters, 12 ml of blood was collected from eight randomly selected rabbits from each group before slaughter. Enzymatic activities of ALT (alanine transaminase), AST (aspartate transaminase), GGT (glutathione hydrolase), ALP (alkaline phosphatase), CK (creatine kinase) and LDH (lactate dehydrogenase) as well as levels of glucose (GLU), uric acid (UA), creatinine (CRE), total cholesterol level (TC), triglycerides (TAG), total protein (TP) and calcium (Ca), phosphorus (P), and magnesium (Mg) were determined by the use of automatic analyzator Unicel DxC 880i (Beckman Coulter, USA) using the commercial kits Labtest according to the methods described by Duncan et al. (1994).

Determining the decomposition changes of lipids

Malondialdehyde (MDA), as a main product of secondary lipid oxidation, was determined in loin and thigh muscle by a spectrophotometric method as a complex with 2-thiobarbituric acid (TBA) at a wavelength of 532 nm by the spectrophotometer (Helios γ; Thermo Spectronic, Cambridge, United Kingdom). Leg and loin meat was frozen at –21 °C 24 h after slaughter and stored for 1 month. The frozen meat was chopped in a frozen meat chopper and ground in a grinder (Ø 4.5 mm). The samples were packed in bags and stored in a refrigerator at 4 °C for 7 days. The results were expressed as mg MDA/kg per sample as the average value of three measurements per sample (Marcincak et al. 2006).

Sensory analysis

The experimental leg muscle samples of rabbits were subjected to sensory analysis, which was carried out in the Specialised Sensory Laboratory at the Institute for the Education of Veterinary Doctors in Košice, Slovakia established according to the general plan for the arrangement of sensory workplaces (ISO 8589:2007 Sensory analysis – General guidance for the design of test rooms). The sensory panel was composed of five trained evaluators aged between 28 and 60 years, who had practical experience in evaluating meat. For assessing experimental samples of rabbit leg muscle, the protocol according to Lawless and Heymann (2010) was followed. The procedure for the preparation of leg meat samples included cooking the meat in boiled water (until a temperature of 80 °C was achieved in the core of the meat) and portioning the cooked meat samples into square cubes (weighing approximately 25 g). The samples were coded with random three-digit numbers and a dish with water for mouth rinsing was provided to the evaluators. The protocol utilises a 9-point hedonic scale to as-

https://doi.org/10.17221/82/2025-CJAS

sess sensory attributes of the meat samples, including colour, aroma, juiciness, tenderness, and overall acceptability. The scale ranges from 1 to 9, where: 1 – extremely undesirable, 2 – very undesirable, 3 – moderately undesirable, 4 – slightly undesirable, 5 – neutral, 6 – slightly desirable, 7 – moderately desirable, 8 – very desirable, 9 – extremely desirable. This structured approach provides a standardised method for quantifying consumer perception and preference regarding key sensory characteristics. The intensity of loin meat juiciness and brittleness attributes was scored on a structured 10 cm line scale anchored as "not perceptible" – at the low end and "intense" at the high end.

Colour measurement

Twenty-four hours after slaughter, the colour of the rabbit meat muscle (the *longissimus lumborum* and the *biceps femoris)* were measured. The colourimetric parameters of the experimental samples were measured using a Chroma meter CR-410 (measuring area Ø 50 mm, illumination D65, standard viewing angle 2°; Konica Minolta, Sensing, Inc., Japan). The colourimetric parameters were processed using the Colour Data Software CM-S100w SpectraMagic™ NX (Konica Minolta Sensing Inc., Osaka, Japan). The colourimetric parameters of the analysed samples were expressed in the CIELab colour space.

The L^* value represents lightness, a^* represents redness [chromaticity from green ($-a$) to red ($+a$)], and b^* represents yellowness [chromaticity from blue ($-b$) to yellow ($+b$)]. The hue angle (h^*) and chroma (C^*) parameters were expressed using a combination of a^* and b^* according to McLaren (1976). Measurements were performed in a laboratory room at 20 ± 2 °C. Before the analysis, the device was calibrated using the attached accessories, the so-called calibration plate (CR-A43, Konica Minolta), which calibrates the device to a white colour standard. Colour parameters were expressed in the Lab and LCh colourimetric spaces according to the International Commission on Illumination (CIE) (2007) and McLaren (1976).

Statistical analyses

The effect of dietary supplements on growth performance, carcass traits, and biochemical parameters was analysed using a one-way analysis of variance (ANOVA) with Tukey's post-hoc test. This part of the analysis was performed using the R-statistics software (R Development Core Team 2021). The effect of dietary supplements on meat colour was examined using the Kruskal-Wallis test (Kruskal and Wallis 1952) with the Dunn post-hoc test (Dunn 1961). Data are presented as means ± standard error of the means (SEM).

RESULTS

Performance, carcass parameters and physicochemical properties of meat

The body weight, total weight gain, and average daily weight gain of rabbits at the end of the fattening period are presented in Table 2. A significantly higher live weight ($P < 0.05$) was observed in the XA experimental group supplemented with 1.0% xanthohumol compared to the control group. Similarly,

Table 2. Comparison of the effects of dietary supplementation with XA and HS on average daily weight gain

Dietary group	Weaning weight (g)	Slaughter weight (g)	Body weight gain during monitoring period (g)	Average daily gain (g)
XA	492	3 375[a]	2 783[a]	44.2[a]
HS	482	2 861[b]	2 375[b]	37.7[b]
C	486	3 118[a]	2 499[b]	39.7[b]
SEM	41.8	129	168	3.1
P-value	0.652	0.045	0.030	0.035

[a,b]Values in column with different superscripts letter are significantly different; Tukey's test, $P \leq 0.05$

C = control group, rabbits fed with complete feed mixture; HS = a group received basal feed + 1.0% humic substances; SEM = standard error of the mean; XA = a group received basal feed + 1.0% xanthohumol

https://doi.org/10.17221/82/2025-CJAS

a statistically significant increase ($P < 0.05$) in total body weight gain and average daily weight gain was recorded in the same group (XA) over the 63-day monitoring period. In contrast, the HS group had significantly lower slaughter weight ($P < 0.05$).

XA supplementation (1%) increased carcass and leg weight ($P < 0.05$) compared to the control group. In the HS group, both monitored parameters (live and carcass weight) were lower ($P < 0.05$) than those in the control group. In the case of leg with bone weight, the values obtained within the experimental group HS were slightly lower than those of the control group, but these differences were not statistically significant ($P > 0.05$) (Table 3).

The effect of XA and HS supplementation in rabbits on the composition of meat from the loin and leg is shown in Table 4. The supplementation of XA to the feed significantly affected the individual parameters of loin and leg meat. In loin and leg meat, protein and BEFFE content increased ($P < 0.05$) compared to control. In addition, leg meat showed a lower content of fat ($P < 0.05$) in the

Table 3. Comparison of the effects of dietary supplementation with XA and HS on live weight, carcass weight, and leg weight with bone at 98 days of age

Dietary group	Carcass weight (CW)	DPC	Weight of leg with bone	
	(g)	(%)	(g)	(% from CW)
XA	1 783[a]	52.8	290[a]	16.3
HS	1 444[c]	50.5	230[b]	15.9
C	1 602[b]	51.4	240[b]	15.0
SEM	73.2	–	23.0	–
P-value	0.035	0.693	0.050	0.614

[a–c]Values in column with different superscripts letter are significantly different; Tukey's test, $P \leq 0.05$

C = control group, rabbits fed with complete feed mixture; DPC = dressing out percentage; HS = a group received basal feed + 1.0% humic substances; SEM = standard error of the mean; XA = a group received basal feed + 1.0% xanthohumol

Table 4. Physicochemical analysis of the loin and leg meat as affected by supplementation with XA and HS at 98 days of age

Samples	Parameters	XA	HS	Control	SEM	*P*-value
Loin meat samples	water (g/100 g)	74.3	74.2	75.3	0.34	0.478
	fat (g/100 g)	1.4	1.7	1.6	0.07	0.546
	ash (g/100 g)	1.71	1.65	1.58	0.13	0.772
	protein (g/100 g)	22.6[a]	22.4[ab]	21.6[b]	0.27	0.045
	collagen (g/100 g)	1.4	1.6	1.5	0.13	0.725
	BEFFE (g/100 g)	21.2[a]	20.8[ab]	20.1[a]	0.11	0.037
	pH	5.57	5.65	5.7	0.08	0.640
Leg meat samples	water (g/100 g)	74.5	75.1	75.1	0.25	0.580
	fat (g/100 g)	1.8[a]	2.2[ab]	2.4[a]	0.14	0.047
	ash (g/100 g)	1.70	1.56	1.67	0.12	0.381
	protein (g/100 g)	21.9[a]	21.1[ab]	20.8[b]	0.22	0.042
	collagen (g/100 g)	1.62	1.75	1.71	0.08	0.735
	BEFFE (g/100 g)	20.28[a]	19.45[ab]	19.1[b]	0.32	0.026
	pH	5.61	5.54	5.65	0.04	0.684

[a,b]Values in rows with different superscripts letter are significantly different; Tukey's test, $P \leq 0.05$

BEFFE = collagen-free muscle protein; C = control group, rabbits fed with complete feed mixture; HS = a group received basal feed + 1.0% humic substances; SEM = standard error of the mean; XA = a group received basal feed + 1.0% xanthohumol

https://doi.org/10.17221/82/2025-CJAS

Table 5. Results of biochemical blood analysis as affected by supplementation with XA and HS at 98 days of age

Parameters	Reference range*	XA	HS	C	SEM	*P*-value
Glucose (mmol/l)	4.16–8.10	6.85	7.02	7.04	0.26	1.000
Urea (mmol/l)	2.10–8.40	4.35^{b}	4.05^{b}	4.93^{a}	0.36	0.014
Creatinine (µmol/l)	44.2–221.0	98.2	96.0	99.2	6.97	0.983
Total proteins (g/l)	54.0–75.0	62.3^{a}	60.4^{b}	57.1^{b}	2.25	0.017
Albumin (g/l)	27.0–50.0	45.7^{a}	43.2^{ab}	40.7^{b}	1.26	0.014
AST (µkat/l)	0.9–2.17	0.71	0.72	0.60	0.08	0.808
ALT (µkat/l)	0.75–1.33	2.17	1.98	1.87	0.24	0.959
GGT (µkat/l)	0–0.12	0.20	0.17	0.18	0.02	0.312
ALP (µkat/l)	0.20–1.60	2.43	2.32	2.22	0.32	0.918
Cholesterol (mmol/l)	0.55–4.44	1.35^{a}	1.22^{a}	1.65^{b}	0.17	0.044
TAG (mmol/l)	–	1.30	1.16	1.48	0.20	0.347
CK (µkat/l)	0–15.97	24.1^{a}	28.5^{b}	31.2^{b}	4.28	0.048
LDH (µkat/l)	–	3.07^{a}	4.74^{b}	4.56^{b}	0.68	0.044
Ca (mmol/l)	2.70–3.50	3.61^{a}	4.18^{b}	3.74^{a}	0.19	0.034
P (mmol/l)	1.30–2.10	2.40^{ab}	2.50^{a}	2.30^{b}	0.11	0.045
Mg (mmol/l)	–	1.20	1.26	1.21	0.05	0.478

*According to Fielder (2022); ^{a,b}Values in rows with different superscripts letter are significantly different; Tukey's test, $P \leq 0.05$
ALP = alkaline phosphatase; ALT = alanine transaminase); AST = aspartate transaminase; C = control group, rabbits fed with complete feed mixture; CK = creatine kinase; GGT = glutathione hydrolase; HS = a group received basal feed + 1.0% humic substances; LDH = lactate dehydrogenase; SEM = standard error of the mean; TAG = triacylglycerols; XA = a group received basal feed + 1.0% xanthohumol

XA than in the control. On the other hand, a concentration of 1.0% HS in the feed did not cause changes in the content of fat, ash, protein, and BEFFE in the loin and leg meat compared with the control and XA groups. The water and pH of meat from both supplemented groups were not affected by the addition of XA and HS to the feed.

By evaluating biochemical parameters from the blood serum of rabbits, both supplemented groups had lower cholesterol than control ($P < 0.05$). Higher albumin levels were recorded in the XA group than in the control group. By comparing the enzymatic activity of CK and LDH, lower values ($P < 0.05$) were recorded in the blood serum of rabbits supplemented with XA than in the control and HS groups. The evaluation of the mineral profile showed increased values of Ca and P in the HS group compared with the control and XA groups ($P < 0.05$) (Table 5).

Assessment of oxidative stability in meat

The results of malondialdehyde determination in the meat are shown in Figure 1. The oxidative stability of the meat was favorably affected in the XA group, as TBA expressed as MDA concentration in kg loin and leg muscle was found to be lower ($P < 0.05$) on the 7th day during cold storage compared to the control group. On the first day after slaughter, there were no significant changes in fat oxidation in the meat.

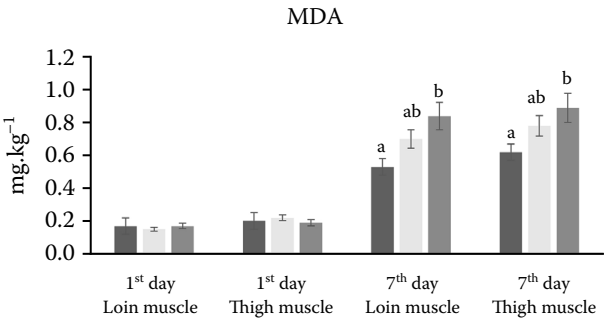

Figure 1. Dietary effects of XA and HS on the oxidative stability of rabbit meat

^{a,b}Values in rows with different superscripts letter are significantly different; Tukey's test, $P \leq 0.05$
C = control group, rabbits fed with complete feed mixture; HS = a group received basal feed + 1.0% humic substances; XA = a group received basal feed + 1.0% xanthohumol

https://doi.org/10.17221/82/2025-CJAS

Sensory evaluation and colourimetric analysis of meat

Table 6 summarises the colour values of the *longissimus lumborum* and *biceps femoris* muscle from the control and supplemented groups 24 h post-slaughter. XA supplementation in the rabbit diet increased ($P < 0.05$) the L^* value in both measured muscles and a^* colourimetric parameter in the *biceps femoris* of the same group. These two values influenced the better visual quality of the evaluated samples.

We noted a slight decrease ($P > 0.05$) in colourimetric redness (C^*) values in leg meat in the HS experimental group compared with in those the control and XA groups. For the other colour components, no statistically significant differences were found in either the middle part or hind part muscles. The results of the sensory properties are presented in Table 7. The total sensory evaluation

Table 6. Instrumental colourimetric analysis of muscles – *longissimus lumborum* and *biceps femoris*

Parameters	XA	HS	Control	SEM	*P*-value
Longissimus lumborum					
L^*	65.9[a]	63.5[ab]	62.2[b]	0.13	0.046
a^*	11.9	11.4	11.3	0.03	0.862
b^*	9.9	10.2	10.7	0.04	0.822
C^*	15.5	15.1	15.5	0.04	0.920
h^*	43.6	43.0	42.5	0.17	0.645
X	34.1	34.4	35.7	0.19	0.438
Y	35.5	33.7	34.2	0.25	0.048
Z	28.0	28.6	29.3	0.16	0.244
Biceps femoris					
L^*	64.7[a]	62.9[b]	62.63[b]	0.28	0.038
a^*	12.0[a]	10.2[b]	11.72[a]	0.18	0.046
b^*	8.06	7.89	9.17	0.15	0.095
C^*	15.4	15.0	15.1	0.27	0.247
h^*	34.5	34.3	33.8	0.29	0.425
X	30.6	32.7	32.1	0.14	0.559
Y	29.2	31.5	30.0	0.13	0.980
Z	26.5	27.1	26.4	0.10	0.522

[a,b]Values in rows with different superscripts letter are significantly different; Tukey's test, $P \leq 0.05$

X, Y, Z – tristimulus values of test stimulans calculated using the colour, Y is the luminance, Z is quasi-equal to blue (of CIE RGB), and X is a mix of the three CIE RGB curves chosen to be nonnegative

C = control group, rabbits fed with complete feed mixture; HS = a group received basal feed + 1.0% humic substances; SEM = standard error of the mean; XA = a group received basal feed + 1.0% xanthohumol

Table 7. Sensory characteristics of leg meat at 98 days of age

Sensory variables	XA	HS	Control	SEM	*P*-value
Appearance	7.70	7.60	7.70	0.63	0.986
Smell	7.70	7.40	7.40	1.19	1.000
Consistency	7.30	7.10	7.30	1.13	0.980
Taste	8.00	8.00	8.00	1.10	1.000
Overall acceptability	8.00	7.80	7.90	0.94	0.996

C = control group, rabbits fed with complete feed mixture; HS = a group received basal feed + 1.0% humic substances; SEM = standard error of the mean; XA = a group received basal feed + 1.0% xanthohumol

https://doi.org/10.17221/82/2025-CJAS

of leg meat included appearance, aroma, consistency, taste, and overall acceptability, which were not affected by dietary supplementation with XA or HS.

DISCUSSION

Using organic and botanical preparations in animal feed as dietary supplements can effectively regulate animal metabolism and influence growth, meat quality, health, and welfare (Zawadzki et al. 2017). The present study investigated the dietary effects of xanthohumol (XA) and humic substances (HS) on growth performance, carcass parameters, and the physicochemical properties of meat in crossbred rabbits. XA, a potent natural antioxidant, helps protect the intestinal mucosa from oxidative damage and pathogens. Additionally, by reducing intestinal peristaltic activity during digestive disturbances, XA may enhance nutrient absorption (Sbardella et al. 2016).

The results of the present study confirm that improved feed intake and nutrient absorption increased body weight at the end of fattening in the XA group supplemented with 1.0% xanthohumol. In addition to the higher final body weight, XA supplementation also positively influenced carcass weight, with increased leg and loin muscle weights compared to the control group. In rabbits, overall live weight is largely determined by the amount of muscle and fat accumulated during the 63-day feeding period. According to Xiccato (1999), the protein content in rabbit meat typically ranges from 18.6% to 22%. Variations in protein levels depend on factors such as breed, age, feed composition, carcass part, and slaughter preparation. In the present study, the protein content in the leg meat of rabbits fed a diet supplemented with 1.0% XA exceeded previously reported levels. XA likely influences the absorption and subsequent distribution of proteins and fats in the body, altering the meat composition.

In the XA-supplemented group of the present study, increased protein and BEFFE (collagen-free muscle protein) content were observed compared to both the control and HS groups, which likely contributed to the higher final body weight at the end of the fattening period. Conversely, a decrease in intramuscular fat content was noted in both the XA and HS groups. This reduction may be attributed to enhanced fat metabolism resulting from the antioxidant and detoxifying effects of XA and HS on liver function. Additionally, the increased fat metabolism was reflected in lower blood cholesterol levels observed in both dietary supplemented groups compared to the control group in the present study. Conversely, some studies have shown that supplementation with XA or HS can reduce body fat and blood cholesterol, but this is sometimes accompanied by reduced body weight or lower weight gains (Miranda et al. 2016). In the study by Ondruska et al. (2012), rabbits fed diets supplemented with HS or a combination of HS and probiotics demonstrated higher live weight in the final phase of fattening (77 days) compared to the control group fed a diet supplemented with HS alone.

The efficacy of humic substances (HS) in enhancing growth performance is highly dependent on their concentration. Previous studies have identified an optimal inclusion range of 0.5% to 1.0%, with efficacy influenced by the purity and composition of the HS preparation, ideally containing a minimum of 40% active HS compounds and subjected to appropriate pre-treatment to ensure bioavailability (Marcincak et al. 2023). Beneficial effects on carcass traits have been consistently observed within a supplementation range of 0.25% to 1.0%. For instance, Jaduttova et al. (2019) reported a significant increase in breast and thigh muscle yields in broilers receiving 1.0% HS, while Hudak et al. (2021) demonstrated that a 0.75% dietary inclusion of HS positively influenced overall carcass weight.

These findings suggest that both the concentration and quality of HS are critical factors in optimizing zootechnical outcomes. Oxidation and post-mortem maturation are key determinants of meat quality, profoundly influencing the physicochemical and sensory attributes of muscle foods. Following slaughter, oxidative processes persist and are a primary cause of quality degradation during meat processing and storage. During the early post-mortem period, muscle fibres undergo complex biochemical transformations as muscle tissue transitions to meat. This conversion is energy-intensive, driven by the catabolism of muscle glycogen. High-energy phosphate compounds facilitate the enzymatic breakdown of glycogen into lactic acid, resulting in a progressive decline in muscle pH. This pH reduction plays a pivotal role in modulating a range of chemical and biochemical path-

https://doi.org/10.17221/82/2025-CJAS

ways that affect meat texture, colour, water-holding capacity, and microbial stability (Simpson 2012). In the present study, supplementation with XA and HS in the feed did not affect the pH values of the loin and thigh muscles. Similar findings were reported by Rezar et al. (2020), who observed no effect of hops or HS supplementation on pectoral muscle pH 24 h post-slaughter.

Preventing lipid oxidation during meat ripening and storage is crucial for maintaining meat quality and ensuring consumer health (Huang and Ahn 2019). To the best of our knowledge, no comprehensive studies have been conducted on the use of hops as a dietary source of antioxidants, nor on their impact on oxidative stress in vivo or on the oxidative stability of rabbit meat. Lipid oxidation leads to the formation of off-flavours and rancidity, whereas protein oxidation can detrimentally impact key quality parameters, including tenderness, juiciness, and water-holding capacity. Therefore, strategies aimed at enhancing redox stability are essential not only improving sensory and nutritional attributes of meat but also for prolonging shelf life and reducing spoilage. Hops are a rich source of polyphenolic antioxidants, which was also confirmed in the present study by their demonstrated free radical scavenging activity. The oxidative stability of meat in the XA-supplemented group was significantly improved on the 7th day of cold storage compared to the control group (Figure 1). Similar results were reported by Hanczakowska et al. (2017), who supplemented pigs' diets with herbal extracts from hops (*Humulus lupulus* L.) at two different doses (500 or 1 000 mg/kg feed). Both groups demonstrated improved oxidative and colour stability of the meat. In another study on Merino lambs, supplementation with hop cones at two different doses (1.5 and 3.0 g hop cones/kg of pelleted total mixed ration) significantly reduced differences in meat chemical composition, colour, and texture ($P < 0.05$), despite no observed differences in meat antioxidant status ($P > 0.10$) (Blanco et al. 2018).

The colour of muscle tissue is one of the key attributes influencing consumer perception of meat and is determined by the concentration and oxidation state of heme pigments. An increase in meat pH reduces its lightness (L^* value), whereas a lower pH increases lightness. The L^* value reflects the visual lightness of meat colour, the a^* value relates to the oxidation state of pigments, intramuscular fat, and myoglobin content, while the b^* value is influenced by the redox state and intramuscular fat content (Rubayet Bostami et al. 2018).

In the present study, the reduced fat content in loin and leg meat from the XA-supplemented group improved visual appearance (L^* value). Additionally, a redder colour in the a^* value was recorded in the leg meat samples of the same group. Comparable findings were reported by Rezar et al. (2020), who observed better lightness (L^*) in chicken meat supplemented with 0.9 g hops/kg of feed on the first day of measurement, with no significant changes in redness (a^*).

However, a decrease in meat yellowness (b^*) was observed in chickens supplemented with both 0.9 g and 3.6 g hops/kg of diet. It is important to note that direct comparison of colourimetric parameters across different meat types are complicated by intrinsic differences in myoglobin content and muscle composition. Previous research by Liu et al. (2009) reported no significant effect of hop supplementation on meat colour in both poultry and rabbits models, suggesting that the effect of hops on pigmentation may by minimal or species-specific.

On the other hand, in the present study no sensory changes were observed in the meat of the supplemented rabbits. For rabbit meat, appearance and texture are key attributes influencing consumer preference; therefore, proper storage conditions are essential to maintain product acceptability. Quantitative descriptive analysis conducted by a panel of trained assessors is an effective method for objectively evaluating and comparing the sensory properties of food products (Lawless and Heymann 2010). In the study by Arino et al. (2007) it was demonstrated that genetic line significantly influences specific sensory attributes associated with rabbit meat tenderness. Similarly, Semjon et al. (2020) reported that dietary supplementation with humic substances positively affected the sensory characteristics of poultry meat. The inclusion of 1.0% HS to the diet broiler chickens led to improved sensory evaluation scores, particularly in terms of meat flavour, indicating a beneficial impact on overall meat quality perception.

CONCLUSION

Applying XA and HS to the feed influenced growth, blood profile, and meat quality in rabbits. Adding 1.0% XA to the diet caused an increase in fi-

https://doi.org/10.17221/82/2025-CJAS

nal live weight and carcass weight, with an increase in average daily gain compared to the control group. In contrast, reduced final weight was recorded in the group treated with 1.0% HS after 63 days of fattening. The reduced final live weight of rabbits in the HS group was also reflected in the reduced fat content in the meat and cholesterol in the blood. In addition, in the present study confirmed a lower fat content in the thigh meat of both groups of rabbits fed diets supplemented with XA or HS. The decreased fat in the meat in both dietary supplemented groups may be due to increased fat metabolism due to the beneficial detoxifying effects of XA and HS on the liver. Due to increased fat metabolism, lower blood cholesterol levels were observed in both dietary supplemented groups than in the control group. In addition, increased values of protein and BEFFE in the loin and thigh meat were confirmed in rabbits fed a diet supplemented with 1.0% XA, which resulted in a higher final live weight. The increased weight and average gain are probably related to the increased appetite for eating the pellets, which were enriched by adding XA. Therefore, its administration positively affects overall metabolism and nutrient utilization. However, the supplementation of XA feed enhances meat oxidative stability, making it more effective than humic substances.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Arino B, Hernndez P, Pla M, Blasco A. Comparison between rabbit lines for sensory meat quality. Meat Sci. 2007 Mar;75(3):494-8.

Blanco C, Bodas R, Moran L, Mateo J, Andres S, Giraldez FJ. Effect of hop (Humulus lupulus L.) inclusion in the diet for fattening lambs on animal performance, ruminal characteristics and meat quality. Food Res Int. 2018 Jun; 108:42-7.

Blasco A, Ouhayoun J. Harmonization of criteria and terminology in rabbit meat research. Revised proposal. World Rabbit Sci. 2010 Jun;4(2):93-9.

Dalle Zotte A, Cullere M, Gleeson EY, Cossu ME. Animal fat and vitamin E in rabbit diets: Total tract apparent digestibility, growth performance, carcass and meat quality traits. Czech J Anim Sci. 2020 Oct;65(10):380-8.

Dalle Zotte A, Cullere M. Rabbit and quail: Little known but valuable meat sources. Czech J Anim Sci. 2024 Feb; 69(2):39-47.

Duncan JR, Prasse KW, Mahaffey EA. Veterinary laboratory medicine: Clinical pathology. 3rd ed. Ames, IA, USA: Iowa State University Press; 1994. 300 p.

Dunn OJ. Multiple comparisons among means. J Am Stat Assoc. 1961 Mar;56(293):54-64.

European Commission. How to stun/kill rabbits on the farm [Internet]. Publications Office, Directorate-General for Health and Food Safety. 2019 [cited 2022 Jan 1]. Available from: https://data.europa.eu/doi/10.2875/05415.

Fielder SE. Serum biochemical analysis reference ranges. MSD veterinary manual [Internet]. 2022 [cited 2024 Nov 20]. Available from: https://www.msdvetmanual.com/reference-values-and-conversion-tables/reference-guides/serum-biochemical-analysis-reference-ranges?mredirectid=3512&mredirectid=521.

Gugolek A, Lorek MO, Kowalska D, Janiszewski P, Daszkiewicz T. Wplyw systemu utrzymania i zywienia na wyniki produkcyjne i stopien zarazenia kokcydiami krolikow bialych nowozelandzkich [Effect of housing and feeding conditions on the production results and coccidiosis infection rates of New Zealand White rabbits]. Rocz Nauk Pol Tow Zootech. 2008 Jan;4(2):39-44. Polish.

Hanczakowska E, Malgorzata S, Grela ER. Effect of dietary supplement of herbal extract from hop (Humulus lupulus) on pig performance and meat quality. Czech J Anim Sci. 2017 Jun;62(7):287-95.

Huang X, Ahn DU. Lipid oxidation and its implications to meat quality and human health. Food Sci Biotechnol. 2019 Jun 7;28(5):1275-85.

Hudak M, Semjon B, Marcincakova D, Bujnak L, Nad P, Korenekova B, Nagy J, Bartkovsky M, Marcincak S. Effect of broilers chicken diet supplementation with natural and acidified humic substances on quality of produced breast meat. Animals (Basel). 2021 Apr 10;11(4):1087.

International Commission on Illumination (CIE). CIE S 014–4:2007. Colorimetry – Part 4: CIE 1976 L*a*b* Colour space. Commission Internationale de l'Eclairage/International Commission on Illumination, CIE Tech. Committee 1–57, ISO 11664-4:2008; 2007.

Jaduttova I, Marcincakova D, Bartkovsky M, Semjon B, Harcarova M, Nagyova A, Vaczi P, Marcincak S. The effect of dietary humic substances on the fattening performance, carcass yield, blood biochemistry parameters and bone mineral profile of broiler chickens. Acta Vet Brno. 2019 Oct;88(3):307-13.

Kruskal WH, Wallis WW. Use of ranks in one-criterion variance analysis. J Am Stat Assoc. 1952 Dec;47:583-621.

https://doi.org/10.17221/82/2025-CJAS

Lawless HT, Heymann H. Sensory evaluation of food: Principles and practices. 2nd ed. New York, USA: Springer Verlag; 2010. 596 p.

Liu HW, Gai F, Gasco L, Brugiapaglia A, Lussiana C, Guo KJ, Tong JM, Zoccarato I. Effects of chestnut tannins on carcass characteristics, meat quality, lipid oxidation and fatty acid composition of rabbits. Meat Sci. 2009 Dec; 83(4):678-83.

Marcincak S, Semjon B, Marcincakova D, Reitznerova A, Mudronova D, Vaskova J, Nagy J. Humic substances as a feed supplement and the benefits of produced chicken meat. Life (Basel). 2023 Apr 1;13(4):927.

Marcincak S, Sokol J, Turek P, Popelka P, Nagy J. Stanovenie malondialdehydu v bravcovom mase s pouzitim extrakcie na tuhej faze a HPLC [Determination of malondialdehyde in pork using solid phase extraction and HPLC]. Chem Listy. 2006 Jan;100(7):528-32. Slovak.

McLaren K. XIII – The Development of the CIE 1976 (L* a* b*) uniform colour space and colour-difference form. J Soc Dyers Colour. 1976 Oct;92(9):338-41.

Miranda CL, Elias VD, Hay JJ, Choi J, Reed RL, Stevens JF. Xanthohumol improves dysfunctional glucose and lipid metabolism in diet-induced obese C57BL/6J mice. Arch Biochem Biophys. 2016 Jun 1;599:22-30.

Ondruska L, Chrastinova L, Rafay J, Pospisilova D, Parkany V. Effect of humic substances and probiotics on growth performance and meat quality of rabbits. Potravin Slovak J Food Sci. 2012 May;6(2):39-41.

R Development Core Team. R: A language and environment for statistical computing [Internet]. Vienna (Austria): R Foundation for Statistical Computing; 2021 [cited 2022 Jan 1]. Available from: https://www.R-project.org/.

Rezar V, Levart A, Cerenak A, Salobir J and Pirman T. Effect of hop (Humulus lupulus) supplementation on performance, oxidative stress, and oxidative stability of broiler chicken meat. Europ Poult Sci. 2020 Feb;84:1-17.

Rubayet Bostami ABM, Mun HS, Yang CJ. Loin eye muscle physico-chemical attributes, sensory evaluation and proximate composition in Korean Hanwoo cattle subjected to slaughtering along with stunning with or without pithing. Meat Sci. 2018 Nov;145:220-9.

Sbardella M, Perina DP, Andrade C, Santos CB, Cairo PLG, Marques ELS, Rezende RP, Costa LB, Miyada VS. Effects of dietary hop β-acids or colistin on the performance, nutrient digestibility, and intestinal health of weanling pigs. Anim Feed Sci Technol. 2016 Jul;217:67-75.

Semjon B, Marcincakova D, Korenekova B, Bartkovsky M, Nagy J, Turek P, Marcincak S. Multiple factorial analysis of physicochemical and organoleptic properties of breast and thigh meat of broilers fed a diet supplemented with humic substances. Poult Sci. 2020 Mar;99(3):1750-60.

Simpson BK. Food biochemistry and food processing. 2nd ed. Iowa, USA: Blackwell Publishing; 2012. 901 p.

The Ministry of Agriculture. The Decree of the Ministry of Agriculture No. 230/1998 Z.z. on livestock breeding and killing of animals for slaughter. Czech Republic; 1998.

Xiccato G. Feeding and meat quality in rabbits: A review. World Rabbit Sci. 1999 Jul;7(2):75-86.

Zawadzki A, Arrivetti LOR, Vidal MP, Catai JR, Nassu RT, Tullio RR, Berndt A, Oliveira CR, Ferreira AG, Neves-Junior LF, Colnago LA, Skibsted LH, Cardoso DR. Mate extract as feed additive for improvement of beef quality. Food Res Int. 2017 Sep;99(Pt 1):336-47.

Received: June 7, 2025
Accepted: August 6, 2025
Published online: August 27, 2025