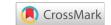


Research Article

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Effects of Xanthohumol supplementation on growth performance and carcass traits of rabbits

Abstract

This study evaluated the effects of xanthohumol (XN) supplementation's effects on growth performance and carcass traits in rabbit diets. Two groups of silver and chinchilla crossbred rabbits were fed pellets enriched with either 0.5% or 1.0% XN during a 63-day fattening period. A control group (C) of rabbits of the same crossbreed and age was fed a basal diet without supplements. Rabbits in the group supplemented with 1.0% XN exhibited higher final and carcass weights at the end of the fattening period, along with increased protein content and collagen-free muscle protein content (BEFFE) in the thigh muscle compared to the control group. In conclusion, the primary benefit of supplementing a rabbit diet with 1% XN lies in its ability to enhance growth performance, driven by increased protein content and reduced fat levels in the meat. Supplementing rabbit diets with hop extract in the form of xanthohumol presents a promising strategy for producing functional foods. This alternative approach to animal nutrition supports growth, promotes overall health, and ensures consumer safety by preventing potential adverse effects.

Keywords: hop extract, rabbit, fattening, weigh, carcas

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Introduction

Rabbit farming has a long tradition in the countries of central and Western Europe. Originally, rabbits and stabled livestock were bred for meat and functioned as consumers of excess feed. Today, rabbit farming has evolved significantly, with breeding practices now focusing on meeting specific requirements for housing, hygiene, and nutrition. These advancements aim to optimize weight gain and achieve maximum growth efficiency within the shortest possible time.¹

To support rabbit fattening, various specialized feed mixtures are produced. Manufacturers with growth stimulants and antibiotics often enrich these to enhance growth rates and maintain optimal health. However, a significant drawback of such mixtures is the continuous use of antibiotics as growth promoters, which can lead to their accumulation in animal tissues. Consuming these animal products may contribute to the rise of antibiotic resistance, a critical global concern.² Currently, efforts are focused on identifying alternative solutions for animal nutrition that promote growth and maintain good health while avoiding any adverse effects on consumers.^{3,4}

Phytogenic additives for animal feed have been receiving attention due to their pharmacological properties holding the potential to replace antimicrobial agents and growth promoters. For farm animals, several studies have demonstrated that the diet has an effective impact on animal performance, intestinal health, fat content, lipid metabolism, meat composition and the oxidative stability of meat.^{5,6} When added to the animal diet, the absorbed active compounds from plant materials can directly reach the muscle tissue or can be metabolized into biological precursors of endogenous antioxidants. In both cases, the bioactive compounds can provide the antioxidant mechanisms required to improve animal health and welfare and to protect meat against oxidation during storage and processing.⁷

Many studies reported that the use of botanicals as dietary supplements in animal feed effectively modulates animal metabolism, affecting animal welfare and meat quality.^{5,8–11} One of the promising

phytogenic additives with strong antioxidant and immunostimulating properties include polyphenols. The health effects of plant products are primarily determined by the presence of metabolites produced by the plant to protect it from external abiotic and biotic factors. Based on their chemical structure, polyphenols are divided into flavonoids and non-flavonoids. The most widespread polyphenols are flavonoids.¹² Hops as a medicinal plant have been used for a relatively long time due to the content of various phenolic compounds. Female inflorescences of Humulus lupulus (hops) are used in traditional medicine and in brewing.¹³

The most abundant prenylated flavonoid in hops is xanthohumol (XN; Figure-1).13 XN, in particular, caught the attention of scientists due to its biological effects.¹⁴ Laboratory studies have shown positive results of xanthohumol as a dietary supplement.^{15,16} It is reported that XN exhibits multiple biological functions, including antioxidant, antiinflammatory, anticarcinogenic, antimicrobial, and chemoprotective effects (Figure 1).¹²

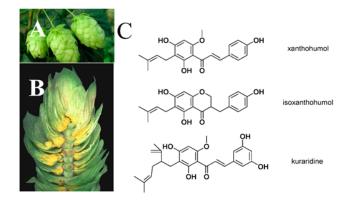


Figure I Female hop flowers (A); hop flowers resin covering flower bracts (B); and structure of xanthohumol, isoxanthohumol, and kuraridine (C). Source: Liu¹³

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Furthermore, it inhibits the process of resorption of calcium from the bones, inhibits the enzymatic activity of lipogenase, has strong phytoestrogenic properties, improves homeostasis, and increases appetite.¹⁴

From the point of view of animal nutrition, dietary hop's positive effects were noted on animal performance and intestinal health.^{5,17} Even though many reports in the literature have been comparing the bacteriostatic efficacy of hop β -acids to the most used synthetic antibiotics in animal feeding trials to evaluate performance, there is little information regarding the metabolic effects following the use of hop as a supplement for animal diet. Therefore, the aim of this study was to evaluate the effects of XN supplementation in feed in the form of pellets on the growth performance and carcass traits of rabbits.

Material and methods

a) Feed supplementation, growth performance and carcass yield of rabbits

In this study, we used 36 silver rabbit and chinchilla crossbred rabbits. Rabbits were bred under the same conditions in the air-conditioned hall. They were placed in standard cages for rabbit breeding ($80 \times 40 \times 66$ cm), and four rabbits were in each cage. During the experiment, light mode was provided for 16 h of light and 8 h of darkness. The average temperature in the hall was 22 ± 4 °C, and the relative humidity was $70 \pm 5\%$. All rabbits were fed standard diet ad libitum. The animal study protocol was approved by the Ethics Commission of University of Veterinary Medicine and Pharmacy in Košice (protocol code EKVP/2024-2).

At the beginning of the experiment, the animals were divided into three groups (n= 12/group) with three replicates in each group: C (control group, standard diet without additives), group XN 0.5 (experimental group 1, received standard feed + 0.5% INHE Xanthohumol, China), group XN 1.0 (experimental group 2, received standard feed + 1% INHE Xanthohumol, China). The addition of XN was incorporated during the production of pellets when mixing the individual components.

The control group was given the pellets 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. During the entire period of fattening (from the 30th to the 93rd day of age), the second and third experimental groups were fed basal diet pellets with 0.5% or 1% of XN (INHE Xanthohumol, Shaanxi In health Nature Industry Co., Ltd., China; 50% share of XN and 10% of hop flavonoids).

At the end of the experiment, after 9 weeks of administration of XN, the final weight was recorded and rabbits were slaughtered.¹⁸ The carcass weight was recorded after slaughtering (decapitation, removal of skin and distal parts of limbs, and evisceration). The yield of the carcass was determined as a ratio of the final body and carcass weight. The thighs with bones were weighed and their percentage values were calculated.

b) Physicochemical analysis of thigh meat samples

For the analysis of meat quality, 8 rabbits of each experimental group were randomly selected. In these samples, ash, water, fat and protein content, collagen, meat protein content without connective tissue content (BEFFE) and pH were evaluated. The thigh meat samples were stored at 4 ± 2 °C until meat quality analysis.

The proportion of individual chemical components in the thigh homogenized meat samples 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 according to Hudák.¹⁹ The pH of the homogenized meat samples was analyzed with a digital inoLab pH 340i meter (Wissenschaftlich-Technische Werkstatten, Germany).

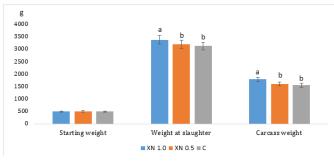
c) Statistical analysis

The results obtained in this experiment were expressed as means and standard deviation of the mean (SD). Analysis of Variance (ANOVA) with Tukey's tests for multiple comparisons of means from all three groups was carried out via the software GraphPad Prism 8.3 (GraphPad Software, San Diego, CA, USA). The effects of rabbit diet supplementation with two different concentrations of XN were set as the main factor identifying which pairs of means in a set of groups are significantly different from each other at a significance level of P < 0.05.

Results and discussion

Numerous studies have shown that incorporating botanical preparations into animal feed as dietary supplements can effectively regulate animal metabolism, enhance animal welfare, and improve meat quality.12-14 Phytogenic feed additives are a promising alternative to antibiotics as growth promoters, offering significant benefits to animal health and productivity.¹⁵

Similar findings were observed in our study, demonstrating the beneficial effects of 1.0% XN supplementation on increasing final body weight in the experimental group of rabbits compared to the control group. Additionally, the same group exhibited a higher carcass weight after 93 days of fattening (Graph 1).



Graph I Comparison of XN dietary supplementation on weight of rabbits

Note: C - control group, rabbits fed with standard feed mixture; XAI.0 - an experimental group received standard feed + 1.0% of xanthohumol; XA0.5 - an experimental group received standard feed + 0.5% of xanthohumol; ^{ab} Means above columns with different superscript letters are statistically different (Tukey's, P<0.05).

Likewise, were recorded a statistically significant increase (P<0.05) in body weight gain during the monitoring period (63 days) with the weight of thigh as well as average daily gain in the same group (XN1.0) (Table 1).

As is known, XN has strong antioxidant effects and protects the intestinal mucosa from oxidative damage and pathogens, while limiting peristaltic activity in digestive disorders, and that some reduction in intestinal motility might lead to better nutrient absorption.^{14,17}

Some authors dealing with possible health benefits achieved by XN supplementation observed a positive effect of xanthohumol on body weight. In the study of Bozkurt²⁰ dietary supplementation of hop extract (1 g/kg) provided a significant improvement to body weight

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gain in broilers. The same effect of XN treatment on body weight was observed in male rats.¹⁵

Phytogenic additives in form of hop extract has beneficial effects on nutrient utilization possibly by stimulating digestive enzymes and improves gastrointestinal morphology. For this reason, there is improved digestibility and absorption of nutrients,²¹ which was also confirmed in our study by the increased weight and average daily gain of rabbits supplemented with 1% XN.

On the other hand, some studies have not recorded significant changes after feeding diets enriched with XN.^{22,23} The results of the study were shown by Kwiecien and Winiarska-Mieczan²¹ that the addition of 2% hops did not increase the body weight of broiler chickens compared to the control group. In a study carried out by Rezar.²³ Consumption of 3.6 g of hops/kg feed did not showed a significant effect on the body weight gain of broilers compared to 0.9 g/kg of the supplemented group.

The interpretation of various findings related to XN supplementation often depends on the health status of the individuals as well as interspecies differences in digestion and nutrient absorption. Rabbits are herbivorous animals with a digestive tract that differs significantly from poultry, allowing them to better utilize nutrients from plant-based feeds.²⁴ Adding XN in the form of pellets to the rabbit diet likely increased their feed intake, which was reflected in higher average daily gains and increased final body weight (Table 1).

Table 2 Comparison of physicochemical analysis of thigh meat samples

 $\label{eq:table_table_table} \begin{array}{c} \textbf{Table I} & \text{Comparison of XN} & \text{dietary supplementation on body weight and} \\ \text{average daily gain of rabbits} \end{array}$

Group	Body weight gain during monitoring period		Average daily gain		Weight of thigh with bone	
	g		g		g	
	x	sd	x	sd	x	sd
XA 1.0	2783.2a	219.7	44.2a	3.2	290.0a	20.98
HS 1.0	2586.2b	170.6	41.8b	2.9	255.0b	26.64
Control	2499.4b	255.9	39.7b	3.8	240.0b	21.47

Note: C - control group, rabbits fed with standard feed mixture; XA1.0 - an experimental group received standard feed + 1.0% of xanthohumol; XA0.5 - an experimental group received standard feed + 0.5% of xanthohumol;^{ab} Means in column with different superscript letters are statistically different (Tukey's, P<0.05).

Different concentrations of XN also affect the components of meat, mainly fat content and total protein content differently.²⁵ The effect of the supplementation of XN in rabbits on the individual components of meat samples from the thigh is shown in Table 2. In thigh meat samples were increased (P < 0.05) protein and BEFFE content compared to control group.

Samples	Parameters	XN I.0		XN 0.5		Control	
		х	sd	x	sd	х	sd
	Water %	74.441	0.298	75.124	0.185	74.342	0.164
	Fat %	1.706 ª	0.199	2.147ª	0.176	3.333 ^b	0.208
	Ash %	1.705	0.081	1.63	0.128	1.689	0.043
Thigh meat samples	Protein %	24.728 ^a	0.371	22.273 ^{a,b}	0.329	21.577 •	0.103
	Collagen %	1.717	0.146	1.621	0.164	1.564	0.113
	BEFFE %	19.627 ^a	0.103	18.274 ^{a,b}	0.171	I7.7I [♭]	0.026
	pН	6.225	0.013	6.2	0.015	6.24	0.012

Note: C - control group, rabbits fed with standard feed mixture; XA1.0 - an experimental group received standard feed + 1.0% of xanthohumol; XA0.5 - an experimental group received standard feed + 0.5% of xanthohumol;²⁶ Means in row with different superscript letters are statistically different (Tukey's, P<0.05).

In addition, in the thigh meat samples, a lower content of fat was confirmed (P < 0.05) in the both XN group compared to control. The water and pH of meat samples from both supplemented groups were not affected by the addition of 1.0% or 0.5% XN to the feed (Table 2).

Zawadzki¹¹ confirmed that addition of XN from hops to broiler diets had been reported to improve the overall redox stability and meat properties of broilers. XN had a significant influence on the concentration of fatty acids like polyunsaturated fatty acids that are relevant for the redox stability of meat. Dietary hop supplementation also showed to stimulate an increase in the level of monounsaturated fatty acids (MUFAs), in breast meat of chicken and may be associated with a better intestinal absorption of MUFAs or to an enhancement in the activity of enzymes responsible for the conversion of saturated fatty acids to monounsaturated fatty acids (Table 2).

One of criteria for meat evaluation is protein content.²⁶ Based on study by authors Xiccato²⁵ states that the protein level in rabbit meat can range from 18.6% to 22%. Differences in proteins the level depends on the breed and age of the animals, the composition of the feed, the part carcass and preparation for slaughter. Protein content in thigh meat from rabbits supplemented of 1.0% XN to the feed in our study was higher as recommended in a previous study. XN is probably able to influence the distribution of fats and proteins in the body and thus also the change in the composition of the meat.

It had been shown that extracts of hops could up regulate the expression of phase II enzymes while increasing the protein, activity and glutathione levels of these enzymes.²⁷ In a series of mice and rat models, XN exerted a triacylglycerol lowering effect and total cholesterol.²⁸ The effects of XN on triacylglycerol concentrations in animals were also observed in the genetically modified mouse after 14 days diets with 0.3% XN powder, when plasma triacylglycerol in animals with XN in the diet was lower than in control animals without the addition of XN.²⁹

Conclusion

The inclusion of XN in rabbit feed positively influenced growth performance and quality parameters of the thigh muscle. Supplementing the diet with 1.0% XN resulted in increased final and carcass weights, as well as higher average daily gains, compared to the control group. Additionally, our study confirmed reduced fat content in the thigh meat of both groups supplemented with XN. This reduction in fat content may be attributed to enhanced fat metabolism, likely due to the detoxifying effects of XN on the liver.

Furthermore, increased BEFFE values in the thigh meat were observed in the group supplemented with 1.0% XN, contributing to their higher final body weight. The improvements in weight and daily gains are likely linked to an increased appetite for the XN-enriched pellets, which enhanced feed intake in the experimental groups. The use of dietary hops for rabbits feed must be considered as a promising strategy to produce a functional food as a alternative solution for animal nutrition that promote growth and maintain good health while avoiding any adverse effects on consumers.

Conflicts of interests

Authors declare that there are no conflicts of interest.

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