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Impact of *Amaranthus hypochondriacus* in nutrition for rabbits on meat quality

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ABSTRACT: Unique properties of *Amaranthus hypochondriacus* have been extensively utilized in the recent time worldwide both in food technologies and husbandry. An intensive growth of *Amaranthus hypochondriacus* plants has been made in Ukraine there and, respectively, production of foods based on amaranth is growing, however no research on its use for feeding rabbits is available. Therefore, the study aimed to review the impact of *Amaranthus hypochondriacus* on slaughter features of rabbits, meat quality including those under storage conditions. The California race rabbits of 60 days age were divided into two groups, 28 animals in each. The rabbits in the study group were fed (by adding to the basal diet) with 20% of amaranth oilcake. The rabbits were slaughtered at age of 120 days. The following key features of meat output and quality performance were determined: pH, moisture content, water-holding capacity, cooking losses, protein and cholesterol content as well as change in pH, microorganisms count, and moisture loss percentage during 9-day storage in chilled state. *Amaranthus hypochondriacus* effect on the rabbit live weight, hot carcass weight, dressing out percentage, percentage ratio of heart, kidney, liver, lung weight to carcass weight, pH, moisture content, water-holding capacity, and meat morphological structure was determined. Reduction in cholesterol level by 15.07% ($p < 0.05$) in the meat of rabbits that were fed with *Amaranthus hypochondriacus* was detected. pH and drip loss percentage were not significantly different among the groups in the shelf-life period. However, it was found out that growth of microorganisms in the meat of rabbits that were fed with *Amaranthus hypochondriacus* was slowed down: microorganisms count was 1.65 and 1.71 ($p < 0.05$) times lower than in the control group on the 6th and 9th days of storage, accordingly. The low-cholesterol and bacteriostatic effects of amaranth oilcake in the rabbit nutrition may significantly increase dietary properties of rabbit meat. Use of amaranth oilcake for rabbit fattening has great potential and further studies including the mechanism of antibacterial effect of *A. hypochondriacus* on the rabbit meat are required

Keywords: amaranth, rabbits, meat quality, cholesterol, microorganisms

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INTRODUCTION

Dietary properties of the rabbit meat are advantageous over that of other animals owing to its higher protein level and lower cholesterol level (Nistor et al., 2013). These rabbit meat properties have been provided both through genetic factors and feeding conditions. Many researchers propose to use various plant additives through rabbit nutrition or partial substitution of feedstuffs aimed to enhance quality performance of meat, decrease sickness rate and fodder cost (Cardinali et al., 2015; Dalle et al., 2016; Cullere et al., 2016; Kone et al., 2016; Dabbouet al., 2017; Duda et al., 2018; Hernández-Martínez et al., 2018; Mancini et al., 2018; Fathi et al., 2019).

High potential capabilities of amaranth (*Amaranthus* spp.) have been acknowledged by the international scientific community for animal feeding, particularly as an alternative source of protein and fibre (Chhayet et al., 2013; Peiretti, 2018; Peiretti et al., 2018). The scientists report on effective inclusion of amaranths: leaf meal, seeds and oilcake (*Amaranthus dubius*, *Amaranthus hypochondriacus*, *Amaranthus caudatus*) to the rabbit nutrition (Bamikole, 2000; Chhay et al., 2013; Molina et al., 2015; Molina et al., 2018).

Amaranth (mainly, *Amaranthus hypochondriacus*) is intensively grown and processed in recent years in Ukraine, however there are no studies of its use in rabbit nutrition (Hoptsii et al., 2018). Potential for the efficient use of vegetative mass and waste from the production of amaranth oil in rabbit breeding requires careful study and research. Research findings regarding use of amaranth in rabbit fattening in various countries are slightly controversial and insufficient. Today, we have not enough data on amaranth effect on change of quality features of the meat of rabbits bred on nutrition with amaranth. Extension of meat shelf-life period is also a relevant issue. Therefore, the study was aimed to review the effect of *Amaranthus hypochondriacus* on shelf-life qualities of rabbit meat.

MATERIALS AND METHODS

The study was carried out at the private rabbit farm Olbest LLC where rabbits of Californian race given by **Veselyi Khutorok Private Company, Novomoskovsk** were bred. Keeping, feeding, care of and all other operations with animals were carried out in conformity with the European Convention for the Protection of Vertebrate Animals used for Exper-

imental and other Scientific Purposes (Strasbourg, 18 March 1986), General Ethical Principles of Animal Experimentation approved by the First National Congress on Bioethics (Kyiv, 20 September 2001), Article 26 of the Law of Ukraine No. 5456-VI of 16.10.2012 'On Animal Protection Against Cruelty', and EU Directive 86/609/EEC of 24 November 1986.

Animals and housing conditions

Clinically healthy fifty-six rabbits of two months old with average live weight of $1,430 \pm 61.3$ g were random selected and divided into two groups, 28 rabbits each. Veterinary examinations of animals were regularly conducted during the study. The control group (I) included 15 males and 13 females, the experimental group (IIa) - 16 males and 12 females. The rabbits were kept in a rabbit warren separately in individual universal galvanized wire hutches during 2 months before being slaughtered, at average temperatures ($19.4^{\circ}\text{C} \pm 0.07$) and relative air humidity ($63.5 \pm 0.04\%$), under 10/12-hour cycle of light and dark.

Feeding animals

Watering and feeding were carried out automatically; rabbits had free access to water and fodder. The first (I), control group of rabbits received basal diet without amaranth, the second (IIa) study group received diet with addition of 20% amaranth oilcake (Table 1). Experimental diet was prepared by partial substitution of wheat and sunflower oilcake in control diet with 20% of *A. hypochondriacus* (diet of IIa group). The basal diet consisted of commercial granulated mixed fodder by Domashenko D.I. (individual entrepreneur from Kryvyi Rih) for fattening the young animals (Recipe K-94-22) according to Smith (1966). The amaranth oilcake that was produced by "Amarant" Trading and Production Farm, LLC (Dnipro) by means of cold pressing of *Amaranthus hypochondriacus* seed was used in production of granulated mixed fodder fed to the rabbits of IIa group. Diets for feeding rabbits of both groups consisted of equivalent shares of crude protein and digestible energy. The dry matter, crude protein, crude fat, crude fibre, ash, calcium and phosphorus were analyzed in diets by the procedures of the national standard DSTU 7693:2015 (2016). The digestible energy (DE) was calculated according with Axelson's formula modified by Grigorev and Volkov: $DE = 0.73 \times GE (1 - CF \times 1.05)$, where 0.73 is the exchangeability coefficient, GE is the gross energy per 1 kg of dry matter of the feed, CF is crude fiber, $(1 - CF \times 1.05)$ - coefficient reduction energy

value by crude fiber (Grigorev et al., 1989). During the experiment feed intake were recorded individually on

a fortnightly basis. Average daily feed intake (ADFI) were calculated.

Table 1. Ingredients and chemical compositions of rabbit nutrition

Indicators	Groups	
	I	IIa
Ingredients, %		
Lucerne	25.00	25.00
Wheat	54.50	42.40
Sunflower oilcake	17.66	9.96
Amaranth oilcake	-	20.00
Vitamin and mineral pre-mix	2.84	2.64
Chemical composition		
Crude protein, %	15.84	15.85
Crude fibre, %	11.96	10.97
Crude fat, %	3.78	3.50
Digestible energy, MJ/kg	11.30	11.09
Ca, %	0.93	0.71
P, %	0.45	0.46

Dressing out animals and determination of meat output

Animals of 120-day age after 12-hour fasting were slaughtered at the company's slaughterhouse using Sprut Krol-100 equipment in accordance with the National Requirements "Rules for pre-dressing out veterinarian animal inspection and veterinary and sanitary expertise of meat and meat products" (2002). The technological process included: electrical stunning, blood draining, skinning, removal of head and limb distal parts, evisceration, and carcass scraping. After weighing and determination of meat pH, the slaughter products were chilled at the temperature of +4°C, relative air humidity 92% for 24 hours, and then chilled carcasses were weighted. Meat productive carcass features were measured or calculated in accordance with Blasco and Ouhayoun (1996): live weight (LW), hot carcass weight (HCW), chilled carcass weight (CCW), dressing out percentage (DoP), drip loss percentage (DLP), percentage in carcass weight: liver (LvP), kidney (KiP), heart (HeP), lung (LuP).

Determination of meat quality performance

Laboratory tests were made in the testing center of Zaporizhzhia Regional State Laboratory at the State Service for Safety of Foods and Consumer Protection accredited in conformity with ISO/IE 17025: 2006, Accreditation Certificate No. 2H305 of the National Accreditation Agency of Ukraine. Quality performance was determined in *Longissimus dorsi* muscles.

Hot carcass pH (15 minutes after dressed out - pH₀) and chilled carcass pH (24 hours - pH₂₄) were measured with portable meat pH-meter with metal pin (Gondo PS-45, China) that was inserted into muscles *Longissimus dorsi* at the level of 5th lumbar vertebra, 5th and 8th thoracic vertebra. Water-producing capacity (WPC) of meat was determined with the method described by Penny (1975) modified with technique by Earl et al. (1996). Ground meat specimens of 3 g weight were wrapped into nylonnet with mesh diameter of 0.5 mm, then placed into a 'basket' of filter paper and inserted into centrifugal test-tubes. Centrifugation was carried out for 15 minutes at 1,400 rpm. Moisture produced of meat was absorbed with filter paper and its amount was determined as weighing difference. Water-holding capacity (WHC) of meat was calculated as difference between 100% and percentage of moisture produced with centrifugation method (WPC). Moisture loss after heat cooking treatment was calculated as difference between fresh meat weight and one after cooking for 1 hour at temperature of 163°C (AMSA, 2015). Meat output after heat treatment was calculated as percentage of cooking loss deducted from 100%. Determination of pH, WPC, moisture loss after heat cooking treatment was made in 3 repetitions. Meat moisture was determined according to DSTU ISO 1442:2005 (2005); protein content - with biuret method (Torten and Whitaker, 2006); cholesterol with spectrophotometric analysis (Li-Hua, 2019).

Meat freshness shelf-life estimation

After carcasses were cut (Blasco and Ouhayoun, 1996), all hind legs one by one were put into clean plastic containers with absorbing insert, weighed and kept storage for 9 days at temperature of +4°C and 80% relative air humidity. On the 1st, 3rd, 6th and 9th day of storage, 14 containers for each period were opened and pH estimated (pH_{1d} , pH_{3d} , pH_{6d} , pH_{9d}) with "PHS-25C" laboratory pH-meter ("BROM" Private Company, Kyiv), measurement accuracy of up to ± 0.05 (GOST P 51478-99 (ISO 2917-74); drip loss percentage (by difference in weight after leg blotting with filter paper) and microorganisms count in tissue smears of surface muscle layers according to National Standard GOST 23392-78. Microorganisms count was calculated in 3 tissue smears (25 microscope fields of view in each) from each sample and arithmetic mean value obtained.

Histomorphology of meat

Portions of *Longissimus dorsi* muscles in both rabbit groups were selected for histomorphology. The material was fixed in 10% neutral buffered formalin. Muscle parts were dehydrated in ethanol (alcohol) solutions of increasing concentration and compressed in histology paraffin (Goralskyj et al., 2011). Serial

histological sections of 3 to 5 microns thick were prepared on a sliding microtome with subsequent coloring with hematoxylin and eosin. Histology preparations were studied with light microscope Leica CX1000 and QWin 3 morphology processing data software.

Statistical analysis

The results were statistically analyzed using the Statistica 10 software. Distribution of the data within groups was evaluated using a Shapiro-Wilk test. All parameters showed a normal distribution. Reliability of the difference between values was verified under T-test (Independent-Samples T-Test). Levene's test was used to test whether variances were homogenous. Data are presented as mean \pm standard deviation. The significance degree between two groups was determined to be $P < 0.05$.

RESULTS

The inclusion of amaranth in the diet did not significantly change the level of ADPI (I: 237.50 ± 11.47 g and IIa: 243.6 ± 9.09 g, at $P < 0.1$).

Effect of dietary inclusion of amaranth as main feedstuff on certain carcass parameters of Californian rabbits are given in Table 2.

Table 2. Effect of diet with use of amaranth on carcass characteristics of rabbits (mean \pm standard deviation)

Indicators	Groups		P value
	I	IIa	
Live weight, g	3543.6 ± 296.62	3417.5 ± 212.17	0.07
Hot carcass weight, g	2057.6 ± 203.96	1991.6 ± 181.40	0.21
Chilled carcass weight, g	2007.2 ± 157.62	1959.4 ± 145.46	0.24
Dressing out percentage, %	58.0 ± 1.11	58.28 ± 1.05	0.33
Drip loss percentage, %	2.45 ± 0.46	1.92 ± 0.48	0.06
HeP, %	0.45 ± 0.21	0.46 ± 0.10	0.08
LvP, %	5.15 ± 0.49	5.45 ± 0.54	0.05
LuP, %	0.68 ± 0.05	0.64 ± 0.06	0.06
KiP, %	0.84 ± 0.05	0.87 ± 0.05	0.11

Notes: HeP - percentage in carcass weight heart; LvP - percentage in carcass weight liver; LuP - percentage in carcass weight lung; KiP - percentage in carcass weight kidney

Live weights of rabbits at 120-day age and dressing out percentage were in conformity to standard race values; however, reliable difference between the groups was not established. Neither was established the effect of feeding *Amaranthus hypochondriacus* on HCW, CCW, LvP, KiP, HeP, LuP. Drip loss percentage after chilling the carcasses in both groups did not exceed 2.5% and the carcasses of control rabbits showed slightly increased value (by 0.53%).

The determined quality performance of rabbit meat (Table 3) showed no significant difference between the groups by contents of moisture, protein and pH, however revealed a highly accurate ($p < 0.05$) decrease in cholesterol content in result of feeding rabbits with *Amaranthus hypochondriacus*. Water-holding capacity of rabbit meat from IIa group was slightly higher than in control group but the difference was insignificant. No difference in heat treated meat output be-

tween the groups was detected.

Monitoring the changes in rabbit meat freshness during the 9-day shelf-life period (Table 4), data showed that meat pH was increasing with time but by

the end of the study the findings did not exceed value of 6.2 and no difference between the experimental and control groups was reported. Drip loss percentage for each three days of shelf-life period varied from 0.62% to 1.27% and had no significant deviation in groups.

Table 3. Qualities of rabbit meat with various nutrition (mean±standard deviation)

Indicators	Groups		P value
	I	IIa	
pH ₀	7.13±0.02	7.12±0.02	0.10
pH ₂₄	5.95±0.04	5.93±0.03	0.26
Moisture, %	71.87±2.37	72.65±2.10	0.44
Water-producing capacity (WPC), %	33.21±3.26	31.56±2.80	0.24
Water-holding capacity (WHC), %	66.79±2.07	68.44±2.68	0.14
Moisture loss after heat cooking treatment, %	29.27±2.08	28.14±1.90	0.22
Meat output after heat cooking treatment, %	70.73±3.36	71.86±2.56	0.41
Protein, %	20.12±1.19	21.46±1.72	0.06
Cholesterol, mg/100 g	51.64±2.46	36.57±2.28*	<0.001

* - means are significantly different between groups

Table 4. Rabbit meat freshness dynamics (mean±standard deviation)

Indicators	Groups		P value
	I	IIa	
pH _{1d}	5.92±0.10	5.89±0.05	0.05
pH _{3d}	5.98±0.05	5.95±0.10	0.07
pH _{6d}	6.02±0.05	6.01±0.05	0.11
pH _{9d}	6.1±0.16	6.08±0.10	0.08
Moisture loss during shelf-life, % day:			
3	0.89±0.16	0.74±0.20	0.06
6	1.27±0.27	1.08±0.28	0.09
9	0.82±0.27	0.62±0.21	0.05
Microbe count in microscope field of view, day:			
1	2.3±1.92	1.45±1.36	0.16
3	2.24±1.66	3.98±1.98	0.05
6	6.91±1.65	4.2±1.62*	< 0.001
9	14.9±4.59	8.7±2.73*	< 0.001

* - means are significantly different between groups

Microorganisms count on the rabbit meat surface layers in both groups on the 9th day after dressing out increased 6-6.5 times compared to the first day, however, in IIa group, this value was in conformity to the fresh meat standards (<10) and in the control group to the low-quality freshness meat (11-30). The group that received *Amaranthus hypochondriacus* reported microorganisms count in meat to range between 1.65 and 1.71 times lower at the 6th ($p<0.05$) and 9th ($p<0.001$) days of shelf-life, accordingly. Dynamics changes for microorganisms count are shown in meat during the shelf-life (Fig. 1).

Figure 2 shows various tendencies for microbe growth during the shelf-life. While the group with amaranth reported the slow reproduction of microorganisms from 1 to 6 day, in the control group this period was 2 times shorter.

Histology of rabbit *Longissimus dorsi* muscle fragments (Fig. 2) established that animals in both groups reported no morphology differences in muscle tissue structure. Animals in I group had more dense located fibers. Nuclei were clearly outlined. Linear fibers prevailed in both animal groups, some were wavy with slight thickening and compression nodes which were

associated with post-mortem changes in muscle tissue. Endomysium, folded in some areas, was clearly visible. Transversal sections showed that muscle fi-

bers were uneven colored, which was due to physical and chemical changes in muscle tissue in the course of maturation.

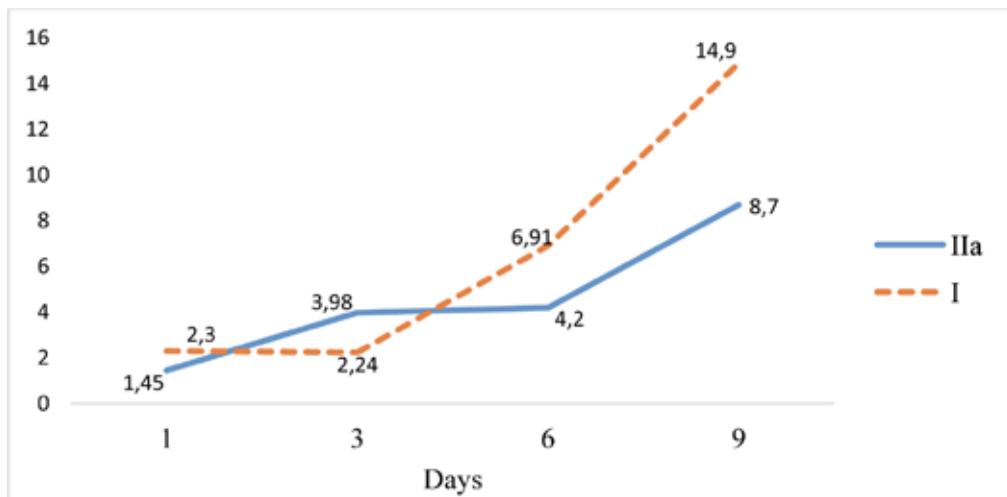


Figure 1. Dynamics of microorganisms count in tissue smears of rabbit meat during shelf-life

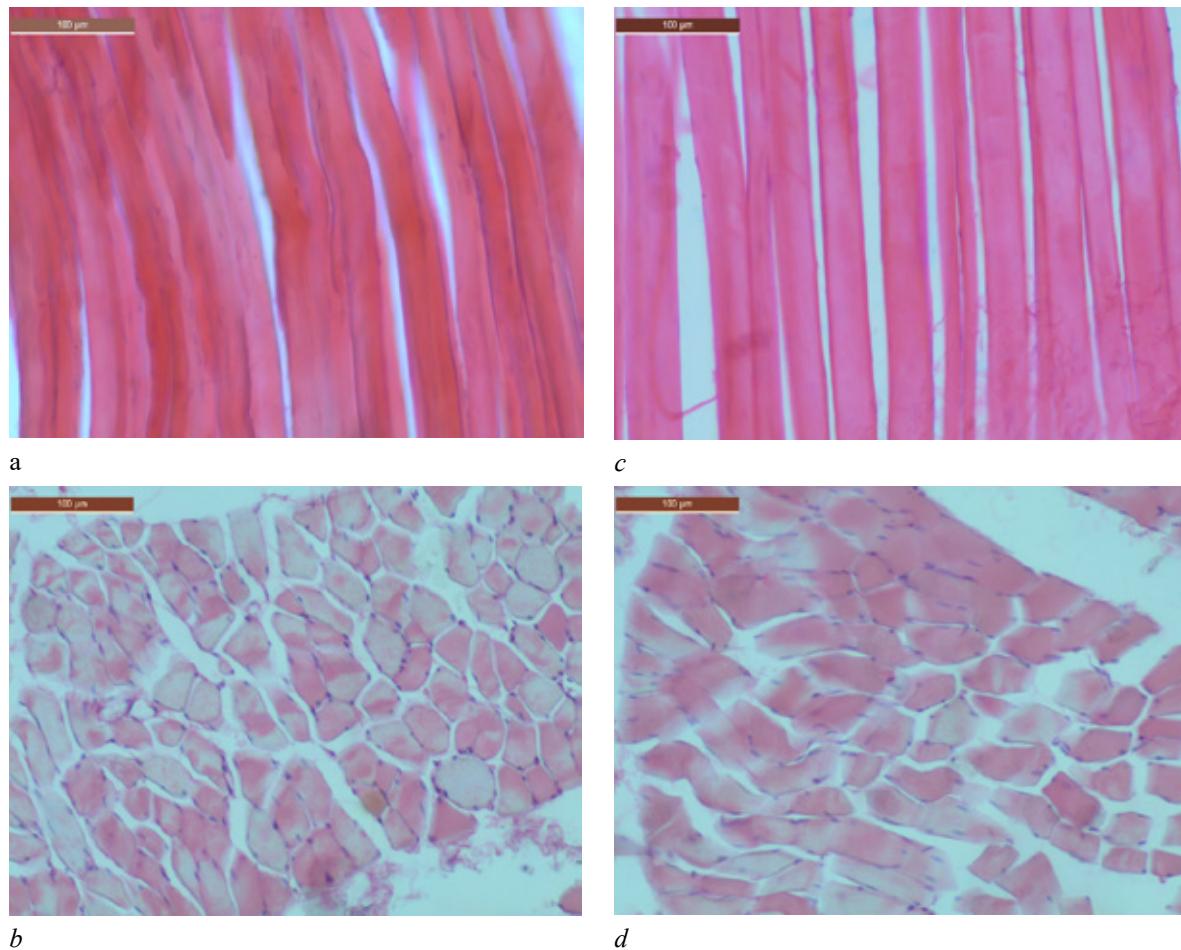


Figure 2. Histology longitude and transverse sections of rabbit muscles *Longissimus dorsi*: a, b - I group; c, d - IIa group. Haematoxylin and eosin, $\times 200$

DISCUSSION

Adding 20% of *Amaranthus hypochondriacus* oil-cake to the rabbit diet did not change meat characteristics of rabbits. Findings of our research also showed no effect of amaranth on the rabbit pre-dressed out live weight, hot carcass weight, dressing out percentage, percentage ratio of heart, kidney, liver, lung weight to carcass weight being coincide with data by Molina et al. (2015) and Mumford et al. (2018) who fed rabbits with sheet amaranth flour *A. dubius*. In contrast, Alfaro et al. (1987) reported an increase in rabbit carcass weight after 15% sheet *Amaranthus hypochondriacus* flour being added to their diet.

The results of our studies and the data of Molina et al. (2018) do not confirm reports of other authors about reducing the weight of rabbits fed with additives of: 20% and 30% barren inflorescences and crude seeds of amaranth (Bamikole et al., 2000); 25%, 50%, 75% of amaranth green mass (Chhay et al., 2013); 30%, 45% and 60% of sheet *Amaranthus hypochondriacus* flour (Alfaro et al., 1987).

Dressing out percentage was almost the same in the groups, 58.0% and 58.28%, accordingly, which is likely explained with genetic factors that have prevailing effect on this value compared to nutrition enriched with plant additives (Daszkiewicz et al., 2012). At chilling of heat carcasses, we reported on a decrease in drip loss percentage by 0.53% in IIa group (with amaranth added) versus I (control) group, however this deviation between the groups was insignificant. In contrast, Molina et al. (2018) determined that dietary addition of amaranth increased moisture loss during chilling of heat rabbit carcasses by 1.21%-1.36%. In both groups of our studies, carcass weight loss after 24-hour chilling were 1.92% and 2.45%, which is in agreement with the findings of Honikel and Hamm (1994), Shevchik et al. (2020) of drip meat losses for 24 hours that may constitute up to 3%.

pH values determined in this study did not change in result by feeding rabbits with amaranth oilcake which is in agreement with data by Molina et al. (2018), Mancini et al. (2018); however, in other studies reported of increase in meat pH for rabbits that were fed with natural plant fodder additives (Cullereet al.,

2016; Zepeda-Bastida et al., 2019).

Determined moisture content in rabbit meat of 71.87 ± 0.24 and $72.65 \pm 0.98\%$ was slightly lower than average rabbit meat moisture values (73.8%) reported by DalleZotte (2014). In addition, we did not find out the effect of amaranth at the meat levels of moisture and protein. Similar findings were obtained by other researchers who also stated that plant fodder additives had no effect on moisture content (Hernández-Martínez et al., 2018) and protein levels (Kone et al., 2016) in the rabbit meat. In contrast, Molina et al. (2018) found out that protein level in meat increased with decrease in moisture in the groups of rabbits fed with amaranth. Cardinali et al. (2015) reported an increase in moisture and protein levels in rabbit meat as result of addition of oregano and rosemary to the rabbit nutrition.

It is well-known that rabbit meat contains cholesterol level lower than meat of other animals with average values as follows: 47.2-61.2 mg/100 g (Dalle Zotte, 2014); 45-67 mg/100g (Dinh et al., 2011), which is similar to cholesterol levels in our studies (51.64mg/100g) in the meat of control group rabbits that were given basal diet. We have found decrease in cholesterol level in meat by 15.07% ($p < 0.05$) as a result of feeding rabbits with amaranth which coincided with statements by Palazzo et al. (2019) who determined cholesterol decrease in rabbit meat by 9.8 and 13% after dietary inclusion of additives with carotenoid pigment and phospholipid complexes. Similarly, it was confirmed by the studies by Plate and Areas (2002) who found out that consumption by rabbits of extruded skimmed amaranth flour reduced the total cholesterol level in blood serum by 50%. Cholesterol lowering action of amaranth is explained by the researches with its phytocomponents as squalen, unsaturated fatty acids, protein, tocotrienols, tocopherols (Caselato and Amaya-Farfan, 2012, Tang and Tsao, 2017).

Water-holding capacity of meat is an important quality that is directly associated with tenderness, juiciness and influences the product output (Qiaofen Cheng and Da-Wen Sun, 2008; Warner, 2014; Lee et al., 2017; Mir Nasiret al., 2017). We did not establish significant changes of feeding amaranth on WHC and meat output after heat treatment which coincided with reports by researches on no effect of plant additives, including amaranth on water-holding capacity of rabbit meat (Alagón et al., 2015; Molina et al., 2018) and boiling losses (Kone et al., 2016; Hernández-Martínez

et al., 2018; Mancini et al., 2018).

No statistically significant difference in meat pH during 9-day shelf-life in between the groups were determined, however we outlined gradual and even increase of pH in both groups: from 5.89 to 6.08 and from 5.92 to 6.1. Increase of pH during storage of rabbit meat in chilled state was explained by Hulot and Ouhayoun (1999) by protein amino acid deamination. On the contrary, pH decrease in the rabbit meat from the 2nd to 8th day of storage was reported by researches as result of ginger powder added to nutrition (Mancini et al., 2018). Drip loss percentage during the meat shelf-life had no significant deviation in groups and varied from 0.62% to 1.27% for 3-day period which coincided with data by Kone et al., 2016, Mancini et al., 2018. Low microorganisms count on the meat surface layers at the beginning of shelf-life indicated that veterinarian and sanitary requirements and technologies for dressing out of rabbits are met (Shevchik et al., 2019). Slow reproduction of microorganisms in the meat of rabbits that were fed with *Amaranthus hypochondriacus* oilcake was reported on the 6th ($p<0.05$) and 9th ($p<0.05$) days of shelf-life which coincided with reports by researches on depression of rabbit meat microflora by adding some plant extracts (Kone et al., 2016), oregano essential oil (Soultos et al., 2009) to nutrition. Emission of 17 phenolic compounds of amaranth leaves and seeds

(Karamaçet al., 2019) and revealed abilities of plant polyphenols to delay growth of some microbes (Papucet al., 2017) may explain the bacteriostatic effect of amaranth. In the future, the mechanism of antibacterial action of amaranth components on rabbit meat needs to be determined in thorough research.

CONCLUSIONS

Research findings have shown that addition of 20% of *Amaranthus hypochondriacus* oilcake to the rabbit diet had no effect on carcass features, pH, moisture content, water-holding capacity, and meat morphological structure. Reduction in cholesterol level by 15.07% ($p < 0.05$) in the meat of rabbits that were fed with amaranth was detected. Such indicators as pH and drip loss percentage did not change significantly in between groups in the shelf-life period. However, microorganisms count in meat of rabbits that were given feed with amaranth was 1.65 and 1.71 ($p < 0.05$) times lower than in the control group on the 6th and 9th days of storage, respectively.

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