Transfer Behavior of Carbendazim from Dried Yingshan Yunwu Tea Leaves into Tea Infusions

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Abstract Tea infusion is the main route of human exposure to pollutants in tea, and it is vital to investigate the transfer rate of pollutants from tea leaves to infusion. Carbendazim is a commonly used systemic fungicide, the transfer behavior of which in Yingshan Yunwu tea is not clear. Hence, this study firstly established the analytical method for determination of carbendazim with great accuracy and precision via modified QuEChERS method and ultra-performance liquid chromatography-fluorescence detection (UPLC-FLD). Then, the transfer behavior of carbendazim from tea leaves and infusion was systematically investigated. Results indicated that water temperature and proportion of tea to water ration would obviously increase the transfer rate of carbendazim, but times of infusion repetition showed negative correlation with the transfer rate of carbendazim. In addition, brewing time seemed to have lesser impact on the transfer rate. This study will be helpful for the risk assessment of carbendazim residue and provide the guidance for tea brewing.

Key words Carbendazim; Yingshan Yunwu tea; Tea fusions; Transfer behavior

DOI:10.19759/j.cnki.2164-4993.2023.05.006

Tea is one of the most consumed drinks in the world because of its special taste and potential health benefits[1-2]. In China, Southern area is hot and moist, which is suitable for tea cultivation, but also favors the growth and distribution of pest and harmful microorganism. Hence, varieties of chemical pesticides were applied in tea plants for avoiding insect attacks and fungal infections, whereas the subsequent pesticides residue in dried tea leaves are of significant concern to consumers. In contrast with edible way of other food, most people drink tea as an infusion which is realized by immersing dried tea leaves in hot water, and faces the exposure of pollutants moving through tea brewing. For this reason, the research of migration patterns of pesticides from leaves into infusions plays a pivotal role in risk assessment of pesticides residue in tea and reasonable application of pesticide in tea cultivation.

Much research has reported the transfer behaviors of varieties of pesticides, such as endosulfan, chlorpyrifos, deltamethrin, fenitrothion, cypermethrin, quinalphos, and dicofol[3-5]. The physiochemical properties of pesticides are the most critical factor affecting their transfer behavior, and different pesticides are diverse in migration patterns. Other than intrinsic factor of pesticides, external factors also play important roles in affecting the transfer behavior of pesticides, such as brewing temperature[6], brewing frequency, the ratio of tea to water and brewing time[7]. Xiao *et al.*[8] found out that the transfer rate of pesticides increased with the rise of temperature, and pesticide solubility was the key factor affecting its migration rate. In addition, the types of tea also affected the transfer behavior. For example, the transfer of organochlorine pesticides from herbal tea leaves to broth was lower compared with the transfers of both black and green teas[9].

Carbendazim is one of the benzimidazole fungicides and applied for control a broad spectrum of fungi pathogens via inhibition of mitotic microtubule formation and cell division[10]. The half-life of carbendazim in soil and water could last for a hundred days, thus it remains persistent in soil[11]. Furthermore, for plant origin, the carbendazim could remain stable after processing by cooking, brewing, pasteurization and sterilization. It has been reported that carbendazim is detected in tea and make it exposure to human[12-13]. Different from other food, tea infusion is the main route of human exposure to pollutants in tea, and it is vital to investigate the transfer rate of pollutants from tea leaves to infusion. However, the transfer behavior of carbendazim in Yingshan Yunwu tea is not clear.

In this study, the analytical method for analyzing carbendazim in Yingshan Yunwu tea leaves and infusion was established to systematically investigate the transfer behavior of carbendazim from tea leaves and infusion. In addition, brewing time, water temperature, brewing frequency and ratio of tea to water were chosen to study their effects on the transfer rate of carbendazim from tea leaves to infusion.

Materials and Methods

Materials and reagents

Standard of carbendazim and sodium chloride (NaCl) was purchased from Aladdin Biochemical Technology Co., Ltd (Shanghai, China). Acetonitrile and methanol with HPLC-grade

Received: July 2, 2023 Accepted: September 6, 2023

Supported by Hubei Key Laboratory of Economic Forest Germplasm Improvement and Resources Comprehensive Utilization, Hubei Collaborative Innovation Center for the Characteristic Resources Exploitation of Dabie Mountains Fund (2021CX06); Teaching and Research Project of Huanggang Normal University (2021CE20).

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were bought from Fisher Scientific (MA, New Jersey, USA). Primary secondary amine (PSA) was provided from Bonna-Agela Technologies (Tianjin, China). Ultrapure water was obtained from a Milli-Q system by Merck Millipore (Merck KGaA, Darmstadt, Germany).

Standard solution preparation

The carbendazim standard stock solution of 100 mg/L was prepared by dissolving 10 mg of carbendazim standard in 100 ml of methanol. Then, a series of working standard solutions were obtained from dilution of stock solution with methanol. All standard solutions were stored in the dark at −20 ℃ for later use.

Sample collection

Pesticide-free Yingshan Yunwu tea was provided by Hubei Zhishun Tea Co., Ltd (Huanggang, China), which was used as blank matrix. Carbendazim-contaminated tea samples were obtained by steeping blank sample into working standard solutions of 100 mg/L and standing for 2 h, following natural drying. Tea infusion was prepared on the basis of the most common method. In briefly, 5 g of dry tea leaves were placed in glass flask with the addition of 150 ml of boiling water at 100 ℃ for brewing 5 min. The contents of carbendazim in tea infusion and tea leaves samples were determined to unveil the distribution of carbendazim between infusion and leaves. In addition, effects of brewing time, water temperature, time of infusion repetition and ratio of tea to water were studied to find out the transfer behavior of carbendazim in tea brewing process.

Sample extraction and clean-up

Tea infusion and leaves samples were treated with QuEChERS procedure for the extraction and clean-up of carbendazim. Briefly, 1 ml of cooling tea infusion was placed in centrifuge tube containing 50 mg of primary secondary amine (40 − 60 μm) absorbent, and then vortexing was performed for 3 min. The tube was centrifuged at 5 000 rpm for 1 min to obtain the supernatant, which was filtered with 0.22 μm nylon microporous membrane and analyzed with ultra-performance liquid chromatography-fluorescence detection (UPLC-FLD). For tea leaves sample, 2 g of sample was weighed in a 15 ml centrifuge tube, and mixed with 10 ml of acetonitrile. After 10 min of sonication, 1 g of NaCl was added following by 5 min of sonication. Then, the tube was centrifuged at 4 000 rpm for 3 min, and 1 ml of supernatant was transferred in another 2 ml centrifuge tube containing 50 mg of PSA absorbent. The centrifuge tube was capped and vortexed for 3 min, and centrifuged at 5 000 rpm for 1min. The supernatant was filtered and analyzed with UPLC-FLD.

UPLC-FLD analysis

The analysis of carbendazim was performed with an Ultimate 3000™ HPLC system and a FLD-3000 fluorescence detector (Thermo Fisher Scientific, USA) with an Inertsil ODS-3 column (4.6 mm i. d. ×250 mm, 5 μm particle size). The mobile phase was composed with 70% acetonitrile and 30% water, the flow rate was 1 ml/min, and the injection volume was 10 μl. The excitation and emission wavelength of carbendazim were 285 and 315 nm, respectively.

Method validation

The stock carbendazim standard solution was diluted to a series of working solutions at varies level (0.5, 1, 5, 10, 15, 25, 50 mg/L) with blank sample extracts, which were determined by UPLC-FLD method. The calibration curves were conducted according to the peak areas of carbendazim and concentration of working solutions. Then, three concentrations of carbendazim (2.5, 5, 25 mg/L) was spiked in the blank samples for evaluating the accuracy and precision of the method, and five replicates were analyzed for every concentration.

Data calculation and analysis

The transfer rate of carbendazim from tea leaves to infusion was calculated based on following equation:

$$Rt\ (\%) = (C_{infusion} \times V_{infusion}) / (C_{leaves} \times m_{leaves})$$

Where Rt (%) is transfer rate, $C_{infusion}$ (mg/L) and C_{leaves} (mg/g) represent the concentration of carbendazim in tea infusion and leaves. $V_{infusion}$ (ml) and m_{leaves} (g) are volume of infusion and weight of leaves.

Results and Discussion

Method validation

The calibration curve, recoveries and precision of carbendazim in tea infusion and leaves are shown in Table 1. As shown, the calibration curves of carbendazim in infusion and leaves were $y = 141\,043x - 52\,180$ and $y = 149\,779x + 259\,676$ with the linear correlation coefficient (R^2) ranging from 0.998 4 to 0.999 4, which indicated that the relation between peak area and carbendazim concentration displayed good linearity. Then, the recoveries of analytes in tea infusion and tea leaves spiked with three concentrations of carbendazim ranged from 82.3% to 105.4% and 86.5% to 92.6%, respectively. In addition, *RSD* of five replicates for every group were all less than 7%. Hence, the data of recoveries and *RSD* elucidated the analysis method was of great accuracy and precision.

Table 1 Calibration curve, recoveries and precision of carbendazim in tea samples

Sample	Calibration curve	R^2	Concentration of carbendazim // mg/L	Recovery // %	*RSD* // %
Tea infusion	$y = 141\,043x - 52\,180$	0.998 4	2.5	95.4	3.9
			5	105.4	3.7
			25	82.3	1.8
Tea leaves	$y = 149\,779x + 259\,676$	0.999 4	2.5	86.5	6.3
			5	92.6	6.9
			25	88.7	3.0

Transfer behavior of carbendazim from dried Yingshan Yunwu tea leaves into tea infusions

Effect of brewing time Brewing time is of great significance during making tea infusion. The effect of brewing time from 5 to 30 min on the distribution and transfer rate of carbendazim was investigated. The mass distribution of carbendazim in tea infusion and remaining leaves with different brewing time was demonstrated in Fig. 1, which indicated nearly all of carbendazim entered infusion and little was in remaining leaves. In addition, the effect of brewing time (5 – 30 min) on the carbendazim distribution was not obvious, which might be attributed to the quick transfer behavior of carbendazim due to its high-water solubility (29 mg/L pH 4; 8 mg/L pH 7)[14]. As shown in Fig. 2, the transfer rates of carbendazim ranging from 77.3% to 92.6% also verified its fast transfer.

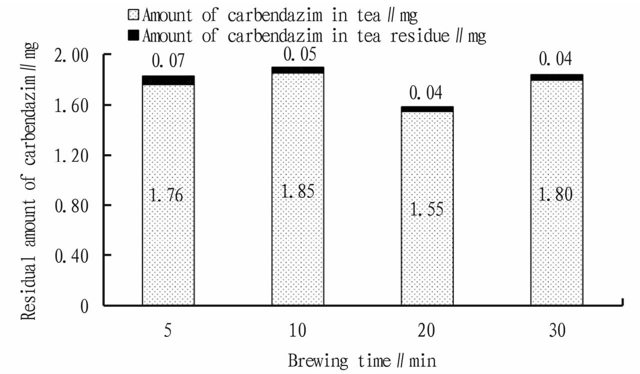

Fig. 1 Effect of brewing time on the distribution of carbendazim in tea infusion and remaining leaves

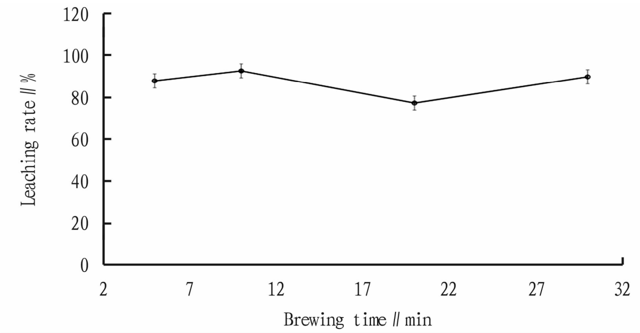

Fig. 2 Effect of soaking time on the transfer rate of carbendazim from tea leaves to infusion

Effect of water temperature Water temperature is of great significant during tea brewing process, and could give different flavors. The ideal temperature for tea varied depending on the type of tea, commonly ranging from 70 to 100 ℃. Thus, the effect of water temperature (70 – 100 ℃) on the transfer behavior of carbendazim was investigated, and 25 ℃ was set as control group. Results in Fig. 3 demonstrated similar trend in Fig. 1, which shown nearly all of the carbendazim entered in infusion and little was in remaining leaves. However, dramatic change of the transfer rate was observed in Fig. 4 when water temperature increased from 25 to 70 ℃. It indicated that water temperature showed positive correlation with transfer rate of carbendazim, which was the same as the results reported by Chen[15].

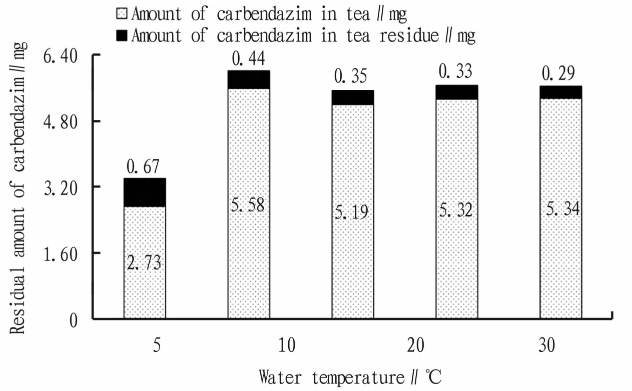

Fig. 3 Effect of water temperature on the distribution of carbendazim in tea infusion and remaining leaves

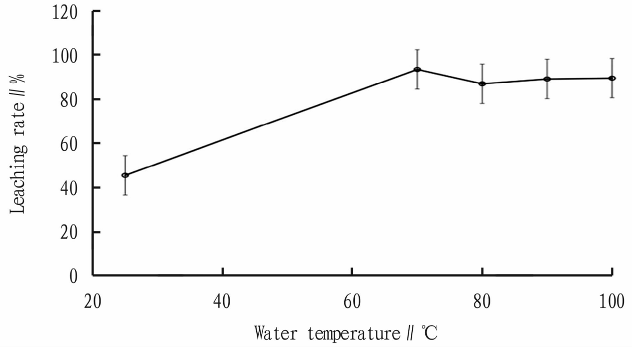

Fig. 4 Effect of water temperature on the transfer rate of carbendazim from tea leaves to infusion

Effect of times of infusion repetition In order to realize favorable taste and aroma, tea was usually brewed twice or more times. Other than the aroma and taste of tea infusion, the time of infusion repetition also affected the transfer rate of carbendazim. As shown in Fig. 5, the detected amount of carbendazim in infusion decreased in the first three brewing and kept stable in the last two brewing. However, the amount of carbendazim in remaining leaves was similar to previous tea leaves after repeatedly brewing. The reason might be carbendazim belong to systemic fungicide[16] and could be absorbed in the tea leaves. Thus, the carbendazim could be still detected in the infusion and remaining leaves even though the brewing process had been repeated for five times.

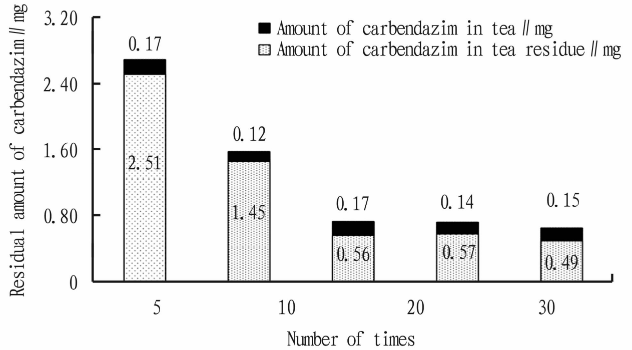

Fig. 5 Effect of times of infusion repetition on the distribution of carbendazim in tea infusion and remaining leaves

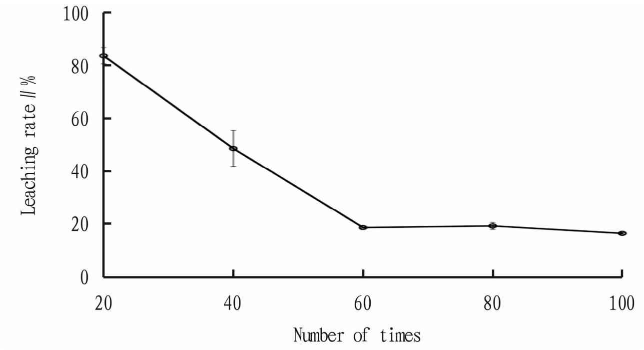

Fig. 6 Effect of times of infusion repetition on the transfer rate of carbendazim from tea leaves to infusion

Effect of tea-to-water ratio The ratio of tea to water is another factor which greatly influenced the taste of infusion, and a commonly used ratio of tea to water is 1 : 50. Hence, this study investigated the effect of ratio of tea to water (1 : 10 – 1 : 100) on the transfer rate of carbendazim from tea leaves to infusion. Results in Fig. 7 and Fig. 8 indicated that both the amounts of carbendazim in tea infusion and transfer rate possessed positive correlation with the ratio of tea to water, while the amount of carbendazim in the remaining leaves decreased with the ratio of tea to water increasing.

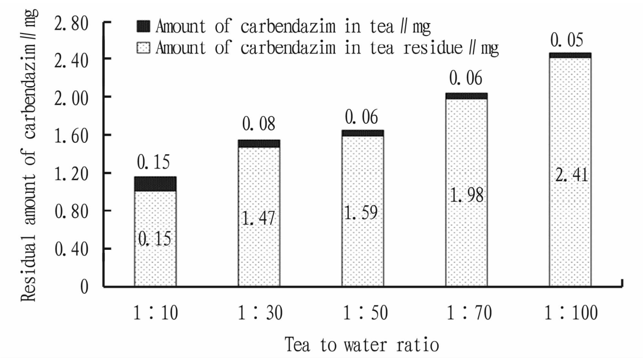

Fig. 7 Effect of proportion of tea to water ratio on the distribution of carbendazim in tea infusion and remaining leaves

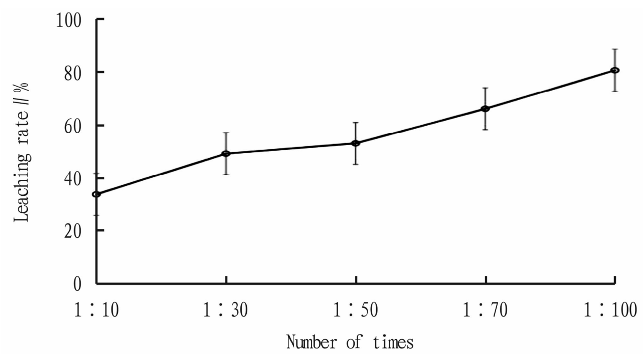

Fig. 8 Effect of proportion of tea to water ratio on the transfer rate of carbendazim from tea leaves to infusion

Conclusions

Tea infusion is the main route of human exposure to pollutants in tea, and it is vital to investigate the transfer rate of pollutants from tea leaves to infusion. Carbendazim, as a commonly used systemic fungicide, the transfer behavior of which in Yingshan Yunwu tea is not clear. Hence, this study firstly established the analytical method for determination of carbendazim with great accuracy and precision. Then, the transfer behavior of carbendazim from tea leaves and infusion was systematically investigated. Results indicated that water temperature and ratio of tea to water would obviously increase the transfer rate of carbendazim, but times of infusion repetition showed negative correlation with the transfer rate of carbendazim. In addition, brewing time seemed to have lesser impact on the transfer rate. This study will be helpful for the risk assessment of carbendazim residue and provide the guidance for the tea brewing.

References

[1] QIU X, WANG J, YU X, *et al*. Aroma formation in Dianhong black tea: Effects of baking[J]. Int J Food Prop, 2017, 20(11): 2724 – 2735.

[2] RAMESHRAD M, RAZAVI BM, HOSSEINZADEH H. Protective effects of green tea and its main constituents against natural and chemical toxins: A comprehensive review[J]. Food Chem Toxicol, 2017(100): 115 – 137.

[3] WAN H, XIA H, CHEN Z. Extraction of pesticide residues in tea by water during the infusion process[J]. Food Additives & Contaminants, 1991, 8(4): 497 – 500.

[4] JAGGI S, SOOD C, KUMAR V, *et al*. Leaching of pesticides in tea brew [J]. J Agric Food Chem, 2001, 49(11): 5479 – 5483.

[5] MANIKANDAN N, SEENIVASAN S, GANAPATHY M N K, *et al*. Leaching of residues of certain pesticides from black tea to brew[J]. Food Chem, 2009, 113(2): 522 – 525.

[6] CHO S-K, ABD EL-ATY AM, RAHMAN MM, *et al*. Simultaneous multi-determination and transfer of eight pesticide residues from green tea leaves to infusion using gas chromatography[J]. Food Chem, 2014 (165): 532 – 9.

[7] MIAO S, WEI Y, PAN Y, *et al*. Detection methods, migration patterns, and health effects of pesticide residues in tea[J]. Compr Rev Food Sci Food Saf, 2023, 22(4): 2945 – 2976.

[8] XIAO JJ, LI Y, FANG QK, *et al*. Factors affecting transfer of pyrethroid residues from herbal teas to infusion and influence of physicochemical properties of pesticides[J]. Int J Environ Res Public Health, 2017, 14 (10): 1157.

[9] WITCZAK A, ABDEL-GAWAD H, ZALESAK M, *et al*. Tracking residual organochlorine pesticides (OCPs) in green, herbal, and black tea leaves and infusions of commercially available tea products marketed in Poland [J]. Food Additives & Contaminants: Part A, 2018, 35(3): 479 – 486.

[10] MAGNUCKA EG, SUZUKI Y, PIETR SJ, *et al*. Action of benzimidazole fungicides on resorcinolic lipid metabolism in rye seedlings depends on thermal and light growth conditions[J]. Pestic Biochem Physiol, 2007, 88(2): 219 – 225.

[11] ARYA R, SHARMA R, MALHOTRA M, *et al*. Biodegradation aspects of carbendazim and sulfosulfuron: Trends, scope and relevance[J]. Curr Med Chem, 2015, 22(9): 1147 – 1155.

[12] WANG D, YANG G, YUN X, *et al*. Carbendazim residue in plant-based foods in China: Consecutive surveys from 2011 to 2020[J]. Environ Sci Ecotechnol, 2024(17): 100301.

[13] LEE K-G, LEE S-K. Monitoring and risk assessment of pesticide residues in Yuza fruits (Citrus junos Sieb. ex Tanaka) and yuza tea samples produced in Korea[J]. Food Chem, 2012, 135(4): 2930 – 2933.

[14] ZHOU L, JIANG Y, LIN Q, *et al*. Residue transfer and risk assessment of carbendazim in tea[J]. J Sci Food Agric, 2018, 98(14): 5329 – 5334.

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growth and improving meat quality, *etc.* As a new multifunctional feed additive, it has been paid more and more attention, but several key problems about its biological activity still need to be solved: ① corresponding relationship between specific biological functions, especially the immunomodulation of *E. ulmoides* leaf extract, and single active compounds in the extract, and its action pathway, ② analysis of the effects of various functional active ingredients on different cultured animals, and ③ optimization of preparation conditions of *E. ulmoides* leaf extract, especially the study on the differences of active components in extracts obtained by different processes and their effects on *in-vitro* and *in-vivo* functions. Therefore, in-depth analysis of the relationship between single active compound components and appropriate compatible combinations, and their biological functions will still be an important direction in the future. With the continuous improvement of research, the application of *E. ulmoides* leaf extract as a multifunctional feed additive in livestock and poultry breeding will receive more and more attention. This paper explored the use of *E. ulmoides* leaves in the growth process of livestock and poultry for reducing animal emissions, improving environmental protection, effectively promoting animal growth, improving animal immunity, and reducing the occurrence of animal diseases, hoping to serve agriculture, rural areas, and farmers, improve quality and efficiency, and assist in rural revitalization.

References

[1] XIE YX, XIAO KN, SU M, *et al.* Research progress and quality markers prediction of Duzhongye (Eucommiae Folium)[J]. Journal of Shandong University of Traditional Chinese Medicine, 2023, 47(2): 243-249. (in Chinese).

[2] ZHANG QC. Study on extraction technology of chemical active components from *Eucommia ulmoides* leaves[J]. Liaoning Chemical Industry, 2022, 51(10): 1379-1381. (in Chinese).

[3] HUANG CF, ZHANG Z, WANG Z, *et al.* Study on difference of chlorogenic acid content in *Eucommia ulmoides* leaves under different harvesting time and optimization of chlorogenic acid extraction process[J]. Feed Research, 2022, 45(15): 84-87. (in Chinese).

[4] YANG YH, LIANG XX, YIN YJ, *et al.* Effect of *Eucommia* leaf extract on the quality and antioxidant performance of pork from fattening pigs [J]. Chinese Journal of Animal Science, 2022, 58(8): 262-269. (in Chinese).

[5] SONG HJ. Effects of *Eucommia ulmoides* and its extracts on animal production and economic benefits[J]. Feed Research, 2022, 45(11): 156-159. (in Chinese).

[6] WANG TT, JIA J, YANG WW. Research progress in the extraction methods of flavonoids from *Eucommia ulmoides*[J]. China Fruit & Vegetable, 2022, 42(5): 34-37. (in Chinese).

[7] ZHANG WJ, XU QY, LIANG Y. Extraction technology of chemical components from Euconnia ulmoides leaves and its application in animal husbandry [J]. Animals Breeding and Feed, 2022, 21(5): 21-25. (in Chinese).

[8] XIANG Q, PENG S, SONG JQ. Application of *Eucommia* leaves extract in animal husbandry[J]. China Feed, 2022(8): 113-116. (in Chinese).

[9] SONG JQ, YAN LN, LI H, *et al.* Effects of the extracts from *Eucommia ulmoides* leaves on the growth, development and immunity of Xiangxi hybrid chicken[J]. China Feed, 2021(24): 29-32. (in Chinese).

[10] HUANG L, ZHOU B, FU ZQ, *et al.* Research progress on the biological functions of extracts from *Eucommia ulmoides* leaf and its application in animal production[J]. Chinese Journal of Animal Science, 2022, 58(6): 60-65. (in Chinese).

[11] LYU GX, HE F, QING J, *et al.* Effect of *Eucommia ulmoides* leaves powder on production performance and egg quality in Hubei tribute chickens[J]. Feed Research, 2021, 44(15): 50-54. (in Chinese).

[12] XU NN. Active constituents of *Eucommia ulmoides* and application in livestock production and disease control [J]. Feed Research, 2021, 44(4): 150-153. (in Chinese).

[13] ZUO XY, WANG FR, XIE ZG, *et al.* Research progress on the application of *Eucommia ulmoides* and its extracts in livestock and poultry production[J]. Guangdong Feed, 2019, 28(11): 32-36. (in Chinese).

[14] PENG MJ, ZHANG ML, WANG ZH, *et al.* Effects of dietary addition of *Eucommia* leaves for antibiotic on growth performance, blood biochemical parameters and immune organs of weaning piglets[J]. Natural Product Research and Development, 2019, 31(4): 675-681. (in Chinese).

[15] JIANG DC, YANG N, SHAN YF, *et al.* *Eucommia ulmoide* leaf and its extract application in livestock and poultry production [J]. China Feed, 2019(3): 57-61. (in Chinese).

[16] ZHOU Y, WEN Y, SHAN HT, *et al.* Research on processing technology of natural plant for livestock breeding[J]. China Animal Health, 2018, 20(4): 40-44. (in Chinese).

[17] KANG KL, CHEN JF, QU XY, *et al.* Effect of *Eucommia ulmoides* leaf on production performance, egg quality and economic benefit of laying hens[J]. China Poultry, 2018, 40(2): 20-24. (in Chinese).

[18] MAO GD, XIAO H. Application of *Eucommia ulmoides* and its extracts in aquatic products[J]. Scientific Fish Farming, 2017(12): 90. (in Chinese).

[19] ZHAO S, DUAN MF, HU HW, *et al.* Function and application of *Eucommia ulmoides* leaves in animal[J]. Feed Review, 2017(3): 27-30. (in Chinese).

[20] ZHU D, ZHANG T, ZHANG PH, *et al.* The functions of Duzhong (*Eucommia ulmoides* Oliver) leaf and its application in livestock and poultry production were reviewed[J]. China Feed, 2013(18): 25-27, 37. (in Chinese).

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU

(Continued from page 29)

[15] CHEN H, PAN M, LIU X, *et al.* Evaluation of transfer rates of multiple pesticides from green tea into infusion using water as pressurized liquid extraction solvent and ultra-performance liquid chromatography tandem mass spectrometry[J]. Food Chem, 2017(216): 1-9.

[16] KOUKOUVINOS G, KARACHALIOU CE, RAPTIS I, *et al.* Fast and sensitive determination of the fungicide carbendazim in fruit juices with an immunosensor based on white light reflectance spectroscopy[J]. Biosensors (Basel), 2021, 11(5).

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU