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Effect of drinking *Houttuynia cordata* Thunb extract supplement on growth performance and colony count outcomes in ICR mice

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Abstract

The purpose of this study was to investigate the safety of *Houttuynia cordata* Thunb extract (HCE) in Institute of Cancer Research (ICR) mice, to determine the effects of the extract on the growth performance and colony count, and to establish the optimal concentration of HCE. In total, 60 five-week-old male ICR mice with an average initial body weight (BW) of 27.24 ± 0.44 g were used in a four-week experiment. Mice were randomly allotted to four treatment groups (five replications per group, three mice per cage): 1) a control (CON) group fed with normal distilled water; 2) treatment group 1 (T1) fed with normal distilled water containing 0.05% HCE; 3) treatment group 3 (T3) fed with normal distilled water containing 0.1% HCE; and 4) treatment group 3 (T3) fed with normal distilled water containing 0.2% HCE. BW, feed intake (FI), and water intake were measured on the first, fourteenth, and eighteenth days. T2 showed a significant improvement ($p < 0.05$) in the feed conversion ratio (FCR) over the experimental period. However, water intake levels did not show significant differences among the groups. In the large intestine and feces, *E. coli* and *Lactobacillus* levels were significantly improved ($p < 0.05$) in the HCE treated group compared to the CON group. Supplying HCE via the drinking water improved the growth performance and colony count in ICR mice. Based on results of this study, utilizing HCE in livestock species is expected to be safe and feasible.

Key words: *Houttuynia cordata* Thunb, *Houttuynia cordata* Thunb extract, mice

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Introduction

Many phytogenic extracts are being studied as additives. Phytogenic extracts can improve antibacterial and antioxidant activities and inflammatory responses (Go et al., 2021; Song et al., 2021). In addition, phytogenic extracts can increase growth performances of broilers. They are also easy to use because there is no residual effect (Hashemi and Davoodi, 2010). *Houttuynia cordata* Thunb (HC) is a perennial herb belonging to the family Trifoliumaceae. It is a plant that grows wild in the southeastern part of Asia and the central region of Korea. Leaves and flowers of HC contain quercitrin, quercetin, rutin, hyperin, isoquercetin, aristolactams, and decanoyl acetaldehyde. These functional

materials have been proven to have antibacterial action against Gram-negative bacteria. They also possess sterilization and bacteriostatic action against *E. coli*, paratyphoid group, gonorrhea, and so on (Kang et al., 1997; Kim et al., 1997; Cho et al., 2008). HC fermented with lactic acid bacteria has excellent antioxidant capacity (Kim et al., 2016). In east Asia, HC is also used for tea, cosmetic, and medicinal ingredient (Choi, 2016). Li et al. (2005) have suggested that water extract of HC might be useful for treating of mast cell-mediated anaphylactic reactions. Seul et al. (2007) have reported that a dietary mixture of *oldenlandiae herba* and HC could mitigate the incidence of diarrhea in calves. However, studies on the effect of supplement HCE on intestinal microflora using animal models are lacking.

Therefore, the purpose of this study was to determine the safety of HCE and its effect on growth performance, colony count, and optimal concentration in Institute of Cancer Research (ICR) mice.

Materials and Method

Ethics

The experimental protocol was approved (CBNUA-1643-21-01) by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea.

Houttuynia cordata Thunb extract (HCE)

HCE was provided by Dronic (Dronic Co., Sungnam, Korea). It mainly contained flavonoids and it was consisted of rutin (95.61%), isoquercetin (1.98%), quercetin (0.89%), quercitrin (1.47%), and afzelin (0.06).

Animals and experimental design

Five-week-old male ICR mice were obtained from DBL (Incheon, Korea). A total of 60 ICR mice with an average initial body weight (BW) of 27.24 ± 0.44 g were used in 4 weeks. The mice were reared in plastic cages in a temperature-controlled room with a 12 h light/12 h dark cycle. In 5 repetitions with 4 treatment groups, mice were randomly assigned to 3 mice per cage. Treatment groups were as follows: the first group (CON) was fed normal distilled water; the second group (T1) was fed normal distilled water with 0.05% HCE; the third group (T2) was fed normal distilled water with 0.1% HCE; the fourth group (T3) was fed normal distilled water with 0.2% HCE. Commercial feed and HCE containing drinking water were provided *ad libitum* for an experimental period.

Sample analysis and measurements

The body weight gain (BWG) was calculated by weighing each participant on the 1st, 14th, and 28th day of the experiment. When measuring body weight, feed intake (FI) was calculated by subtracting the remaining amount from the feeding amount, and the feed conversion ratio (FCR) was calculated by dividing the BWG by the FI. Similarly, drink is measured by subtracting the remaining amount from the serving amount.

Samples for colony count were collected form the large intestine and feces at the end of the experiment. After homogenization by suspending in aseptic distilled water, samples were used for measuring the number of viable

microorganisms by serial dilution from 10^{-2} to 10^{-6}. In order to measure the colony count of *Lactobacillus* and *E. coli*, de MAN, ROGOSA and SHARPE (MRS agar) for *Lactobacillus*, and MacConkey agar for *E. coli* were used, and *E. coli* was cultured at 37℃ for 20 hours, and *Lactobacillus* was cultured for 48 hours.

Statistical analysis

All data were analyzed of variance (ANOVA) for a completely random design using the general linear model (GLM) procedure of SAS software (SAS Institute, Cary, NC, USA). Duncan's multiple range test identified significant differences ($p < 0.05$) among treatments. All statistical analysis differences were taken to be significant at $p < 0.05$, and $p < 0.01$ were considered clearly significant.

Result and Discussion

Growth performance and water intake

Table 1 shows effects of HCE in drinking water for four weeks on growth performance of ICR mice. The T2 group has significantly higher FCR ($p < 0.05$) and BWG ($p < 0.05$) than other treatment groups at 0 - 1 weeks and 0 - 2 weeks. During the entire experimental period, the T2 group showed a significantly improved ($p < 0.05$) FCR than other treatment groups. In the present study, FCR and BWG showed quadratic effects (Q, $p < 0.01$) according to the amount of HCE at 0 - 1weeks and 0 - 2 weeks.

HCE did not significantly affect the amount of drinking water intake (Table 2). However, water intake was increased linearly (Lin, $p < 0.01$) with increasing concentration of HCE.

Cho et al. (2012) have reported that HC can improve the growth performance of weanling-growing pigs. Hah et al. (2010) have reported that when broilers are challenged with aflatoxin B1 toxin, a dietary mixture of activated carcoal and HC could improve feed efficiency by reducing aflatoxin B1 toxin. When 1 $g \cdot kg^{-1}$ flavonoid rutin additive is used, BW, BWG, and FCR of broilers are improved (Hassan et al., 2019). Flavonoids present in HC exhibit a wide range of biological effects, including anti-inflammatory, anti-allergic, anti-virus, anti-bacteria, and anti-oxidation activities (Jian and Xiao, 1986; Narayana et al., 2001; Havsteen, 2002). Flavones may also promote growth in animals by raising insulin-like growth factor 1 concentrations (Ouyang et al., 2016). It is thought that HC can improve the growth performance of mice due to the above positive effects. However, research about the effect of HC on growth performance of mice is insufficient. Thus, additional research is needed.

Colony count

Tables 3 and 4 show the effect of HCE on *E. coli* and *Lactobacillus* counts in the large intestine and feces. At the 2nd and 4th weeks, the T2 group showed significantly decreased ($p < 0.05$) number of *E. coli* in the large intestinal compared to other treatment groups. It showed a decreasing (Q, $p = 0.01$) trend when the amount of HCE added was increased. The T1 group showed a significant increase ($p = 0.01$) in the number of *Lactobacillus* in the large intestinal at the 2nd week. At the 4th week the T1 group had the highest number of *Lactobacillus* ($p < 0.05$). However, there was no significant difference from the other treatment groups.

Table 1. Effect of drinking *Houttuynia cordata* Thunb extract supplement on growth performance in ICR mice.

Item	Treatment[z]				SE	p-value	Linear	Quadratic
	CON	T1	T2	T3				
BW								
Initial	81.73	81.72	81.71	81.65	2.62	1.00	0.99	0.98
1 w	91.90	89.86	95.16	93.44	3.68	0.77	0.98	0.42
2 w	95.41	92.46	99.20	95.67	3.75	0.66	0.83	0.46
3 w	101.75	91.94	103.14	97.68	5.07	0.42	0.98	0.70
Final	104.05	94.49	106.55	99.70	5.08	0.37	0.84	0.67
0 - 1 w								
BWG	10.17ab	8.14b	13.45a	11.79ab	1.41	0.07	0.93	0.04
FI	83.62	83.64	85.49	86.04	3.70	0.95	0.84	0.56
FCR	9.15b	12.54a	6.97b	7.46b	0.87	< 0.01	0.15	< 0.01
1 - 2 w								
BWG	3.51	2.60	4.04	2.23	3.51	0.09	0.11	0.73
FI	80.76	84.11	83.10	84.46	3.83	0.90	0.96	0.64
FCR	27.43	38.40	33.82	48.25	6.95	0.21	0.28	0.14
0 - 2 w								
BWG	13.68ab	10.74b	17.49a	14.02ab	1.60	0.04	0.64	0.08
FI	84.27	86.71	87.14	86.69	4.01	0.96	0.86	0.69
FCR	12.98b	17.18a	10.25b	12.67b	0.99	< 0.01	0.79	< 0.01
2 - 3 w								
BWG	4.27	2.83	4.37	3.30	0.87	0.53	0.78	0.97
FI	93.90	89.24	86.97	82.92	5.07	0.50	0.62	0.16
FCR	52.30	36.05	30.87	34.05	10.71	0.51	0.69	0.24
3 - 4 w								
BWG	2.31ab	2.55ab	3.41a	2.02b	0.40	0.11	0.03	0.67
FI	88.61	87.56	86.27	84.05	3.95	0.86	0.68	0.43
FCR	40.64ab	38.46ab	29.81b	76.26a	14.49	0.14	0.03	0.24
2 - 4 w								
BWG	6.57	5.38	7.79	5.32	6.57	0.24	0.24	0.83
FI	182.51	176.80	173.24	166.96	8.88	0.66	0.64	0.24
FCR	33.09	32.98	26.68	33.99	3.43	0.42	0.23	0.58
0 - 4 w								
BWG	21.08	16.68	24.08	19.38	2.48	0.22	0.61	0.45
FI	351.92	337.44	339.09	334.89	16.67	0.89	0.99	0.58
FCR	18.29ab	20.95a	15.06b	18.09ab	1.26	0.02	0.61	0.06

ICR, Institute of Cancer Research; BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; HCE, *Houttuynia cordata* Thunb extract; SE, standard error.

[z] CON, fed with normal distilled water; T1, fed with normal distilled water containing 0.05% HCE; T2, fed with normal distilled water containing 0.1% HCE; T3, fed with normal distilled water containing 0.2% HCE.

a, b: Means within a row with different letters are significantly different at $p < 0.05$.

Table 2. Effect of drinking *Houttuynia cordata* Thunb extract supplement on water intake in ICR mice.

Item	Treatment[z]				SE	p-value	Linear	Quadratic
	CON	T1	T2	T3				
0 - 2 w								
Drink	304.00	298.00	291.00	291.00	14.01	0.90	0.99	0.46
HCE	0.00d	0.16c	0.29b	0.58a	0.02	< 0.01	< 0.01	< 0.01
2 - 4 w								
Drink	108.75	121.25	101.25	97.50	13.27	0.61	0.52	0.29
HCE	0.00c	0.06b	0.10b	0.20a	0.02	< 0.01	< 0.01	0.37
0 - 4 w								
Drink	436.00	411.25	410.00	403.75	20.37	0.69	0.99	0.34
HCE	0.00d	0.22c	0.41b	0.81a	0.02	< 0.01	< 0.01	< 0.01

ICR, Institute of Cancer Research; HCE, *Houttuynia cordata* Thunb extract; SE, standard error.

[z] CON, fed with normal distilled water; T1, fed with normal distilled water containing 0.05% HCE; T2, fed with normal distilled water containing 0.1% HCE; T3, fed with normal distilled water containing 0.2% HCE.

a - d: Means within a row with different letters are significantly different at $p < 0.05$.

Table 3. Effect of drinking *Houttuynia cordata* Thunb extract supplement on large intestinal colony count in ICR mice.

Item (log_{10} CFU·g^{-1})	Treatment[z]				SE	p-value	Linear	Quadratic
	CON	T1	T2	T3				
E. coli								
2 w	8.97a	8.57b	7.93c	8.52b	0.09	0.01	0.13	0.01
4 w	5.66a	5.70a	4.71b	5.52a	0.18	0.03	0.28	0.02
Lactobacillus								
2 w	8.77b	9.63a	9.30a	8.71b	0.12	0.01	0.23	0.01
4 w	10.23	10.10	10.06	9.94	0.11	0.45	0.57	0.18

ICR, Institute of Cancer Research; HCE, *Houttuynia cordata* Thunb extract; SE, standard error.

[z] CON, fed with normal distilled water; T1, fed with normal distilled water containing 0.05% HCE; T2, fed with normal distilled water containing 0.1% HCE; T3, fed with normal distilled water containing 0.2% HCE.

a - c: Means within a row with different letters are significantly different at $p < 0.05$.

Table 4. Effect of drinking *Houttuynia cordata* Thunb extract supplement on fecal colony count in ICR mice.

Item (log_{10} CFU·g^{-1})	Treatment[z]				SE	p-value	Linear	Quadratic
	CON	T1	T2	T3				
E. coli								
2 w	8.44	8.48	8.46	8.50	0.14	0.99	0.90	0.87
4 w	6.49	6.95	6.37	7.08	0.21	0.18	0.19	0.60
Lactobacillus								
2 w	8.30	8.72	7.68	8.07	0.55	0.63	0.98	0.37
4 w	9.81b	10.36a	10.12ab	10.35a	0.13	0.04	0.07	0.42

ICR, Institute of Cancer Research; HCE, *Houttuynia cordata* Thunb extract; SE, standard error.

[z] CON, fed with normal distilled water; T1, fed with normal distilled water containing 0.05% HCE; T2, fed with normal distilled water containing 0.1% HCE; T3, fed with normal distilled water containing 0.2% HCE.

a, b: Means within a row with different letters are significantly different at $p < 0.05$.

In this study, adding HCE to drinking water showed a significant effect on *E. coli* and *Lactobacillus* concentration in intestine and feces. Similarly, Wang et al. (2018) have reported that dietary HC supplement in high fat diet could reduce *E. coli* concentration in mice. In addition, Kim et al. (2010) have suggested that the mechanism of action of *E. coli* reduction upon HC addition involves a multifaceted process including inhibition of cell wall synthesis, impaired folate synthesis, and alteration of outer membrane permeability through modulation of multiple drug efflux pumps. The production of quercetin from rutin in HC can affect intestinal flora. Decanal, endobornyl acetate, fenchene, decarnoic acid, and decanoyl acetaldehyde show strong antibacterial activities against *E. coli* (Kang et al., 1997; Tamura et al., 2007; Weng et al., 2017). Quercetin supplementation can also enhance populations of *Bacteroides*, *Bifidobacterium*, *Lactobacillus*, and *Clostridia* and significantly reduce populations of *Fusobacterium* and *Enterococcus* (Lin et al., 2019).

Conclusion

Supplementation of HCE in drinking water showed a positive effect on growth performance and colony count in mice. When looking at the mice FCR and colony count of large intestine, 0.1% HCE supplementation can be seen as the optimal concentration. Through this study, HCE is thought that it will be possible to develop HCE supplements through additional research using other livestock models.

Conflict of Interests

No potential conflict of interest relevant to this article was reported.

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