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Health benefits of co-supplementing mealworm protein hydrolysate and cranberry fruit extract

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PAPER

Abstract

The demand for valuable protein sources is increasing. The mealworm has been highlighted as a good source of protein. Nevertheless, beneficial effects of mealworm such as the antioxidative and/or anti-inflammatory effects are rarely studied. It is well-known that cranberry fruit has a strong antioxidant effect. The biologically active compounds in mealworm and cranberry could boost the antioxidative and/or anti-inflammatory effects. The current study investigated the interactive effects of mealworm protein hydrolysate (MWPH) and cranberry fruit extract (CFE) in mammals. We evaluated growth performance, relative organ weight, immune responses, antioxidant enzyme activities, blood properties, and fecal microflora. A 2 × 2 factorial experimental design was used. The co-supplementation of MWPH and CFE improved serum glutathione peroxidase. MWPH affected a lower serum IL-1β and fecal *Clostridium* density. The co-supplementation appeared more effective in terms of good health and potentially the prevention of disease.

Keywords: cranberry; extracts; immunity; inflammation; mealworm; microbiota

Introduction

Demand for protein is expected to increase in the future, owing to the increasing global population. Recently, insects have been recognized as the best alternatives to meet protein requirements (Van Huis, 2013). The edible larvae of the common pest insect *Tenebrio molitor* (yellow mealworm; YMW) distributed worldwide are a good source of protein, fat, vitamins, and minerals (Kim *et al*., 2014). YMWs contain high-quality protein (Shockley and Dossey, 2014), and contain more essential amino acids than soybeans (Yi *et al*., 2013). In addition, they have higher unsaturated fatty acid content than meat, and are relatively rich in vitamin A and iron (Rumpold and Schlüter, 2013). Recent studies have reported that mealworms can partially replace soybean and fish meal as a livestock protein feed source, and can improve the growth and productivity of various animals, including chicken and fish (Bovera *et al*., 2015; Ido *et al*., 2019). Hence, YMWs are extensively used in livestock feed due to the richness of amino acids and proteins (Hong *et al*., 2020). The YMW is also attracting attention as a useful raw material for the production of physiological active peptides. Physiologically active peptides are natural antioxidants, generally defined as a peptide with physiological activity and low molecular weight, which helps in their easy absorption into the body (Arihara *et al*., 2001). Their bioactive peptide contents and chitin were known to possess antioxidant and antimicrobial properties (Di Mattia *et al*., 2019; Matheswaran *et al*., 2019). Nevertheless, the antioxidant and antimicrobial

effects of YMWs were reported in mostly in vitro studies. A few fish feed research demonstrated the antioxidant enzyme activities in rainbow trout (Henry *et al*., 2018) and olive flounder (Jeong *et al*., 2021). There is a limited study regarding the antioxidant effects of YMW dietary supplementation in mammals. Recently, Ringseis *et al*. (2021) reported that the 10% YMW supplementation to growing pigs did not show a significant alternation in the antioxidant enzymes catalase (CAT), glutathione peroxidase (GPX), and superoxide dismutase (SOD) in the liver and of GPX and SOD in gastrocnemius muscle. Therefore, it is tempting to boost the antioxidant effect of YMW by co-supplementing with natural antioxidants.

Cranberries are a group of evergreen dwarf shrubs in the family Ericaceae, belonging to the subgenus Oxycoccus of the genus Vaccinium. Fresh cranberries, which are red in color and have a sour and sweet taste, are mainly cultivated in the cool climates of northern United States, Canada, and Chile. These berries have several biological functions that help prevent chronic disease in humans (Rupasinghe *et al*., 2015). Cranberries are also rich in phenolic compounds that exert strong antioxidant effects (Cho *et al*., 2012). Cranberry fruit extracts (CFE) contain a good source of polyphenols such as proanthocyanidins, flavanols (Vvedenskaya and Vorsa, 2004), and quercetin (Duthie *et al*., 2006; Zheng and Wang, 2003), which is attributed to the strong antioxidant properties. In addition, cranberries are rich in anthocyanins, water-soluble red pigments of the flavonoid family that exhibit anticancer, antioxidant, and anti-aging effects (Song *et al*., 2018). So far, the antioxidant and anti-inflammatory effect of CFE in mammals are also limited. Therefore, we hypothesized that the combination of YMW and cranberry as a dietary supplement would improve health in mammals. In the present study, we aimed to verify the in vivo efficacy of a diet supplemented with YMW and CFEs either alone or in combination. Toward this, the effects of feeding mealworm protein hydrolysate (MWPH) and cranberry fruit extract (CFE) on mouse growth, organ weight, COX-2 and NF-kB expression, blood parameters, and fecal microflora were investigated.

Materials and Methods

Ethical endorsement

The experimental protocol and procedures used in this study were approved by the Institutional Animal Care and Use Committee of Dankook University (DK-1-2136).

Preparations of MWPH and CFE

The MWPH and CFE used in this study were purchased from Jeonbuk Institute for Food-Bioindustry (Jeonju, Korea). The MWPH was prepared as follows: 40 kg defatted mealworms were mixed with 360 L water, and the pH was adjusted to 6.5–7.0 using 1 N NaOH. Next, 0.01% (w/w) formulations of each of Alcalase and Flavorzyme were added, and the mixture was hydrolyzed at 50°C for 3 h while stirring at 40 rpm. Subsequently, the mixture was heated at 85°C for 30 min to deactivate the enzymes, followed by centrifugation at 152 rpm under 130 L/h flow rate conditions. Next, microbial cells were removed by following vibrating membrane separation process, after which ultrafiltration was performed to obtain hydrolysate with molecular weight of 100 kDa and 30 kDa. The final hydrolysate was mixed with malto-dextrin at 1:1 ratio and freeze-dried at –60°C before use (Figure 1). The composition of amino acids in unhydrolyzed mealworm and MWPH were analyzed.

The CFE was prepared in three different extract methods, namely, hot water, methanol and HCl, and Ethanol. The water extraction was carried at 80°C water for 3 h and, secondly, cranberry fruit was extracted by 80% methanol and 0.3% HCl for 5 h. The third method is as follows: 5-fold volume of 70% ethanol was added to 100 g cranberry fruit and extracted for 5 h using a reflux condenser. This extract was concentrated under reduced pressure at 40 ± 1°C and freeze-dried before use (Figure 1).

Animals and experimental design

A total of 40 mice (BALB/c female inbred, 42 days old) with an initial average body weight of 29.4 ± 0.1 g were purchased from Central Lab. Animal Inc. (Seoul, Korea) and fostered in ventilated cages with a 12 h daylight (06:00–18:00) and 12 h dark cycle. The mice were allowed free access to water and food throughout the study.

Mice were randomly assigned to one of the four experimental treatments (one replication; 10 mice per treatment). The experimental treatments were arranged in a 2 × 2 factorial design with two levels of MWPH (0 and 2%) and two levels of CFE (0 and 400 ppm). CFE was added to the basal diet, while MWPH substituted 2% of the casein protein in the basal diet. Basal diets were prepared based on the American Institution of Nutrition (AIN-93G) guidelines for the nutritional requirements of mice (Table 1).

Sampling and analysis

The amino acid compositions of unhydrolyzed mealworm and MWPH were quantified in an amino acid analyzer (Sykam S-433 D, Sykam GmbH, Eresing, Germany). The amino acid compositions of the samples were compared with

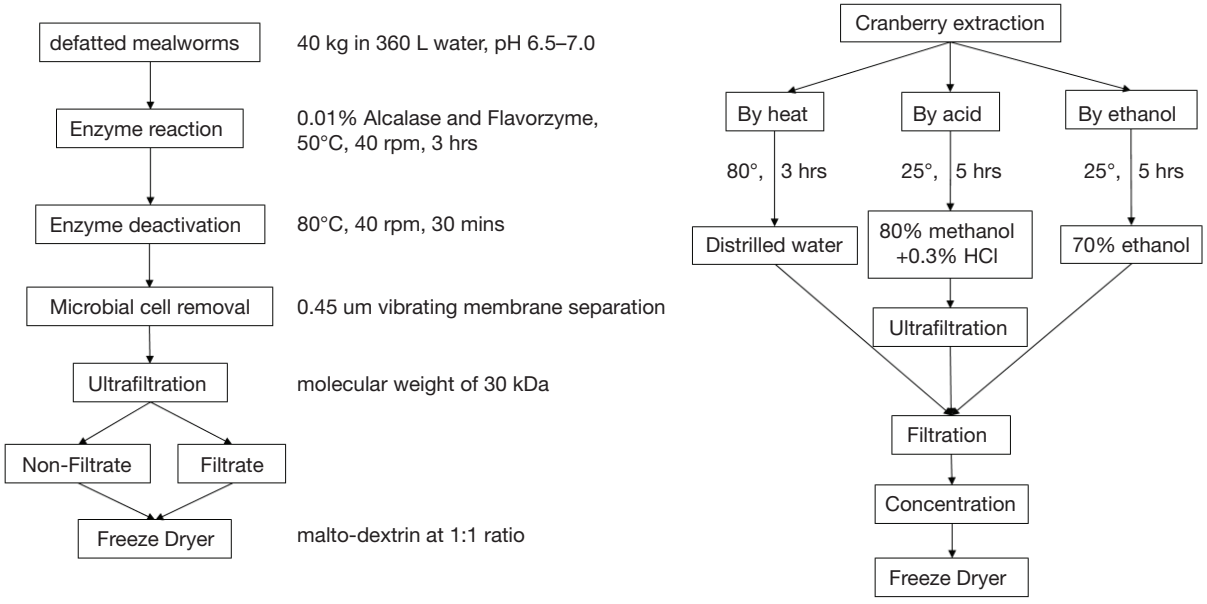

Figure 1. The process of cranberry extraction mealworm protein hydrolysate used in the current research.

Table 1. Composition of the basal diets (on as-fed basis).

	YMW–		YMW+	
Ingredients, g/kg	**CFE–**	**CFE+**	**CFE–**	**CFE+**
Casein	200	200	180	180
Sucrose	100	100	100	100
Dextrose	132	132	132	132
Corn starch	398	398	398	398
Cellulose	50	50	50	50
Soybean oil	70	70	70	70
Mineral mix	35	35	35	35
Vitamin mix	10	10	10	10
L-cystine	3	3	3	3
Choline bitartrate	2.5	2.5	2.5	2.5
Yellow mealworm	–	–	20	20
Cranberry fruit extract	–	0.4	–	0.4
Total	1000.0	1000.4	1000.0	1000.4

those of standards (Sigma Aldrich, St. Louis, MO, USA). The chemical compounds of CFEs from different extract methods were analyzed by HPLC using an Agilent 1100 HPLC system equipped with quaternary pumps, an auto-sampler, and a diode array detector set at 520 nm.

The body weight (BW) of each mouse was measured at the beginning and end of the study. Body weight gain (BWG), feed intake (FI), and feed efficiency (FE) were assessed at the end of the study. On day 42, all mice were placed in metabolic cages and fresh fecal samples were collected. On the same day, blood samples were drawn by using EDTA-treated tubes, stored at 37°C for 2 h, and centrifuged at 3500 rpm for 15 min at 4°C. Then, the supernatants were aspirated and stored at 4°C until further analysis. Serum level of pro-inflammatory cytokines (interleukin-6 [IL-6], IL-1β, and tumor necrosis factor-α [TNF-α]) were measured using a commercial ELISA kit (ELISA MAK; BioLegend, San Diego, CA) and absorbance was measured at 450 nm using a spectrophotometer. Antioxidant enzyme (superoxide dismutase [SOD] and glutamic peroxidase [GPx]) activity was analyzed using specific enzyme assay kits (Cayman Chemical, Michigan, USA). Following blood sampling, mice were anesthetized in chambers saturated with isoflurane and euthanized by cardiac puncture. Kidneys, spleens, and livers were harvested carefully for further analysis, and organ weight was calculated as a percentage of live weight.

DNA extraction

The QIAamp DNA Stool kit (Qiagen) was used to extract DNA from frozen stool samples according to the manufacturer's instructions. Briefly, the procedure involved lysis of the bacterial cells within the fecal material in ASL buffer, adsorption of impurities that inhibit EX reagent, and purification of the DNA on a spin column. ASL buffer was specially developed to remove inhibitory substances from stool samples. The DNA was eluted in a final volume of 200 µL and stored at –20°C.

The total volume of each amplification reaction mixture was 25 µL, consisting of 1 × TaqMan Universal PCR Master Mix (Applied Biosystems), both primers (each at 300 nM concentration), 200 nM TaqMan MGB probe, 60 ng purified target DNA, and bovine serum albumin at a

final concentration of 0.1 μg/μL (New England Biolabs). Amplification (2 min at 50°C, 10 min at 95°C, followed by 45 cycles of 15 s at 95°C and 1 min at 60°C) and detection were carried out on an ABI Prism 7900 sequence detection system (Applied Biosystems). Total bacteria were used as an endogenous control to normalize target gene expression (Table 2).

Quantitative real-time (qRT)-PCR

To measure the expression of COX-2 and NF-kB, mice were euthanized at the end of the experiment. Kidney samples were frozen in liquid nitrogen and stored at –80°C. Total RNA was isolated from the kidneys using TRIzol (Invitrogen, Carlsbad, CA, USA). For qRT-PCR, total RNA (1 μg) was used as a template for cDNA synthesis performed using the Maxima First-Strand cDNA Synthesis Kit (Life Technologies, Carlsbad, CA, USA). Sequence-specific primers (Table 3) were designed using Primer3 (http://frodo.wi.mit.edu/), and the reaction was performed on the CFX real-time PCR system (Bio-Rad, Hercules, CA, USA) under the following conditions: 94°C for 3 min, followed by 40 cycles of 94°C for 30 s, 59–61°C for 30 s, and 72°C for 30 s. Target gene expression levels were normalized to that of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase and were calculated using the 2–ΔΔCt method (Livak and Schmittgen, 2001).

Table 2. Group-specific primers based on 16S rDNA sequences used in this study.

Target bacteria		Sequences (5′ – 3′)
Total bacteria	F	ACTCCTACGGGAGGCAG
	R	GTATTACCGCGGCTGCTG
Bifidobacterium	F	CGCGTCYGGTGTGAAAG
	R	CCCCACATCCAGCATCCA
Lactobacillus	F	GAGGCAGCAGTAGGGAATCTTC
	R	GGCCAGTTACTACCTCTATCCTTCTTC
Clostridium	F	GGGGGTTTCAACACCTCC
	R	GCAAGGGATGTCAAGTGT
Firmicutes	F	GGAGYATGTGGTTTAATTCGAAGCA
	R	AGCTGACGACAACCATGCAC
Bacteroidetes	F	GAAGGTCCCCCACATTG
	R	CGCKACTTGGCTGGTTCAG

Statistical analysis

All statistical analyses were performed in a 2 × 2 factorial design using ANOVA tests (SAS Inst. Inc., Cary, NC, US). Statistical significance was set at $P < 0.05$.

Results

Active compounds and amino acids

The quantity of amino acids in unhydrolyzed mealworm and MWPH were estimated. Both did not contain threonine, histidine, isoleucine, tryptophan, and taurine. The MWPH had higher quantity of amino acids compared to unhydrolyzed mealworm (Table 4).

The quantity of active compounds from cranberry fruits was compared based on the three different extract methods. The ethanol methods showed the highest quantity of active compounds; thus, CFE from ethanol was used for the animal trial. The CFE from ethanol methods

Table 4. The quantity of amino acid from unhydrolyzed mealworm and mealworm protein hydrolysate (MWPH) (mg/g dry weight).

Amino acids		Unhydrolyzed mealworm	MWPH
Essential	Threonine	0.00	0.00
	Methionine	7.49 ± 1.26	13.6 ± 1.74
	Lysine	20.5 ± 1.96	34.6 ± 2.68
	Histidine	0.00	0.00
	Isoleucine	0.00	0.00
	Leucine	0.24 ± 0.06	0.93 ± 0.07
	Phenylalanine	0.16 ± 0.05	0.54 ± 0.06
	Tryptophan	0.00	0.00
	Valine	0.17 ± 0.06	0.57 ± 0.05
Nonessential	Arginine	0.23 ± 0.04	0.63 ± 0.06
	Taurine	0.00	0.00

The values represent mean ± SEM, n = 5.

Table 3. List and sequences of primers used in this study.

Gene symbol	Description	Accession no.	Forward primer (5′ -> 3′)	Reverse primer (5′ -> 3′)
Cox-2	cyclooxygenase-2	NM011198	CTCACGAAGG AACTCAGCAC	GGATTGGAAC AGCAAGGATT
Nf-Kb1	nuclear factor of kappa light polypeptide gene enhancer in B cells 1	NM008689	GTCTGCCTCTC TCGTCTTCC	CAGTGGGCTG TCTCCAGTAA
Gapdh	glyceraldehyde-3-phosphate dehydrogenase	NM001289726	CGAGACCCCA CTAACATCAA	GGTTCACACC CATCACAAAC

contained 80.6 and 2.917 μg/mL of total anthocyanin and proanthocyanin, respectively (Table 5).

Weight gain, feed intake, and feed efficiency

After 6 weeks, neither MWPH- nor CFE-supplemented diets showed a significant difference in BWG, FI, or FE compared to the control. In addition, there was no significant interactive effect of MWPH and CFE supplementation on BWG, FI, and FE (Table 6).

Organ size

There were no differences in the relative weights of the kidney and liver between mice fed diets with MWPH or CFE and those fed unmodified diets. However, the relative weight of the spleen increased with supplementation of MWPH ($P < 0.05$). No interactive effects were observed of MWPH and CFE supplementation on the relative organ weights (Table 7).

COX-2 and NF-kB expression

The expression of COX-2 and NF-kB was not influenced by supplementation with MWPH or CFE. Also, there was no significant interactive effect of MWPH plus CFE supplementation on the expression of COX-2 and NF-kB (Table 8).

Serum cytokine and antioxidant enzyme levels

Serum IL-1β concentration was lower in mice fed the diet containing MWPH than in those fed diets lacking MWPH ($P < 0.05$). The GPx concentration in mice fed diets containing MWPH or CFE was higher than that in mice fed diets lacking MWPH or CFE ($P < 0.05$). The concentrations of IL-6, TNF-α, and SOD were not influenced by MWPH or CFE supplementation. In addition, there was not an interaction between MWPH and CFE supplementation on serum levels of cytokines and antioxidant enzymes ($P < 0.05$; Table 9).

Fecal bacteria

There were no differences in bacterial counts of Bifidobacterium, Lactobacillus, Bacteroidetes, and Firmicutes in mice fed diets containing MWPH or CFE, but Clostridium counts decreased with the supplementation of MWPH ($P < 0.05$). There was no significant interactive effect of MWPH and CFE supplementation on fecal bacteria (Table 10).

Table 5. Chemical compounds of cranberry fruit extracts from different extract methods.

Compounds	Unit	Hot water	Acid	Ethanol
Cyanidin 3-Gluciside Chloride	mg/g	0.000	0.125 ± 0.08	0.000
Cyanidin 3-Galactoside Chloride	mg/g	0.226 ± 0.08	0.692 ± 0.12	0.737 ± 0.17
Cyanidin Chloride	mg/g	0.022 ± 0.001	0.030 ± 0.001	0.029 ± 0.001
Proanthocyanin	μg/mL	0.000	0.000	2.917 ± 0.07
Total anthocyanin	μg/mL	0.000	0.000	80.56 ± 3.45
Total polyphenol	μg/mL	5.658 ± 0.35	9.693 ± 0.42	13.14 ± 0.49

The values represent mean ± SEM (dry weight), n = 6.

Table 6. Effects of the MWPH and CFE supplementation on growth performance in mice1.

	MWPH −		MWPH +			MWPH		CFE		P-value		
Items	CFE−	CFE+	CFE−	CFE+	SEM	−	+	−	+	MWPH	CFE	MWPH × CFE
Initial BW, g	29.3	29.5	29.5	29.3	0.45	29.4	29.4	29.4	29.4	0.888	0.986	0.847
Finial BW, g	32.6	33.7	32.3	32.1	0.60	33.2	32.2	32.5	33.0	0.524	0.767	0.634
BWG, g	3.30	4.20	2.80	2.80	0.29	3.80	2.80	3.10	3.50	0.062	0.467	0.464
FI2, g	113	123	126	132	2.63	118	129	120	128	0.031	0.098	0.669
FE3	0.029	0.034	0.022	0.021	0.004	0.032	0.020	0.026	0.028	0.066	0.752	0.505

MWPH = mealworm protein hydrolysate, CFE = cranberry fruit extract, + or − = supplemented with or without 2% MWPH and 400 ppm CFE, respectively.
BWG, body weight gain; FI, feed intake; FE, feed efficiency.

Table 7. Effects of the MWPH and CFE supplementation on relative organ weight in mice1.

	MWPH –		MWPH +			MWPH		CFE		P-value		
Items	CFE–	CFE+	CFE–	CFE+	SEM	–	+	–	+	MWPH	CFE	MWPH × CFE
Kidney (R), %	0.52	0.54	0.59	0.55	0.009	0.53	0.57	0.55	0.55	0.127	0.812	0.115
Kidney (L), %	0.53	0.53	0.61	0.55	0.01	0.53	0.58	0.57	0.54	0.156	0.382	0.266
Spleen, %	0.25	0.24	0.27	0.32	0.01	0.25	0.30	0.26	0.28	0.015	0.461	0.109
Liver, %	4.09	4.26	4.35	4.24	0.06	4.18	4.29	4.22	4.25	0.373	0.787	0.292

MWPH = mealworm protein hydrolysate, CFE = cranberry fruit extract, + or – = supplemented with or without 2% MWPH and 400 ppm CFE, respectively.

Table 8. Effects of the MWPH and CFE supplementation on COX2 and NF-kB expression in mice1.

	MWPH –		MWPH +			MWPH		CFE		P-value		
Items	CFE–	CFE+	CFE–	CFE+	SEM	–	+	–	+	MWPH	CFE	MWPH × CFE
COX-2	1.00	0.88	1.19	1.16	0.13	0.94	1.18	1.10	1.02	0.426	0.803	0.873
NF-κB	1.00	0.60	0.65	0.64	0.11	0.80	0.65	0.83	0.62	0.553	0.422	0.451

MWPH = mealworm protein hydrolysate, CFE = cranberry fruit extract, + or – = supplemented with or without 2% MWPH and 400 ppm CFE, respectively.
COX-2, cyclooxygenase-2; NF-κB, nuclear factor kappa B.

Table 9. Effects of the MWPH and CFE supplementation on serum profiles of mice1.

	MWPH –		MWPH +			MWPH		CFE		P-value		
Items	CFE–	CFE+	CFE–	CFE+	SEM	–	+	–	+	MWPH	CFE	MWPH × CFE
IL-6, pg/mL	130.4	75.73	50.82	46.30	30.3	103.1	48.60	90.6	61.0	0.418	0.657	0.706
IL-1β, pg/mL	54.94	65.46	33.67	28.60	4.95	60.20	31.14	44.30	47.03	0.001	0.697	0.276
TNF-α, pg/mL	12.86	9.05	10.46	10.02	0.76	10.95	10.23	11.65	9.53	0.643	0.184	0.286
SOD, U/mL	4.50	7.27	8.36	7.62	0.78	5.89	7.99	6.43	7.44	0.199	0.525	0.277
GPx, U/mL	498.9	612.5	936.2	1382	98.1	555.7	1159	717.6	997.4	0.001	0.012	0.103

MWPH = mealworm protein hydrolysate, CFE = cranberry fruit extract, and + or – = supplemented with or without 2% MWPH and 400 ppm CFE, respectively.
IL-6, interleukin-6; IL-1β, Interleukin-1β; TNF-α, tumor necrosis factor-α, SOD, superoxide dismutase; GPx, glutathione peroxidase.

Table 10. Effects of the MWPH and CFE supplementation on fecal microbiota in mice1.

	MWPH –		MWPH +			MWPH		CFE		P-value		
Items	CFE–	CFE+	CFE–	CFE+	SEM	–	+	–	+	MWPH	CFE	MWPH × CFE
Bifidobacterium spp., log10	9.12	9.31	9.24	9.32	0.15	9.22	9.28	9.18	9.32	0.328	0.945	0.537
Lactobacillus spp., log10	8.25	8.48	7.79	8.20	0.13	8.36	8.00	8.02	8.35	0.205	0.258	0.749
Clostridium spp., log10	9.79	9.96	8.36	9.03	0.22	9.88	8.70	9.08	9.50	0.003	0.187	0.414
Bacteroidetes, %	48.4	49.4	49.5	49.2	0.32	48.9	49.4	49.0	49.3	0.539	0.664	0.381
Firmicutes, %	51.6	50.6	50.5	50.8	0.32	51.1	50.6	51.0	50.7	0.539	0.664	0.381

MWPH = mealworm protein hydrolysate, CFE = cranberry fruit extract, + or – = supplemented with or without 2% MWPH and 400 ppm CFE, respectively.

Discussion

The study was carried out to investigate the healthy benefits of co-supplementing MWPH and CFE in mice. Organ weight can be an important indicator of pathology, and measurements of the liver, kidneys, and spleen are recognized as indicators of toxicity and immunity in vivo. Since there was no difference in the liver and kidney weights of mice in this study, our data suggest that neither MWPH nor CFE had any harmful effects in the mice. However, MWPH supplementation increased spleen weight in this study. It is generally known that immunopotentiators increase the weight of the thymus and spleen (Chen *et al*., 2012). Since the spleen is largely composed of immune cells, it can be inferred that MWPH supplementation increases spleen immune cell.

The protein complex NF-κB is involved in inflammatory response regulation, immune modulation, apoptosis, cell proliferation, and epithelial differentiation among other processes. It regulates the expression of various genes and forms the central axis of the intracellular signaling system. The cytokines TNF-α, IL-1, IL-6, and the inducible enzyme COX-2 are regulated by NF-κB (Liu *et al*., 2017). In a previous in vitro study based on lipopolysaccharide-activated macrophages, mealworm larval protein inhibited the expression of iNOS and COX-2 proteins in a concentration-dependent manner (Seo *et al*., 2019). However, this effect was not observed in the mice in our study.

Cytokines are relatively small molecular weight immune proteins that play an important role in cell signaling (Arango Duque and Descoteaux, 2014). Cytokines involved in inflammatory processes are classified as either inflammatory or anti-inflammatory. Inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, are involved in immune-related disorders, such as rheumatoid arthritis, atopic dermatitis, and asthma (Girodet *et al*., 2016; Sato *et al*., 1999; Turner *et al*., 2014). Anti-inflammatory cytokines such as IL-10 and TGF-β protect against these diseases by performing functions such as the induction of B cells proliferation and tissue repair (Girodet *et al*., 2016). In this study, IL-1β levels were significantly lower in the MWPH supplementation group. These results suggest that MWPH exerts anti-inflammatory effects via regulating cytokine expression and may help suppress the conversion to chronic inflammation due to enhanced immune function.

Antioxidants are known to slow aging by scavenging cellular oxidants and active radicals in the body (Kim *et al*., 2003). Several physiologically active peptides act as natural antioxidants and function in hypertension relief, immune regulation, pain relief, and antibacterial action (Xing *et al*., 2021). They are usually composed of 3–20 amino acids with a low molecular weight that makes it easy to absorb them into the body (Kim *et al*., 2013). Previous studies have reported that protein hydrolysate from mealworm larvae (Yu *et al*., 2017) and *Protaetia brevitarsis* larvae (Lee *et al*., 2017) increased antioxidant activity. Therefore, in the present study, the increase in GPx activity in mouse blood can be considered as the effect of physiologically active peptides from MWPH.

Cranberries exhibit high antioxidant activity, which is closely related to flavonoid pigments such as anthocyanins, flavonols, and proanthocyanidins (Brown and Shipley, 2011; Feghali *et al*., 2012). The antioxidant capacity of proanthocyanidins is reportedly reported to be stronger than that of vitamins C and E and catechins (Ariga, 2004). In this study, GPx levels were higher in mice fed on CFE, which is likely due to the abundant polyphenolic compounds in CFE.

Furthermore, CFE can contribute to gut microbiota regulation. The positive effects of polyphenols derived from many plant sources on the gut microbiota have already been investigated (Anhê *et al*., 2017; Heyman-Lindén *et al*., 2016; Roopchand *et al*., 2015). However, in our study, CFE did not affect fecal microbiota in mice fed on CFE-supplemented diet when compared with mice fed on nonsupplemented diet.

In this study, the number of Clostridium bacteria was significantly lower in the mice fed on MWPH supplemented diet, showing that MWPH supplementation inhibits the growth of harmful microorganisms in the intestine. The outer shell of mealworm larvae is mainly composed of Brown mealworm larvae that develop an exoskeleton during metamorphosis into pupae. It has been reported that chitin can be used as a source of dietary fiber that provides a favorable environment for the growth of beneficial bacteria in the gastrointestinal tract (Hamed *et al*., 2016; Ringø *et al*., 2012). We therefore expect that chitin contained in MWPH may have influenced our results.

Conclusions

Combination of MWPH and CFE could be advantageous for boosting anti-inflammation via regulating cytokine activation. The combination significantly reduced the expression of IL-1 as compared to individual supplements. Besides, MWPH-supplemented diet improved the immune function and reduced harmful gut bacteria in mice. The effect of CFE supplementation on mice increased levels of antioxidant enzymes in serum. This study provides preliminary data for the further evaluation of dietary supplementation with MWPH and CFE. In future studies, it is necessary to investigate the relative effects of these two additives at various feed levels.

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