Dietary supplementation with housefly (Musca domestica) maggot meal in growing beagles: hematology, serum biochemistry, immune responses and oxidative damage*

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Abstract
The present study was carried out to evaluate the hematology, serum biochemistry, immune responses and oxidative damage of growing beagles fed a diet supplemented with housefly (Musca domestica) maggot meal (MM). Weanling beagles (initial body weight 2.69 ± 0.17 kg) were fed a control diet (0% MM) or experimental diet (5% MM) for 42 days. The results indicated that the diet supplemented with 5% MM had no significant effects on the hematology and serum biochemistry of growing beagles (P>0.05). Meanwhile, neither the serum concentrations of lysozyme and C-reactive protein nor the serum antibody responses to canine distemper virus and canine parvovirus were influenced by dietary MM supplementation (P>0.05). However, dogs in the experimental group had lower serum levels of malondialdehyde and protein carbonyl than those in the control group (P<0.05). These findings demonstrated that MM could be used as an alternative protein source in growing beagles without any adverse effects on hematology, serum biochemistry and immune responses. Furthermore, dietary MM could alleviate oxidative damage in growing beagles.

Key words: protein source, blood profile, immune response, oxidative damage, housefly maggot meal

The combination of increasing living standards and health benefits of pet ownership contributes to the rising demand for dogs as companions. The global number of domestic dogs has been estimated at 703.3 million (Hughes and Macdonald,

*This research was financially supported by the Basic Research Project of Sichuan Province (A-2017N-29; A-2018N-24), Science and Technology Plan Project of Sichuan Province (2018JY0659), Scientific and Technical Research Project of Sichuan Provincial Administration of TCM (2018QN041). The authors would like to thank the personnel of these teams for their kind assistance.
2013), resulting in increasing competition for protein sources among pet food, human food and livestock feeds. Because of the limited amount of agricultural land, new methods of protein production are urgently needed. Recently, insects have received much attention because of their use as a land-saving approach for protein production (Sánchez-Muros et al., 2014; Lei et al., 2019). The possible use of insect meals in livestock and fish has been widely studied (Sánchez-Muros et al., 2014). However, to date, limited information is available regarding the use of insect meals in dog diets. In vitro studies showed that the protein quality, based on the amino acid (AA) profile and digestibility, of several insect larvae meals was high for dogs (Bosch et al., 2014, 2016). In addition, Lisenko et al. (2018) reported that 3 insect meals, speckled cockroach (*Nauphoeta cinerea*), Madagascar cockroach (*Gromphadorhina portentosa*), and superworm (*Zophobas morio*), could be well accepted by adult beagles without negative effects on nutrient digestibility, fecal metabolites, and the microbiota. Furthermore, Lei et al. (2019) found that dietary black soldier fly (*Hermetia illucens*) larvae meal had beneficial effects on beagle dogs by improving the apparent total tract digestibility of dry matter (DM) and crude protein (CP), in addition to anti-inflammatory and antioxidative effects. Additionally, it was reported that insects could be used as alternatives to commercial aromatic additives in dog nutrition (Kierończyk et al., 2018).

As an insect protein source, housefly (*Musca domestica*) maggot meal (MM) is produced by culturing housefly larvae in organic waste, which plays an important role in recycling many forms of accumulated nutrients in the environment (Hussein et al., 2017). In addition, MM has high nutritional value. The levels of CP and ether extract in MM are in the range of 37.5%–63.9% and 9.0%–26.0% (in DM), respectively (Ogunji et al., 2007; Hwangbo et al., 2009; Dong et al., 2013; Bosch et al., 2014; Sánchez-Muros et al., 2014; Wang et al., 2017). MM also has a well-balanced AA profile (Hussein et al., 2017). Additionally, MM is rich in B complex vitamins, phosphorus and trace elements (Teotia and Miller, 1973). Hence, MM seems to have an immense potential as a good protein source. However, there have been only a few in vitro studies evaluating the quality of MM protein as a potential ingredient in dog food. Bosch et al. (2014) reported that MM had high AA scores for dogs. Additionally, it was found that the nutrient digestibility of MM for dogs was all above 90% in an in vitro assay (Bosch et al., 2016). These results indicated that MM might be a good source of high-quality protein for dogs, which warrants investigation.

It is well known that all ingredients used in feeds must maintain animal health. The hematology and serum chemistry analyses are useful for assessing the physical health status of animals. To date, no studies are available regarding the effects of dietary MM on the hematology and serum chemistry of dogs. Studies have shown that dietary MM had no negative influence on the hematology and serum chemistry of broilers, Nile tilapia (*Oreochromis niloticus*), rabbits and rats (Awoniyi et al., 2004; Ogunji et al., 2008; Iheukwumere et al., 2009; Marcel et al., 2011; Duwa et al., 2014; Mbiba et al., 2019). Improvements of hematology were observed in African catfish (*Clarias gariepinus*) and Egyptian sole (*Solea aegyptiaca*) with MM supplementation in diets (Okore, 2016; Saleh et al., 2016). However, Li et al. (2019) found
an increase in serum aspartate aminotransferase (AST) activity caused by dietary MM in bullfrog *Rana (Lithobates) catesbeiana*. Therefore, the effects of dietary MM on hematology and serum chemistry differ among animals. The present study focused on the effects of MM on the hematology and serum clinical chemistry of beagles.

Generally, animal health depends on immunity. Insects and insect derivatives have a great potential in improving animal immunity. Since ancient times, insects and their derivatives have been widely used in folk medicine worldwide (Ratcliffe et al., 2011). Currently, there are approximately 300 medicinal insects distributed in 70 genera, 63 families, and 14 orders (Feng et al., 2009). The housefly is one of these 300 medicinal insects. In China, since the 14th century, housefly larvae have been used clinically to cure malnutritional stagnation, skin scald, osteomyelitis, decubital necrosis, eczema and lip boil (Jiang, 1999). To date, studies have shown that whole housefly larvae and their components, such as chitin, the chitin derivative – chitosan and antimicrobial peptides have antibacterial, antifungal, antiviral and antitumor activities, both *in vitro* and *in vivo* (Gu et al., 2006; Hou et al., 2007; Ai et al., 2013; Chu et al., 2013; Wang et al., 2013; Chen et al., 2015). In addition, the lauric acid, an antiviral and antibacterial substance (Gasco et al., 2018), was shown to represent approximately 70% of total fatty acid in housefly larvae (Odesanya et al., 2011). All of these facts indicated that MM could have a great potential in improving animal immunity. In fish, studies have shown that survival rate of fish after bacterial challenge was improved by feeding with housefly larvae, pupae and MM (Ido et al., 2015; Pei et al., 2019; Xiang et al., 2019). In addition, Li et al. (2019) found an upsurge in serum lysozyme activity by increasing dietary MM levels in bullfrogs. To date, there is no information regarding the effect of MM on the immunity of dogs, which warrants further study.

On the other hand, animal health is also threatened by reactive oxygen species (ROS). ROS are normal by-products of metabolic processes. When the rate of ROS exceeds that of their removal, oxidative damage to membrane structures and cell functions occurs (Storz and Imlay, 1999). Alleviation of oxidative damage by nutrients offers a positive means to improve animal health. To date, little information is available regarding the relationship between MM and oxidative damage in dogs. Studies have shown that extracts of housefly maggots have ROS scavenging activity *in vitro* (Zhang et al., 2016; Li et al., 2017) and in cells (Ai et al., 2013). Hence, MM might be able to alleviate oxidative damage by enhancing the ROS scavenging ability in animals. However, whether this is true in dogs is unknown, and awaits additional investigation.

The present study investigated the nutritional value of MM and the effects of dietary MM on hematology, serum biochemistry, immune responses and oxidative damage of growing beagles. Growing dogs have an underdeveloped digestive system, which makes them highly sensitive to environmental factors, including feed ingredients (Buddington et al., 2003). Therefore, the data obtained here would be useful for testing the safety of MM as a protein source in dog feed.
Material and methods

All procedures used in this study were approved by the Animal Care Advisory Committee of the Sichuan Academy of Chinese Medicine Sciences (SCAC-MS-20180406).

Experimental diets and animals

MM used in this study was purchased from Anhui Jingde Green Spring Ecological Breeding Farm, Xuancheng, China. Maggots were produced by culturing housefly larvae in wheat bran at a temperature of 27°C±1 and moisture of 600–700 g kg⁻¹. The maggots matured within 3–4 days and then they were harvested, oven-dried and milled to form MM. Before the diet formulation, the nutrient composition of MM and white fish meal (WFM) (Table 1) was determined using the methods recommended by the Association of Official Analytical Chemists (AOAC, 2006). Diets were formulated to meet the nutrient recommendations for the growth of dogs according to the Association of American Feed Control Officials (AAFCO, 2014). The composition of the control diet (CD) is given in Table 2. The experimental diet (ED) was prepared by replacing 5% of the WFM with MM in the CD (Table 2). The diets were made into pellets, dried at 60°C and stored in vacuum bags until use. Water at 37°C was added to the feed to soften the pellets before feeding. The nutrient composition of CD and ED was determined according to AOAC (2006) (Table 2).

| Table 1. Nutritive value of the maggot meal (MM) and white fish meal (WFM) used in this study |
|------------------------------------|---------------------------------|-------------------|
| Components                         | MM (g kg⁻¹)                    | WFM (g kg⁻¹)     |
| Dry matter                         | 931.7                          | 915.8             |
| Crude protein                      | 564.2                          | 660.4             |
| Ether extract                      | 200.3                          | 78.7              |
| Crude ash                          | 78.6                           | 139.5             |
| Crude fiber                        | 71.4                           | 10.6              |
| Gross energy (kJ g⁻¹)              | 32.4                           | 25.4              |

*The values are the results of a study conducted by Wang et al. (2017).

| Table 2. Composition and nutritive value of diets for beagles used in the study |
|------------------------------------|---------------------------------|-------------------|
| Ingredients (g kg⁻¹ in dry matter) | Diets                           |                   |
|                                    | control diet (CD)               | experimental diet (ED) |
|                                    | 1                               | 2                 | 3                 |
| White fish meal                   | 200.0                           | 150.0             |
| Maggot meal                       | 0                               | 50.0              |
| Soybean meal                      | 220.0                           | 220.0             |
| Corn gluten meal                  | 30.0                            | 30.0              |
| Corn                               | 210.2                           | 196.3             |
| Rice                               | 130.0                           | 130.0             |
Table 2 – contd.

| 1   | 2   | 3   |
|-----|-----|-----|
| Wheat bran | 30.0 | 30.0 |
| Soybean oil | 150.0 | 150.0 |
| Ca(H_2PO_4)_2 | 15.0 | 28.0 |
| Sodium chloride | 1.8 | 2.7 |
| Potassium chloride | 6.0 | 6.0 |
| Choline chloride (50%) | 2.0 | 2.0 |
| Vitamin premix\textsuperscript{a} | 2.5 | 2.5 |
| Mineral premix\textsuperscript{b} | 2.5 | 2.5 |

Chemical composition (g kg\textsuperscript{-1} in dry matter)

|                | 1      | 2      | 3      |
|----------------|--------|--------|--------|
| Crude protein (CP) | 276.6  | 271.3  |
| Ether extract (EE) | 182.0  | 187.0  |
| Crude ash        | 57.4   | 52.1   |
| Crude fiber      | 20.4   | 20.1   |
| Nitrogen free extract (NFE) | 347.2  | 332.4  |
| Metabolizable energy (kJ g\textsuperscript{-1})\textsuperscript{c} | 15.5   | 15.5   |

\textsuperscript{a}Provided per kilogram of control and experimental diets: vitamin A, 5,000,000 IU; vitamin D\textsubscript{3}, 500,000 IU; vitamin E, 50,000 IU; thiamine, 2,250 mg; riboflavin, 5,200 mg; pantothenic acid, 12,000 mg; niacin, 13,600 mg; pyridoxine, 1,500 mg; folic acid, 0.216 mg; vitamin B\textsubscript{12}, 0.028 mg.

\textsuperscript{b}Provided per kilogram of control and experimental diets: iron, 88,000 mg; copper, 12,400 mg; manganese, 7,200 mg; zinc, 100,000 mg; iodine, 1,000 mg; selenium, 0.350 mg.

\textsuperscript{c}Estimated as follows: metabolizable energy (kJ g\textsuperscript{-1}) = (3.5×CP% + 8.5×EE% + 3.5×NFE%)/100×4.186 (NRC, 1985).

Weanling beagles (6 wk old; 6 females and 6 males), in good general health and vaccinated according to current guidelines, were obtained from the Institute of Laboratory Animals of Sichuan Academy of Medical Sciences, China. The feeding trial was also conducted there. Dogs were housed one per kennel (1.5×1.0 m) in a temperature-controlled room (24ºC±2) on a 12 h light/12 h dark cycle. Dogs were fed the CD for 2 weeks to acclimatize them to the experimental conditions.

**Feeding trial**

During the 2-week acclimation period, all dogs received a combined booster vaccine against canine distemper virus (CDV) and canine parvovirus (CPV) as part of normal veterinary care on day 7 (Khoo et al., 2005). No apparent vaccination-related changes were observed. After the 2-week acclimation, three dogs of each sex with an initial body weight of 2.69±0.17 kg were randomly assigned to one of the two dietary treatment groups. Dogs were individually fed twice daily (09:00 and 16:00) for 6 weeks. The feed ration was adjusted every week with a slight increase in feed allowance according to Dobenecker et al. (2013). Water was available ad libitum.

**Amino acid analysis**

The AA content in MM and WFM was determined by ion exchange chromatography using an auto amino acid analyzer (L-8800, Hitachi, Japan) after one of
3 hydrolysis procedures (AOAC, 2006). Most of the AA were analyzed after hydrolyzing with 6 mol/L HCl at 110°C for 24 h. For the analysis of sulfur-containing amino acids, the oxidation process was employed before the acid hydrolysis using performic acid. The determination of tryptophan was performed after alkaline hydrolysis. The AA scores (AAS) were calculated using the following equation:

\[
\text{AAS} = \frac{\text{mg of AA in 1 g of test protein}}{\text{mg of AA in 1 g of reference protein}} \times 100
\]

(Kerr et al. 2013). The reference pattern used was the minimal requirements for growth of dogs according to AAFCO (2014).

**Blood sampling and analysis**

At the end of week 6, blood samples were collected via the jugular vein from each dog after a 12 h fast. Blood samples were collected into two types of vacutainer tubes: one containing EDTA-K2 and the other with no additive. Serum samples were obtained by centrifuging the tubes without additive, and stored at –80°C until analysis.

Hematology parameters including red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), white blood cells (WBC), neutrophil granulocytes (NEUT), lymphocytes (LYM), monocytes (MON), mean corpuscular volume (MCV) and platelets (PLT) were analyzed using a hematology analyzer (MEK-7222K, Nihon Kohden, Japan). Serum biochemical parameters including total protein (TP), albumin (Alb), globulin (Glob), albumin/globulin (A/G) ratio, AST, alanine transaminase (ALT), alkaline phosphatase (ALP), creatinine (Crea), total cholesterol (TC), creatine kinase (CK) and glucose (Glu) were assessed using biochemistry analyzer (Coulter-AU480, Beckman, USA). The sodium, potassium, calcium and chloride ions in serum were evaluated using an electrolyte analyzer (AVL9181, Roche, USA).

Serum lysozyme (LZM) activities and C-reactive protein (CRP) levels were measured by using LZM test kits and CRP test kits (Shanghai Enzyme-linked Biotechnology), respectively. Serum antibody responses to the CDV and CPV vaccine were evaluated by using ELISA-test kits (Shanghai Enzyme-linked Biotechnology). The levels of contents of malondialdehyde (MDA) and protein carbonyl (PC) were measured by procedures described by Zhang et al. (2008) and Baltacloglu et al. (2008), respectively.

**Statistical analysis**

The results are presented as means ± SDs. The data were analyzed with a two-tailed t-test to determine differences between the two groups. The statistical software used was SPSS (version 18.0: SPSS, Chicago).

**Results**

Table 1 and Table 3 summarize the nutrient composition and AA profile of the MM used in this study. The MM was rich in CP (564.2 g/kg DM) and ether extract (200.3 g/kg DM). All essential amino acids (EAAAs) needed by dogs were observed in the MM. Among the EAAAs, phenylalanine-tyrosine was the most abundant
Housefly maggot meal in dogs

The AA scores ranged from 87.73 to 201.24. Only three EAs had AA scores below 90 (isoleucine, methionine-cystine and threonine).

Table 3. Essential amino acid profile and essential amino acid scores of maggot meal (MM), white fish meal (WFM), soybean meal (SBM) and poultry by-product (PBP)

| Item               | Essential amino acid profile [g/100 g crude protein (CP)]a | Essential amino acid scoresb |
|--------------------|-----------------------------------------------------------|-------------------------------|
|                    | MM (CP 56.4%) | WFM (CP 66.0%) | SBM (CP 44.5%) | PBP (CP 59.0%) | MM (CP 56.4%) | WFM (CP 66.0%) | SBM (CP 44.5%) | PBP (CP 59.0%) |
| Arginine           | 4.87          | 5.57           | 7.37           | 6.59           | 109.67         | 125.38          | 165.84          | 148.35          |
| Histidine          | 2.75          | 2.36           | 2.76           | 2.27           | 140.48         | 120.79          | 141.34          | 116.14          |
| Isoleucine         | 2.82          | 4.63           | 4.56           | 3.81           | 89.31          | 146.84          | 144.56          | 120.85          |
| Leucine            | 5.28          | 7.57           | 7.80           | 7.12           | 92.12          | 132.06          | 136.01          | 124.16          |
| Lysine             | 7.39          | 7.74           | 6.27           | 4.81           | 184.77         | 193.44          | 156.74          | 120.34          |
| Methionine         | 2.23          | 2.95           | 1.44           | 1.73           | 143.57         | 189.82          | 92.46           | 111.14          |
| Methionine-Cystine | 2.73          | 3.88           | 2.97           | 3.41           | 87.73          | 124.60          | 95.35           | 109.50          |
| Phenylalanine      | 6.33          | 4.03           | 5.26           | 3.46           | 171.53         | 109.19          | 142.55          | 93.73           |
| Phenylalanine-Tyrosine | 11.63      | 7.28           | 9.55           | 6.31           | 201.24         | 126.06          | 165.30          | 109.13          |
| Threonine          | 4.09          | 4.27           | 3.98           | 3.56           | 88.58          | 92.38           | 86.05           | 77.00           |
| Tryptophan         | 1.08          | 1.15           | 1.28           | 0.78           | 121.63         | 129.47          | 144.10          | 87.71           |
| Valine             | 4.79          | 5.31           | 4.70           | 4.68           | 158.34         | 175.86          | 155.40          | 154.79          |

aThe essential amino acid profile of soybean meal and poultry by-product is from NRC (2006).
bCalculated as described in Kerr et al. (2013) using minimal requirements for growth of dogs according to AAFCO (2014).

Table 4. Body weight and feed intake of dogs fed the control and experimental dietsa

| Item               | Unit | Control group | Experimental group | P-value |
|--------------------|------|---------------|--------------------|---------|
| Initial body weight | kg/dog | 2.68±0.18     | 2.70±0.16          | 0.903   |
| Final body weight  | kg/dog | 4.89±0.46     | 4.97±0.54          | 0.810   |
| Feed intake        | kg/dog | 6.04±0.38     | 6.09±0.20          | 0.787   |

aValues are the mean ± SDs (n = 6).

All dogs were clinically healthy throughout the experimental period. The dogs ate most of the offered feed, and there were rarely leftovers. No significant difference was observed in feed intake (P>0.05; Table 4). The final body weight of the dogs was close to the body weight of beagles observed at this growth stage in other studies (Hawthorne et al., 2004; Dobenecker et al., 2013). No significant difference in body weight was observed between the groups (P>0.05; Table 4). Hematology parameters, including RBC, HGB, HCT, WBC, NEUT, LYM, MON, MCV and PLT were not significantly affected by diet (P>0.05; Table 5). Moreover, diets did not have a significant impact on the serum biochemistry of the beagles (P>0.05; Table 6), including on TP, Alb, Glob, A/G ratio, AST, ALT, ALP, Crea, TC, CK and Glu.
Furthermore, serum electrolyte values, including the levels of sodium, potassium, calcium and chloride were also not significantly influenced by diet (P>0.05; Table 7). Serum concentrations of LZM and CRP were not significantly different between the two groups (P>0.05; Table 8). At the same time, serum levels of CDV antibody and CPV antibody in the experimental group were not significantly different from those in the control group (P>0.05; Table 8). However, as shown in Table 9, dogs fed the ED had lower serum levels of MDA and PC than dogs fed the CD (P<0.05; Table 9).

Table 5. Selected blood haematology parameters of dogs fed the control and experimental diets

| Item | Unit | Control group | Experimental group | P-value | Reference range for adult dogs |
|------|------|---------------|--------------------|---------|-------------------------------|
| RBC  | ×10¹²/L | 6.2±0.4       | 6.1±0.4            | 0.793   | 5.1–8.5                      |
| HGB  | g/L   | 131.3±8.7     | 132.0±3.0          | 0.874   | 120.0–180.0                  |
| HCT  | L/L   | 0.42±0.03     | 0.42±0.01          | 0.603   | 0.35–0.55                    |
| WBC  | ×10⁹/L | 11.9±2.2      | 12.1±2.0           | 0.907   | 6.0–18.0                     |
| NEUT | ×10⁹/L | 5.2±0.3       | 5.3±0.20           | 0.434   | 3.6–13.0                     |
| LYM  | ×10⁹/L | 2.6±0.2       | 2.6±0.3            | 0.918   | 0.8–5.8                      |
| MON  | ×10⁹/L | 1.2±1.1       | 1.2±0.3            | 0.893   | 0.0–1.6                      |
| MCV  | fL    | 67.5±1.1      | 68.9±2.4           | 0.250   | 62.0–76.0                    |
| PLT  | ×10⁹/L | 364.7±23.8    | 359±19.6           | 0.690   | 180.0–500.0                  |

RBC: red blood cells; HGB: hemoglobin; HCT: hematocrit; WBC: white blood cells; NEUT: neutrophil granulocytes; LYM: lymphocytes; MON: monocytes; MCV: mean corpuscular volume; PLT: platelet.

*Values are the mean ± SDs (n = 6).

Table 6. Selected serum biochemical traits of dogs fed the control and experimental diets

| Item | Unit | Control group | Experimental group | P-value | Reference range for adult dogs |
|------|------|---------------|--------------------|---------|-------------------------------|
| TP   | g/L  | 54.9±1.2      | 54.5±1.7           | 0.633   | 54.0–75.0                     |
| Alb  | g/L  | 27.7±0.3      | 27.3±0.4           | 0.146   | 32.0–44.0                     |
| Glob | g/L  | 27.2±1.0      | 26.3±1.9           | 0.347   | 22.0–31.0                     |
| A/G ratio | – | 1.02±0.02 | 1.05±0.08 | 0.498 | 1.00–2.00 |
| AST  | U/L  | 40.4±4.9      | 42.5±5.2           | 0.522   | 0–40.0                        |
| ALT  | U/L  | 36.3±3.7      | 38.1±5.2           | 0.534   | 0–80.0                        |
| ALP  | U/L  | 201.2±26.6    | 210.0±25.1         | 0.570   | 0–90.0                        |
| Crea | µmol/L | 38.5±4.0     | 38.2±1.1           | 0.853   | 65.0–110.0                    |
| TC   | mmol/L | 3.4±0.6      | 3.5±0.2            | 0.675   | 3.4–10.0                      |
| CK   | U/L  | 396.0±21.9    | 388.2±18.3         | 0.553   | 0–200.0                       |
| Glu  | mmol/L | 5.9±0.3      | 6.0±0.3            | 0.570   | 3.6–6.6                       |

TP: total protein; Alb: albumin; Glob: globulin; A/G ratio: albumin – globulin ratio; AST: aspartate transaminase; ALT: alanine transaminase; ALP: alkaline phosphatase; Crea: creatinine; TC: total cholesterol; CK: creatine kinase; Glu: glucose.

*Values are the mean ± SDs (n = 6).

*Rørtveit et al. (2015).*
### Table 7. Serum electrolyte values of dogs fed the control and experimental diets

| Item    | Unit | Control group | Experimental group | P-value | Reference range |
|---------|------|---------------|--------------------|---------|-----------------|
| Sodium  | mmol/L | 150.5±1.6    | 151.2±0.2          | 0.332   | 141.0–152.0     |
| Potassium | mmol/L | 5.7±0.2   | 5.6±0.5           | 0.502   | 4.4–5.4         |
| Calcium | mmol/L | 2.82±0.09 | 2.82±0.07        | 0.949   | 2.25–2.83       |
| Chloride | mmol/L | 115.9±1.3 | 115.2±2.2        | 0.570   | 105.0–115.0     |

*aValues are the mean ± SDs (n = 6).  
*bRørtveit et al. (2015).  

### Table 8. Concentrations of lysozyme, C-reactive protein, antibodies against canine distemper virus and canine parvovirus in serum samples of dogs fed control and experimental diets

| Item     | Unit | Control group | Experimental group | P-value |  
|----------|------|---------------|--------------------|---------|
| LZM      | ng/mL | 11.4±0.5   | 12.1±0.4           | 0.067   |
| CRP      | µg/mL | 10.9±0.3  | 11.2±0.5           | 0.190   |
| CDV      | ng/mL | 161.6±15.1 | 146.5±11.1        | 0.077   |
| CPV      | ng/mL | 355.6±30.0 | 325.7±18.5        | 0.064   |

LZM: lysozyme; CRP: C-reactive protein; CDV antibody: anti-canine distemper virus antibody; CPV antibody: anti-canine parvovirus antibody.  
*aValues are the mean ± SDs (n = 6).  

### Table 9. Levels of malondialdehyde and protein carbonyl in serum samples of dogs fed control and experimental diets

| Item | Unit | Control group | Experimental group | P-value |
|------|------|---------------|--------------------|---------|
| MDA  | nmol/mg protein | 2.6±0.3   | 2.2±0.2           | 0.018   |
| PC   | nmol/mg protein | 4.2±0.3  | 3.8±0.3           | 0.040   |

MDA: malondialdehyde; PC: protein carbonyl.  
*aValues are the mean ± SDs (n = 6).  

### Discussion

In recent years, substantial research has been conducted on the utilization of MM as a protein source in livestock and fish feeds. In this study, we evaluated the nutritional value of MM and the effects of dietary MM on the hematology, serum biochemistry, immune responses and oxidative damage of beagles, which may provide some insight into the utilization of MM as a feed ingredient for dogs.

Protein is a key nutrient for animals. Especially for growing puppies, who require proteins for the development of bone, muscle and many other important structures (Gessert and Phillips, 1956). Based on these facts, the protein content is an important criterion for feed protein sources. In this study, the CP content of MM was high (564.2 g/kg DM), and was in agreement with that reported by Awoniyi et al. (2004), Khan et al. (2016) and Wang et al. (2017). This value was slightly lower than the protein content present in fish meal (Esteban et al., 2007), but was higher than that in soybean meal (Bosch et al., 2014). Studies have shown that the protein content
of MM ranges from 37.5% to 63.9% (Ogunji et al., 2007; Hwangbo et al., 2009; Dong et al., 2013; Bosch et al., 2014; Wang et al., 2017). The various protein contents in MM could be related to differences in substrates used for housefly maggot production, the age of the maggot and methods of MM production (Fasakin et al., 2003; Hwangbo et al., 2009; Hussein et al., 2017). In addition, AA composition is another important parameter to evaluate the quality of protein sources (Bosch et al., 2016). The present study showed that MM had all the EAAs, among which phenylalanine-tyrosine was present at the highest concentration. Compared with the AAFCO (2014) nutrient profiles, the least abundant EAA in terms of dog requirements was methionine-cystine. However, our results showed that the methionine-cystine AA score was still 87.73. These results suggested that MM might be a high-quality protein source that could be used in dog food. A comparison of MM and other common protein ingredients in dog diets (WFM, soybean meal, poultry by-product) shows large differences in the AA profile between them. MM had higher levels of phenylalanine and phenylalanine-tyrosine, but lower levels of isoleucine and leucine than other products. These AA profile differences should be considered when making diet formulations.

It is well known that any protein source used in animal feeds must maintain the physical health of the animals. In this study, all dogs were clinically healthy throughout the experimental period. The physical response of animals to feed can be indicated by hematology and serum biochemical parameters. The present study found that hematology, serum biochemical parameters and the serum electrolyte levels of beagles were not significantly influenced by MM. In addition, most of these parameters were within the reference ranges (Rørtveit et al., 2015), except for Alb, Crea, ALP and CK. The reason for these four parameters out of the reference ranges might be the differences in the growth stage of dogs. It is well known that age has a significant effect on hematology and serum biochemical parameters in dogs (Rørtveit et al., 2015). The levels of Alb, Crea, ALP and CK here were similar to those observed in puppies in a study conducted by Rørtveit et al. (2015). Therefore, it can be concluded that 5% MM in the diet had no adverse effect on the hematology and serum biochemical parameters of growing beagles, implying that MM could maintain physiological homeostasis in dogs. Similar results were also observed in broilers, Nile tilapia, rabbits and rats (Awoniyi et al., 2004; Ogunji et al., 2008; Iheukwumere et al., 2009; Marcel et al., 2011; Duwa et al., 2014; Mbiba et al., 2019). Furthermore, in African catfish and Egyptian sole, Okore (2016) and Saleh et al. (2016) found that dietary MM could improve hematology parameters. All these studies suggested that MM had no adverse effects on these animals’ physical health.

In animals, physical health is closely related to non-special immunity. LZM is a key protein of the host non-special immune system (Callewaert and Michiels, 2010). Serum LZM activity is commonly used for evaluating the effect of nutrients on the non-special immunity of animals (Cho and Lee, 2012). In this study, serum LZM activity showed no significant difference between the control and experimental groups, indicating that MM did not attenuate the non-special immunity of growing beagles. This was consistent with the results of studies carried out on Nile tilapia and barramundi (Lates calcarifer) (Lin and Mui, 2017; Wang et al., 2017). However, Li
et al. (2019) found that dietary MM increased the serum LZM activity of bullfrog. The reason for these disparities needs to be further studied. CRP is a general indicator of inflammation, which plays an important role in non-special immunity (Satyaraj et al., 2013). In this study, there was no significant difference in CRP levels between the two groups, suggesting no significant variation in the non-special immune system. In addition, all CRP levels were within the normal range (0.8–16.4 µg/mL) for dogs (Satyaraj et al., 2013), indicating that dietary MM did not induce inflammation in growing beagles. In addition to non-special immunity, specific immunity is also critical in determining animal health. To date, little information is available regarding the effect of dietary MM on specific immunity of animals. In this study, the levels of antibodies against CDV and CPV were used to measure the specific immune function of beagles (Satyaraj et al., 2013). Our study found that there was no significant difference in the levels of antibodies against CDV and CPV between the two groups. All these results suggested that 5% MM supplementation in the diet had no negative effect on the immunity of growing beagles.

It is well known that oxidative damage caused by excessive ROS, is a major threat to animal health. Lipid peroxidation and protein oxidation products, such as MDA and PC, can reflect oxidative damage in living organisms (Ghosh et al., 2008). In the present study, the MDA and PC levels were decreased by dietary MM. To date, reports on the effect of MM on oxidative damage in animals are scarce. Li et al. (2019) reported that dietary MM inclusion alleviated liver lipid peroxidation in bullfrogs. Our results first showed that in addition to lipid peroxidation, dietary MM could also alleviate protein oxidation in animals. The reason for this alleviation of oxidative damage might be due to the improved activities of antioxidant enzymes. In animals, antioxidant enzymes play an important role in preventing oxidative damage by ROS removal. Studies have shown that dietary MM inclusion increases antioxidant enzyme activities in fish and bullfrogs (Ogunji et al., 2007; Dong et al., 2013; Lin and Mui, 2017; Li et al., 2019). However, further research is needed to verify this hypothesis in dogs.

In conclusion, the results of this study demonstrated that growing beagles could tolerate 5% MM in their diets without adverse effects on hematology, serum chemistry and immune responses. Furthermore, dietary MM alleviated oxidative damage in beagles. All these findings suggested that MM could be used as a high-quality protein source in growing dogs.

Acknowledgments
This research was financially supported by the Basic Research Project of Sichuan Province (A-2017N-29; A-2018N-24), Science and Technology Plan Project of Sichuan Province (2018JY0659), Scientific and Technical Research Project of Sichuan Provincial Administration of TCM (2018QN041). The authors would like to thank the personnel of these teams for their kind assistance.
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Received: 25 XI 2019
Accepted: 6 IV 2020