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Abstract

This study investigated if the intake of goat meat affects muscle atrophy and changes gut microbiota in animal models. Muscle atrophy-induced mice (C57BL/6N; 5-week-old) by dexamethasone were treated with a standard chow diet (DEX) and goat meat (DEX+G) for 18 days. Muscle atrophy-uninduced mice were treated with the standard chow diet (CON). The relative muscle mass (gastrocnemius, soleus, and quadriceps femoris) to the body weight of the mice, levels of serum biochemical markers, expression levels of muscle atrophy-related proteins, and cross-sectional area (CSA) of muscle fibers were measured in the mice. The gut microbiota was also analyzed. The relative mass of the gastrocnemius muscle was higher in the DEX+G than in the DEX. However, improvement related to muscle mass was not observed in serum biochemical markers. Of the three examined proteins in gastrocnemius muscle, MuRF1 and GDF-8 expression levels were lower in the DEX+G than in the DEX group. The average CSA of gastrocnemius muscle fiber was higher in the DEX+G group than in the DEX group, but it was lower in the DEX+G group than in the CON group. The goat meat treatment changed the composition of some gut bacteria in muscle atrophy-induced mice. In summary, goat meat intake might have a mild effect on improving gastrocnemius muscle mass and CSA, potentially related to lowered MuRF1 and GDF-8 expression and changes in gut microbiota. The current findings from a mouse model indicate that goat meat treatment has only a mild effect on limited factors. Therefore, further research is necessary.

Keywords: Muscle atrophy, goat meat, gut microbiota, skeletal muscle

Introduction

The goat is the most widely distributed livestock species globally, and its numbers have steadily increased over the past decade (FAOSTAT, 2020). Global meat consumption is also expected to increase, and the goat industry has the potential for similar growth (Mazhangara et al., 2019). In the past, goats were recognized mainly as a health supplement, but more recently, they have been consumed for their meat (Hwang et al., 2019). While the functions of goat meat were only described in classical Eastern medical literature, the nutritional and physiological activities of goat meat have recently been studied with modern science (Kim et al., 2019).

Muscle atrophy is a reduction in the skeletal muscle and may be caused by various factors, including muscle disuse, aging, and starvation (Jackman and Kandarian, 2004). In muscle atrophy, the fatigue resistance is lower, and the muscle fiber is reduced in diameter (Jackman and Kandarian, 2004). The World Health Organization (WHO) classified sarcopenia, the age-related loss of muscle, as a disease and suggested that it should be managed (Anker et al., 2016; Cao and Morley, 2016). Several meta-analysis studies have found that the prevalence of sarcopenia has increased in older adults around the world (Yuan and Larsson, 2023). To prevent muscle atrophy, adequate intake of dietary nutrients and increasing the protein anabolic capacity of skeletal muscle are necessary (Deutz et al., 2014; Bowen et al., 2015).

Wall and Van Loon (2013) found that supplementation with dietary protein and essential amino acids can help preserve muscle mass during disuse-induced muscle atrophy. Similarly, dietary protein supplementation during muscle disuse atrophy in healthy older men did not result in muscle loss during short-term disuse (Dirks et al., 2014). In addition, Thalacker-Mercer et al. (2007) suggested that inadequate protein intake during age-related muscle atrophy may downregulate transcripts encoding essential muscle proteins and protein

synthesis. There is also a possibility of gut microbiota being changed by the dietary protein (Albracht-Schulte et al., 2021; Wu et al., 2022).

Therefore, the objective of this study was to evaluate the effect of goat meat treatment on muscle atrophy and changes in gut microbiota in animal models.

Materials and Methods

1. Preparation of goat meat diet

The 12-month-old female goat meat used in the diet preparation was provided by Gaon Agricultural Corporation (Gangjin, Jeollanam-do, Republic of Korea). Each cut of goat meat (forelegs, hind legs, loin, and ribs) was cubed into 2 cm × 2 cm × 2 cm, and the cube samples were placed into sterilized bags and boiled in an 80°C water bath until the core temperature reached 77°C (Son et al., 2014). The cooked meat was stored in a -80°C deep freezer for one day and freeze-dried for 48 h in a freeze-dryer. The freeze-dried meat was ground into powder, and the powdered goat meat mixture of the four cuts (1:1:1:1) was added to the 2018S (2018S Teklad Global 18% Protein Rodent Diet; Envigo, Madison, WI, USA) at 8%. It simulated the inclusion of goat meat in a regular diet pattern. The concentration of goat meat in the diet corresponds to the average daily intake of red meat per person (69.5 g for a 60 kg Korean), according to the 2016 Korea National Health and Nutrition Examination Survey (KCDC, 2018). The dose was converted for mice according to the clinical trial guidelines of the U.S. Food and Drug Administration (FDA, 2005).

2. Animal experimental design

Five-week-old male C57BL/6N mice (Raon Bio, Yongin, Gyeonggi-do, Republic of Korea) were housed under a 12-h light/dark cycle with a constant temperature (23±1°C) and a humidity (55±5%). After one week of acclimation, the mice were treated with an intraperitoneal injection

of dexamethasone (dissolved in saline; D2915, Sigma-Aldrich, St. Louis, MO, USA) to induce muscle atrophy at 10 mg/kg/day (Hah et al., 2020), and the other mice were treated with saline for 14 days. The saline-treated mice were then fed with a normal diet (2018S) for control (CON; n=8), and the dexamethasone-treated mice were fed with a normal diet (DEX; n=7) and goat meat (DEX+G; n=8) for 18 days. The scheme of the animal experiment is described in Fig. 1, and the nutritional information of each diet is shown in Table 1. The animal experiment was approved by the Institutional Animal Care and Use Committee of Sookmyung Women's University (approval number: SMWU-IACUC-2301-025).

3. Measurement of body weight and relative mass of skeletal muscle

The body weights of the mice were measured daily during the muscle atrophy induction period and weekly during the dietary period. Also, the final weight of the mice was measured on day 18 of the dietary period. After the sacrifice of the mice by inhalation of isoflurane (Terrell™ isoflurane, Piramal Critical Care, Bethlehem, PA, USA) after 18-h fasting, skeletal muscle tissues were collected from the hindlimbs and weighed. The isolated skeletal muscle tissues were gastrocnemius, soleus, and quadriceps femoris. The relative mass of the skeletal muscles to the body weight of the mice were calculated with the following equation: relative mass of skeletal muscle to body weight (%) = skeletal muscle weight (g)/body weight (g)×100 (Kim et al., 2015). The obtained skeletal muscle tissues were stored at -80℃ for further analysis.

4. Serum biochemistry analysis

Blood samples were obtained from the posterior aorta of mice at sacrifice on day 18. The blood samples were left at room temperature for 30 min and then centrifuged at 2,339×*g* and 4℃ for 10 min to separate the serum. The serum was stored at -20℃ until analysis. The levels of serum creatine kinase (CK), lactate dehydrogenase (LDH), and creatinine were measured with Hitachi

Automatic Biochemical Analyzer (Hitachi 7180, Hitachi, Tokyo, Japan) in the KP&T (Cheong-ju, Chungcheongbuk-do, Republic of Korea).

5. Protein analysis

The protein of mouse gastrocnemius muscle tissue was extracted with PRO-PREP™ protein extraction (iNtRON Biotechnology, Seongnam, Gyeonggi-do, Republic of Korea), and protein concentration was quantified with DC™ Protein Assay Kit I (BioRad, Hercules, CA, USA) according to the manufacturer's instructions. The 15 μg of total protein samples were electrophoresed on 12% sodium dodecyl sulfate polyacrylamide gel at 120 V for 1 h, and the separated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Cytiva, Marlborough, MA, USA) at 60 V for 2 h 30 min. The membranes were incubated in 5% skim milk in Tris-buffered saline with 0.1% Tween-20 at 25℃ for 1 h for blocking. The proteins were then treated with the primary antibodies of GAPDH (GTX100118, GeneTex, Irvine, CA, USA), MuRF1 (sc-398608, Santa Cruz Biotechnologies, Inc., Santa Cruz, CA, USA), MAFbx (sc-166806, Santa Cruz Biotechnologies, Inc.), and GDF-8 (myostatin; sc-134345, Santa Cruz Biotechnologies, Inc.) at 4℃ for overnight, followed by the treatment of goat anti-mouse IgG (H+L)-HRP (Invitrogen, Carlsbad, CA, USA) and goat anti-rabbit IgG (H+L)-HRP (GenDEPOT, Katy, TX, USA) as the secondary antibodies. The antigen/antibody complexes were detected using enhanced chemiluminescence (ECL) solution (Dongin LS, Seoul, Republic of Korea) at room temperature for 1 min and then visualized by a biomolecular imaging system (Amersham™ ImageQuant™ 800, Cytiva). The intensity of the bands was quantified with GelQuaunt software v.2.7 (DNR Imaging system Ltd., Jerusalem, Israel), and the concentrations of each protein were normalized to the expression level of GAPDH.

6. Histological analysis

For histological analysis, three mice, which had results closed to the average of relative mass of gastrocnemius muscle and expression levels of muscle atrophy-related proteins in gastrocnemius muscle in each treatment, were selected with comprehensive analysis. The gastrocnemius muscles (n=3/group) were frozen-sectioned and stained with hematoxylin and eosin (H&E). The stained tissues were observed using a digital slice scanner (PANNORAMIC SCAN II, 3DHISTECH Ltd., Budapest, Hungary), and the cross-sectional area (CSA; μm^2) of 100-200 fibers for each sample was measured with the image analysis program (Image-Pro®, Media Cybernetics, Inc., Silver Spring, MD, USA). The average CSA of muscle fibers in each group was then calculated. This analysis was performed in T&P BIO (Gwangju, Gyeonggi-do, Korea).

7. Gut microbiota analysis

Fecal samples were collected directly from the anus into sterilized tubes after immobilizing the mice with a hand on day 0 of the dietary period. The fecal samples on day 0 and fecal samples from the cecum after sacrifice on day 18 of the dietary period were used for DNA extraction with DNeasy PowerSoil Pro Kit Protocol (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Quant-iT™ PicoGreen™ dsDNA Assay Kit (Invitrogen) was used to quantify the extracted DNA. From the extracted DNA sample, the V3-V4 regions of the 16S rRNA region in genomic DNA were amplified by PCR with Illumina 16S Metagenomic Sequencing Library Preparation protocols, and the pair-end (2×300 bp) sequencing was performed with the MiSeq™ platform (Illumina, San Diego, USA) in Macrogen (Seoul, Republic of Korea). The sequencing adapter and primer forward/reverse sequences were trimmed with the Cutadapt (v.3.2) program. The DADA2 (v.1.18.0) package in the R (v.4.0.3) program was used to correct errors in amplicon sequencing. The consensus method of DADA2

was then used to remove chimeric sequences and construct amplicon sequence variants (ASVs). The ASVs sequences were assigned taxonomy information for the most similar organisms based on the reference DB (NCBI 16S Microbial DB) using BLAST+ (v.2.9.0). The abundance and taxonomy information of the ASVs were used to analyze the diversity of the gut microbiota with QIIME (v.1.9). The alpha diversity was analyzed by the Shannon index and Chao1 values (Finotello et al., 2018). The beta diversity was analyzed based on weighted and unweighted UniFrac distance and visualized by principal coordinate analysis (PCoA) plot (Lozupone et al., 2011). To analyze the differences in gut microbiota among the groups on day 18, linear discriminant analysis effect size (LEfSe) analysis was performed with the phyloseq package in R (v.4.3.1) software at a significance level of $p<0.05$ and a linear discriminant analysis (LDA) score threshold of > 2.0.

8. Statistical analysis

Statistical analysis of the data was performed with SAS® OnDemand for Academics (SAS Institute Inc., Cary, NC, USA). For weekly body weight of mice, relative mass of skeletal muscle to body weight, serum biochemical markers, protein expression levels, histological analysis, and diversity and abundance of gut microbiota, differences among the three groups (CON, DEX, and DEX+G) were determined by pairwise t-test comparing least squares means at $\alpha=0.05$ with the general linear model. In addition, the data between pairs of two groups (CON vs. DEX, CON vs. DEX+G, and DEX vs. DEX+G) were compared with the student's *t*-test or Wilcoxon rank-sum test, depending on the normality of the data. A comparison of the relative abundance of gut microbiota within each group between day 0 and day 18 was evaluated using a paired t-test and Wilcoxon signed-rank test, depending on the normality test of the data.

Results and Discussion

1. Relative mass of skeletal muscle to body weight

Because dexamethasone used to induce muscle atrophy decreased skeletal muscle mass and weight loss (Choe, 2005; Lee et al., 2023), the weights of mice were measured. The weights of mice were significantly decreased ($p<0.05$) in the muscle atrophy-induced groups compared to the CON group during muscle atrophy induction with dexamethasone. On day 7 of the dietary period, both the DEX and DEX+G groups had similar body weight to the CON group, and all groups had similar body weight at the end of the dietary period (Fig. 2A). This result indicates that dexamethasone administration caused weight loss, but it was recovered during the dietary period.

About 40% of the human body mass is composed of skeletal muscle, and it has an important role in physical activity and energy expenditure (Wang and Pessin, 2013). In particular, the gastrocnemius muscle is often analyzed in studies of skeletal muscle function because it could be a representative of the strength and skeletal muscle mass of the body (Edström and Ulfhake, 2005; Xie et al., 2021). Also, the soleus and quadriceps femoris muscles that make up the hindlimb are often analyzed in animal models of muscle atrophy (Yamamoto et al., 2010; Sakai et al., 2019). The relative mass of skeletal muscles (gastrocnemius, soleus, and quadriceps femoris muscle) to body weight are shown in Fig. 2B-2D. The relative mass of the gastrocnemius muscle to body weight was lower ($p<0.05$) in the DEX (1.30±0.11%) group than the CON (1.41±0.05%) group, and it became higher ($p<0.05$) in the DEX+G (1.45±0.07%) group compared to the DEX group (Fig. 2B). The relative mass of gastrocnemius muscle was similar to the CON (Fig. 2B). For soleus muscle, there were no differences in the relative mass among the treatments (Fig. 2C). The relative mass of quadriceps femoris muscle of the DEX (0.94±0.18%) group was lower ($p<0.05$) than that of the CON (1.22±0.18%) group, but the relative mass of the DEX+G (1.16±0.26%) group was

not different from that of the DEX group (Fig. 2D). However, the relative mass of quadriceps femoris muscle in DEX+G was similar to that of the CON (Fig. 2D). This result shows that dexamethasone induced muscle atrophy in the gastrocnemius and quadriceps femoris muscles, and goat meat treatment in the muscle atrophy-induced group (DEX+G) made the relative mass higher than the DEX only in gastrocnemius muscle. Therefore, this result suggests that consumption of goat meat might help mildly restore muscle mass only in gastrocnemius muscle, not in other muscles, in muscle atrophy-induced mice.

2. Levels of biochemical markers in mice serum

According to Brancaccio et al. (2010), CK and LDH are enzymes that are released into the blood when muscle tissue is damaged and can be used as serum biomarkers to identify muscle damage. Creatinine is a product of the breakdown of creatine phosphate in the muscle (Patel et al., 2013). Thus, the levels of these biochemical markers in blood were measured and are presented in Fig. 3A-3C. The levels of CK, LDH, and creatinine in the DEX+G group were not different from those in the DEX group. The level of CK was higher ($p<0.05$) in the CON group than those in DEX and DEX+G groups (Fig. 3A). This result might be caused by variation among individual mice and hemolysis that occurred in the mice during blood collection. In hemolyzed samples, adenylate kinase released from red blood cells during their destruction might have interfered with the measurement of CK (Greenson et al., 1989). Meanwhile, the levels of LDH and creatinine were not different among the treatments (Fig. 3B-3C). Even though the differences in relative mass of gastrocnemius muscle were observed between DEX and DEX+G, the levels of biochemical markers were not different between the groups. It might be caused by variation among individual mice or insufficient muscle damage to cause the production of the biomarkers beyond the threshold.

3. Protein expression level in gastrocnemius muscle

The expression levels of proteins associated with muscle atrophy were analyzed in mouse gastrocnemius muscle (Fig. 4A-4C). The expression of MuRF1 was not different between CON and DEX, but it was lower (p<0.05) in the DEX+G group compared to the DEX group (Fig. 4A). The expression of MAFbx was not different among all treatments (Fig. 4B). The expression of GDF-8 protein was lower (p<0.05) in the DEX+G group compared to the DEX group, and it was similar to that of the CON group (Fig. 4C). MuRF1 (muscle RING finger 1) and MAFbx (muscle atrophy F-box) are E3 ubiquitin ligases that are major factors in causing atrophy of skeletal muscle by promoting the degradation of proteins (Bonaldo and Sandri, 2013; Kang et al., 2023). These proteins promote skeletal muscle atrophy under various stress conditions, including aging, increased glucocorticoids, inflammatory cytokine expression, and oxidative stress at the cellular level (Bodine and Baehr, 2014). Kim et al. (2021) found increased protein expression of MuRF1 and MAFbx in the muscle atrophy-induced group with dexamethasone compared to the control group. However, Alev et al. (2022) showed that rats induced with muscle atrophy using dexamethasone and then subjected to passive recovery tended to regain muscle mass. In our study, the mice in the DEX group were fed a normal diet without continuous dexamethasone treatment during the dietary period, which may have passive recovery and influence the expression of MuRF1 and MAFbx. In addition, the expression of MuRF1 and MAFbx might not be influenced by the applied concentration of dexamethasone. These reasonings might explain why there was no difference in the protein expression of MuRF1 and MAFbx between the CON and DEX groups. GDF-8, a member of the transforming growth factor-β (TGF-β) family, is a factor that regulates skeletal muscle growth by decreasing Akt/mTOR/p70S6K signaling (Sharma et al., 2001). Inactivation of GDF-8 can lead to skeletal muscle hypertrophy. In contrast, overexpression of GDF-8 can cause muscle atrophy (Rodriguez et al., 2014).

This result suggests that improved relative mass in gastrocnemius muscle in the DEX+G group might be related to the regulations of some muscle atrophy-related proteins, such as MuRF1 and GDF-8. However, the interpretation needed to be expanded more fundamentally. Therefore, the CSA of the gastrocnemius muscle was further analyzed to evaluate if the improved relative muscle mass was associated with an increased CSA.

4. Cross-sectional area of gastrocnemius muscle fiber in mice

The muscle fiber size was observed to be larger in the DEX+G group than in the DEX group (Fig. 5A). The CSA of gastrocnemius muscle fibers was higher ($p<0.05$) in the CON group than in the DEX and DEX+G groups (Fig. 5B). In a comparison between the two-muscle atrophy-induced groups, CSA was higher ($p<0.05$) in the DEX+G group than those in the DEX group (Fig. 5B). The decrease in the number and size of muscle fibers is an important characteristic of skeletal muscle atrophy (Hah et al., 2020; Jo et al., 2021), and thus, the CSA of muscle fibers can be an indicator to determine muscle atrophy and the recovery (Wang et al., 2022). This result suggests that treatment of goat meat in muscle atrophy-induced mice may increase the CSA of gastrocnemius muscle fiber. However, this increase was not as large as in the CON group. It may be associated with the improved relative muscle mass as described above.

5. Gut microbiota

5.1. Diversity of gut microbiota

Alpha diversity indicates the diversity of microbes in the community and is represented by Chao1 indicates species diversity, and the Shannon index considers the number of species and species evenness of the gut microbiota (Masetti et al., 2018). On day 0 and day 18, there were no differences in Chao1 and Shannon indices among the treatments. Overall, there was

a slight increase in alpha diversity on day 18 compared to day 0, regardless of the treatment group (Fig. 6A-6B). These results suggest that the administration of dexamethasone and the consumption of goat meat may not have a significant impact on change in the diversity of the gut microbiota. Beta diversity represents the diversity of the microbiota between samples within a comparison group (Koleff et al., 2003). On day 0, clusters of gut bacterial communities appeared similar in all groups (Fig. 6C). On day 18, the communities of the DEX+G group were clustered closer to the CON group than the DEX group (Fig. 6D).

5.2. Taxonomic composition of gut microbiota

The relative abundance at the phylum level on day 18 compared to day 0 was decreased in Bacillota in the CON group (Fig. 7A). The relative abundance was increased in Bacteroidota and decreased in Bacillota in the DEX group (Fig. 7B). However, no obvious changes of the relative abundance in Bacteroidota and Bacillota were observed in the DEX+G group (Fig. 7C). For the family level, the relative abundance of Akkermansiaceae was highly increased on day 18 in CON group compared to the DEX and DEX+G groups (Fig. 7D-7F). Comparison of the DEX and DEX+G groups showed that Lachnospiraceae decreased in the DEX group (Fig. 7E -7F). In Fig. 7G-7I, the relative abundance at the genus level is depicted. On day 18, the relative abundances of *Prevotellamassilia* were higher in the DEX and DEX+G groups than in the CON group (Fig. 7G-7I). *Prevotellamassilia* was lower in the DEX+G group than in the DEX group (Fig. 7H-7I). At the species level, the relative abundance of *Akkermansia muciniphila* was higher in the CON group compared to those of the DEX and DEX+G groups on day 18 (Fig. 7J-7L). Differences in gut microbiota on day 18 were investigated by LEfSe analysis of gut microbiota (Fig. 8). The LDA score of 14 bacteria was more than 2.0. Actinomycetes (class), Bifidobacteriales (order), and Bifidobacteriaceae (family) characterized the DEX group from CON and DEX+G. For the genus level,

Ligilactobacillus, *Luoshenia* and *Papillibacter*, *Bifidobacterium*, and *Marasmitruncus* characterized the CON, the DEX, and the DEX+G groups, respectively. In addition, five species (*Ligilactobacillus apodemi*, *Luoshenia tenuis*, *Papillibacter cinnamivorans*, *Christensenella hongkongensis*, and *Acetivibrio thermocellus*) were identified in the CON group, and one species (*Marasmitruncus massiliensis*) was identified in the DEX+G group. These results indicate that the taxonomic compositions of microbiota generally varied among the treatments. The composition of the gut microbiota is influenced by various ingredients consumed. The ingredients in goat meat are diverse and might vary among individual goats, sexes, ages, species, feed, etc. Thus, if an analysis is conducted on the relationship between gut microbiota and muscle atrophy without considering these factors in experimental design, using the result for the practical implication may not be appropriate, and the interpretation of the result from the analysis may include too many assumptions. Therefore, the relationship between muscle atrophy and gut microbiota was not analyzed in this study; only the effects of DEX and DEX+G treatments on changes in gut microbiota were analyzed as the optimum approach under given conditions.

4. Conclusions

In the mouse model, of the three muscles examined, the relative mass of only the gastrocnemius muscle was higher in the DEX+G than in the DEX, and it was similar to the CON group. However, obvious relations between muscle damage-related biomarkers in blood and improving the relative muscle mass were not observed. Of the three muscle atrophy-related proteins in the gastrocnemius muscle, the expressions of MuRF1 and GDF-8 were lower in the DEX+G than in the DEX. The CSA of the gastrocnemius muscle was higher in the DEX+G group than in the DEX group, but it was lower in the DEX+G group than in the CON group. Muscle atrophy induced by dexamethasone and goat meat treatment resulted in changes in gut

microbiota. These results show that goat meat treatment might improve mildly relative muscle mass and CSA in only gastrocnemius muscle in muscle atrophy-induced mice not in other muscles, and it might be related to lowered expression of MuRF1 and GDF-8 in the gastrocnemius muscle, but some biomarkers were not different between DEX and DEX+G. Hence, drawing a general conclusion with the information described above may not be appropriate before further study. The current findings are based on a mouse model and indicate that goat meat treatment shows only a mild effect on improving relative muscle mass and CSA only in the gastrocnemius muscle, but not in other muscles. Additionally, some factors were not different between DEX and DEX+G groups. Therefore, further research is necessary to assess the more apparent effect of consuming goat meat on muscle atrophy, especially in humans.

Conflict of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

Conceptualization: Lee J, Oh J, Yoon Y. Formal analysis: Lee J, Oh J. Methodology: Lee J, Oh J. Investigation: Lee J, Oh J. Writing – original draft: Lee J. Writing – review & editing: Kang J, Yoon Y.

Ethics Approval

This study was conducted in accordance with the ethical guidelines and was approved by the Institutional Animal Care and Use Committee (IACUC) of Sookmyung Women's University, under approval number SMWU-IACUC-2301-025.

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Table. 1. Nutritional information of 2018S (normal diet) and 2018S supplemented with powdered goat meat diet at 8% (goat meat diet)

Nutrition facts	Normal diet	Goat meat diet*
Calories (kcal/100 g)	338.18	360.52
Water (%)	5.24	6.82
Crude protein (%)	17.92	21.41
Crude fiber (%)	3.26	3.01
Crude fat (%)	4.93	10.23
Crude ash (%)	5.18	4.79

*Simulation for the inclusion of goat meat in a regular diet pattern

Figure legends

Fig. 1. Scheme of animal experimental to examine the effect of goat meat treatment on improving muscle mass in muscle atrophy-induced mice (five-week-old male C57BL/6N mice) with dexamethasone (10 mg/kg/d). CON: mice intraperitoneally injected with saline, followed by treatment with normal diet [standard chow diets (2018S)] during the dietary period; DEX: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with normal diet; DEX+G: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with goat meat during the dietary period.

Fig. 2. Changes in body weight of mice during the experimental period (A), relative mass of gastrocnemius (B), soleus (C), and quadriceps femoris (D) muscles to body weight in mice. Different letters indicate a significant difference ($p<0.05$) among treatments, and significant difference between two groups was indicated with asterisk symbol ($p<0.05$). CON: mice intraperitoneally injected with saline, followed by treatment with normal diet [standard chow diets (2018S)] during the dietary period; DEX: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with normal diet; DEX+G: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with goat meat during the dietary period.

Fig. 3. Levels of creatine kinase (A), lactate dehydrogenase (B), and creatinine (C) in mice serum. Different letters indicate a significant difference ($p<0.05$) among all treatments. CON: mice intraperitoneally injected with saline, followed by treatment with normal diet [standard chow diets (2018S)] during the dietary period; DEX: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with normal diet;

DEX+G: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with goat meat during the dietary period.

Fig. 4. Protein expression levels of MuRF1 (A), MAFbx (B), and GDF-8 (C) in gastrocnemius muscle of mice. Different letters indicate a significant difference ($p<0.05$) among all treatments and significant difference between two groups was indicated with asterisk symbol ($p<0.05$). CON: mice intraperitoneally injected with saline, followed by treatment with normal diet [standard chow diets (2018S)] during the dietary period; DEX: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with normal diet; DEX+G: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with goat meat during the dietary period.

Fig. 5. Representative muscle fiber cross-sections by histopathology at 40× magnification (A) and the average cross-sectional area (CSA) of gastrocnemius muscle fibers (B) in mice. Different letters indicate a significant difference ($p<0.05$) among all treatments and significant difference between two groups was indicated with asterisk symbol ($p<0.05$). CON: mice intraperitoneally injected with saline, followed by treatment with normal diet [standard chow diets (2018S)] during the dietary period; DEX: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with normal diet; DEX+G: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with goat meat during the dietary period.

Fig. 6. Chao1 (A) and Shannon indices (B) for alpha diversity, and beta diversity on day 0 (C) and day 18 (D) in mice. CON: mice intraperitoneally injected with saline, followed by

treatment with normal diet [standard chow diets (2018S)] during the dietary period; DEX: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with normal diet; DEX+G: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with goat meat during the dietary period.

Fig. 7. Relative abundance at the phylum level (A-C), family level (D-F), genus level (G-I) and species level (J-L) in the gut microbiota of mice for each treatment on day 0 and day 18 during the dietary period. The three figures for each level show the CON, DEX, and DEX+G groups in that order. CON: mice intraperitoneally injected with saline, followed by treatment with normal diet [standard chow diets (2018S)] during the dietary period; DEX: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with normal diet; DEX+G: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with goat meat during the dietary period.

Fig. 8. Linear discriminant analysis Effect Size (LEfSe) for the bacterial communities on day 18. CON: mice intraperitoneally injected with saline, followed by treatment with normal diet [standard chow diets (2018S)] during the dietary period; DEX: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with normal diet; DEX+G: mice intraperitoneally injected with dexamethasone during muscle atrophy induction, followed by treatment with goat meat during the dietary period.

Fig. 1

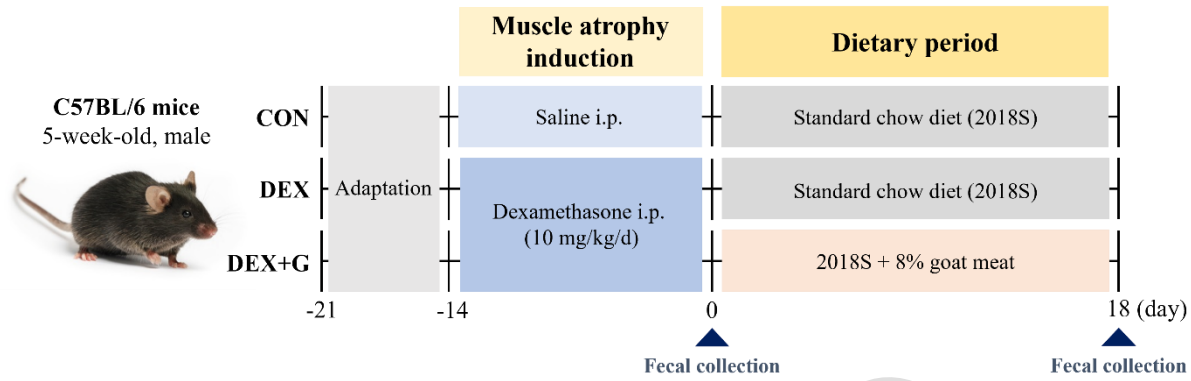


Fig. 2

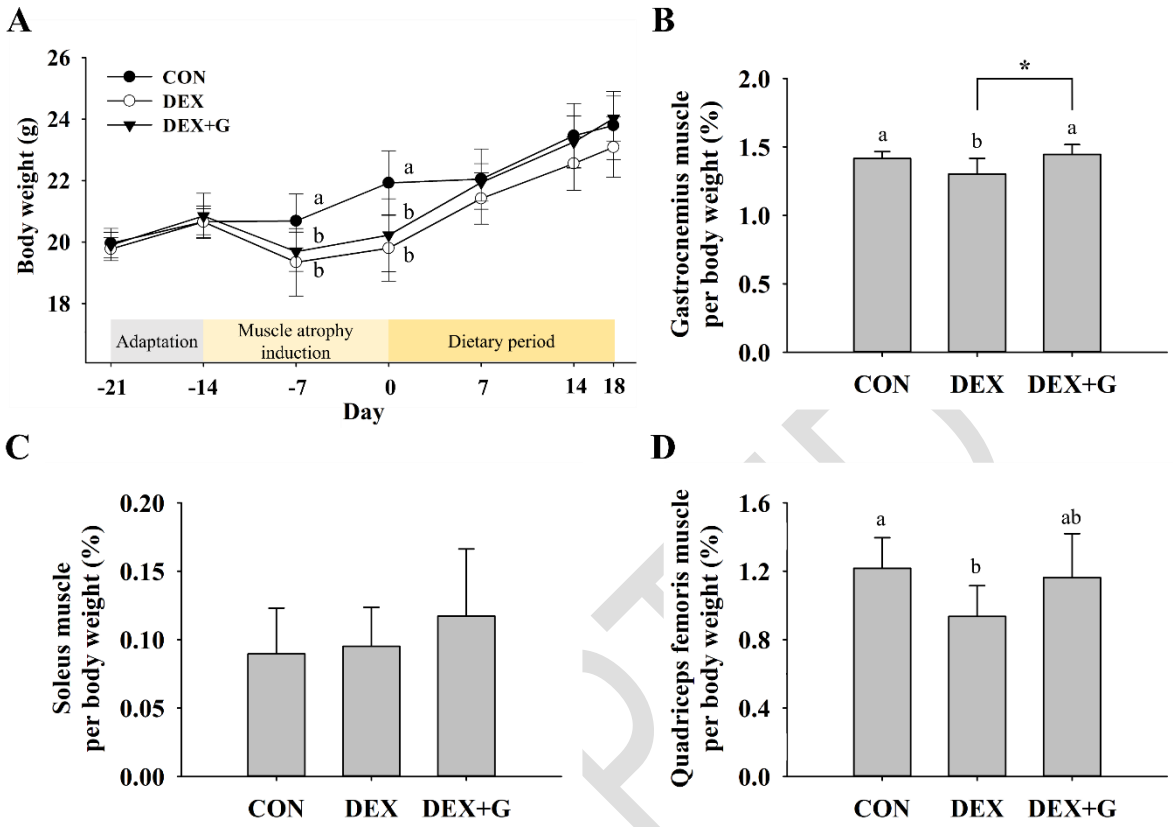

A
Body weight (g)
CON
DEX
DEX+G
Adaptation
Muscle atrophy induction
Dietary period
Day
B
Gastrocnemius muscle per body weight (%)
CON
DEX
DEX+G
C
Soleus muscle per body weight (%)
CON
DEX
DEX+G
D
Quadriceps femoris muscle per body weight (%)
CON
DEX
DEX+G

Fig. 3

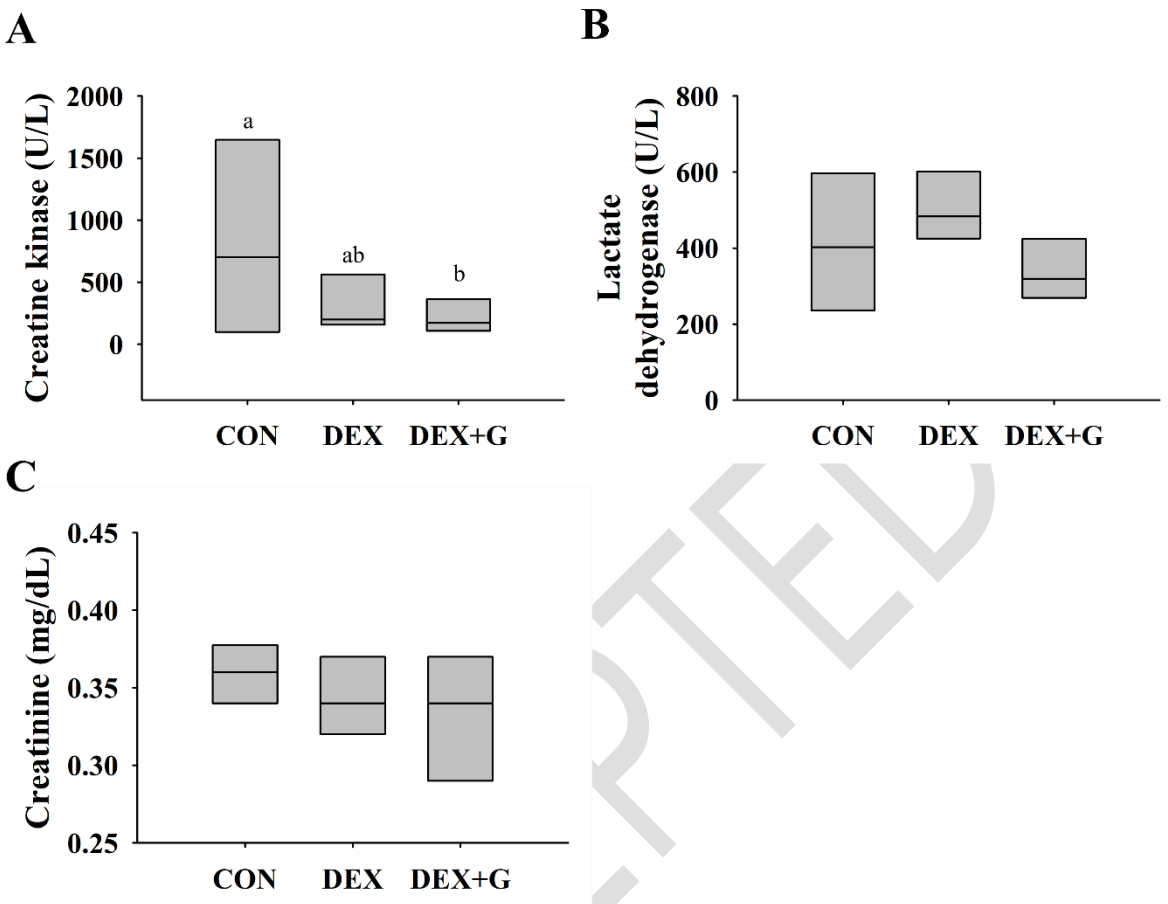
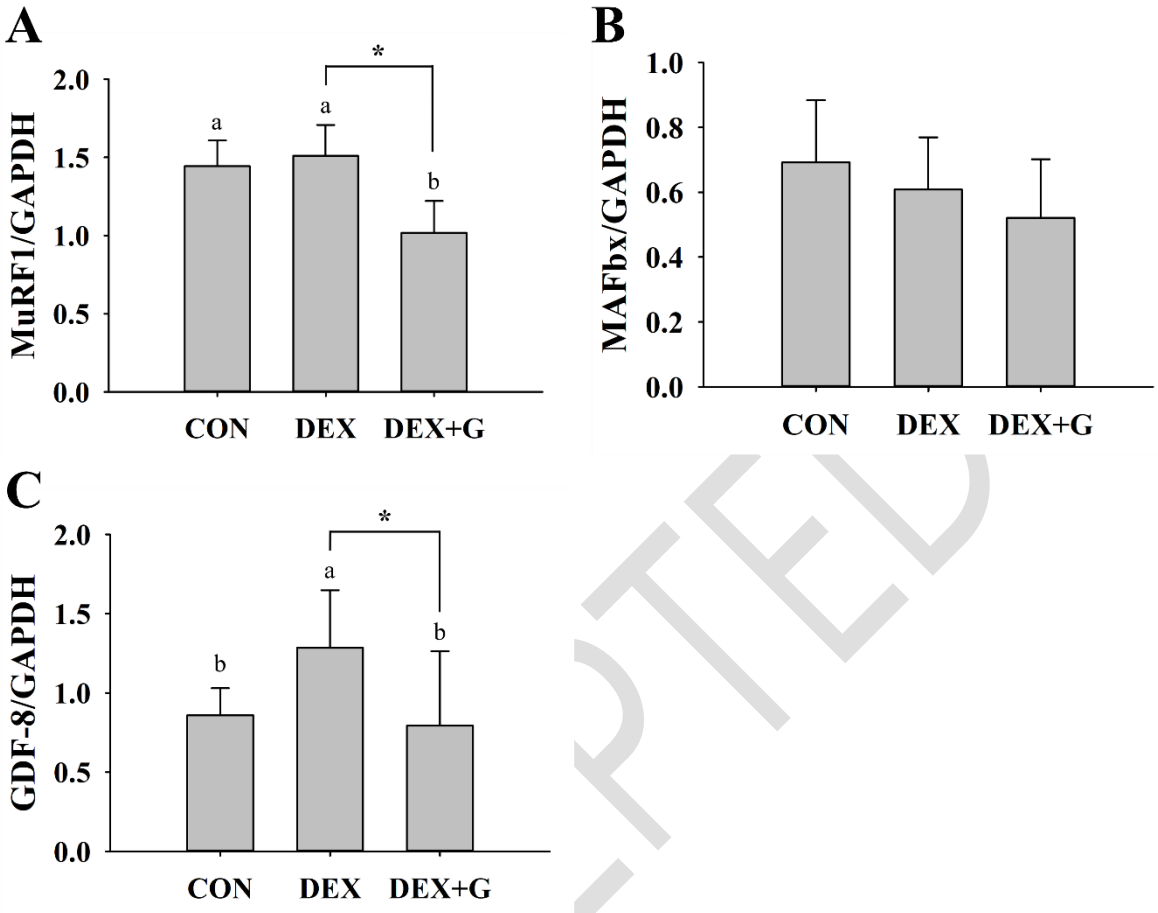


Fig. 4



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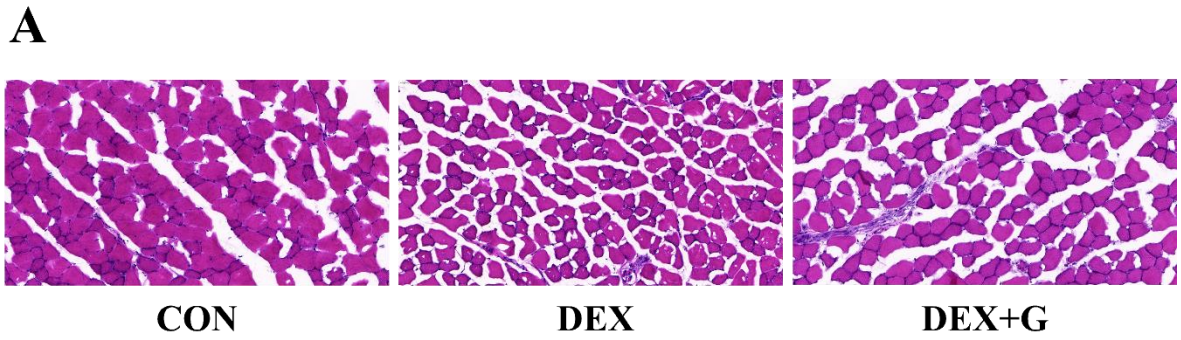
A
CON
DEX
DEX+G

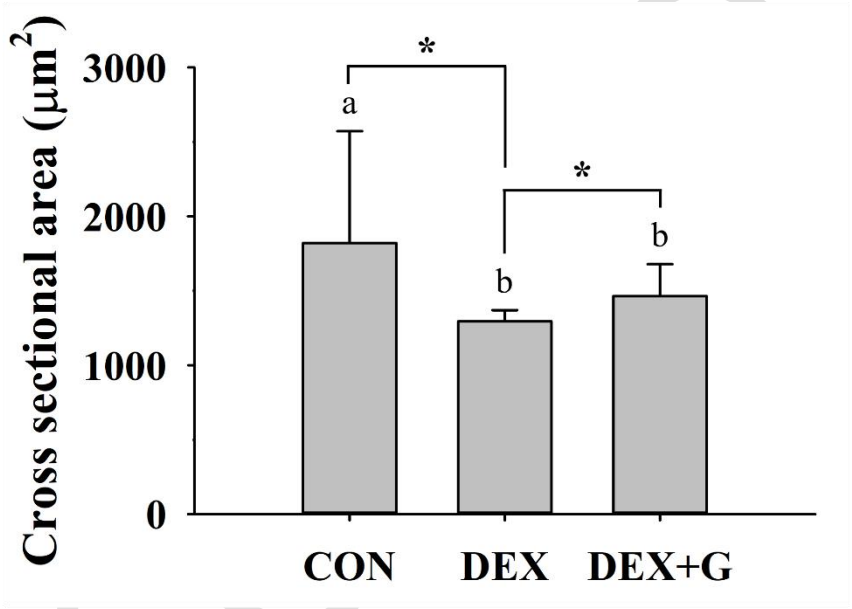
B
Cross sectional area (μm²)
3000
2000
1000
0
a
b
b
*
*
CON
DEX
DEX+G

Fig. 6

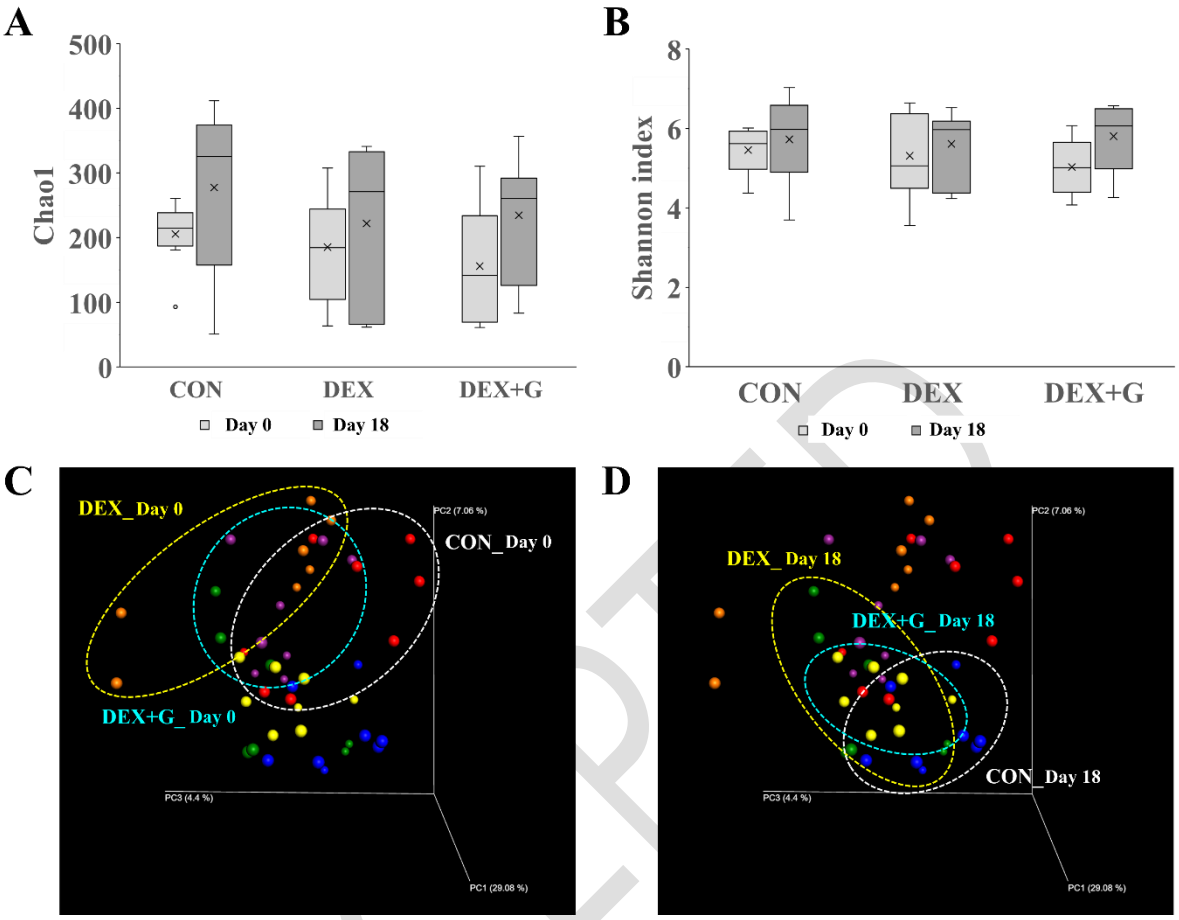

Fig. 7

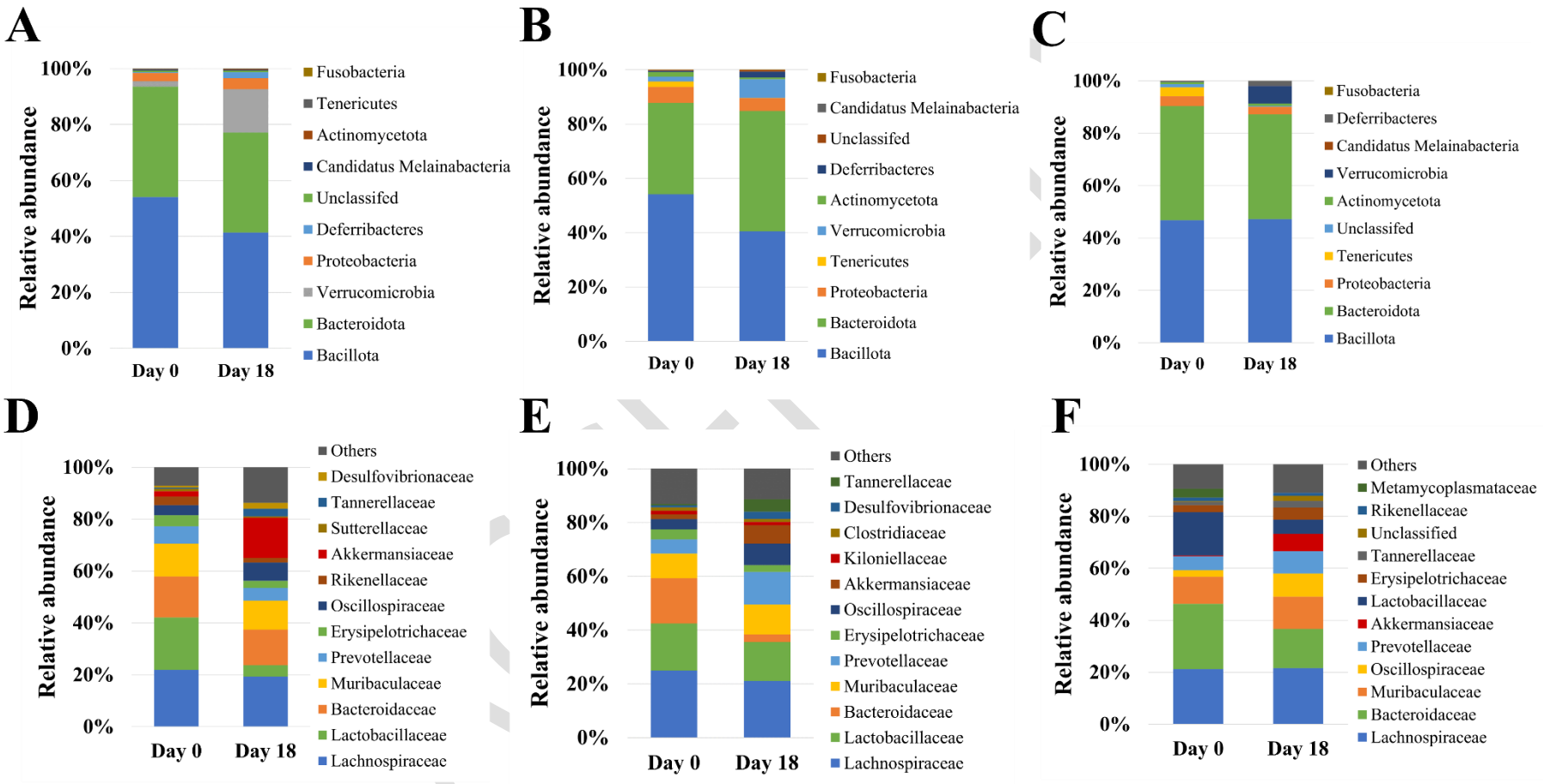
A
100%
80%
60%
40%
20%
0%
Relative abundance
Day 0
Day 18
Fusobacteria
Tenericutes
Actinomycetota
Candidatus Melainabacteria
Unclassifed
Deferribacteres
Proteobacteria
Verrucomicrobia
Bacteroidota
Bacillota
B
Fusobacteria
Candidatus Melainabacteria
Unclassifed
Deferribacteres
Actinomycetota
Verrucomicrobia
Tenericutes
Proteobacteria
Bacteroidota
Bacillota
C
Fusobacteria
Deferribacteres
Candidatus Melainabacteria
Verrucomicrobia
Actinomycetota
Unclassifed
Tenericutes
Proteobacteria
Bacteroidota
Bacillota
D
Others
Desulfovibrionaceae
Tannerellaceae
Sutterellaceae
Akkermansiaceae
Rikenellaceae
Oscillospiraceae
Erysipelotrichaceae
Prevotellaceae
Muribaculaceae
Bacteroidaceae
Lactobacillaceae
Lachnospiraceae
E
Others
Tannerellaceae
Desulfovibrionaceae
Clostridiaceae
Kiloniellaceae
Akkermansiaceae
Oscillospiraceae
Erysipelotrichaceae
Prevotellaceae
Muribaculaceae
Bacteroidaceae
Lactobacillaceae
Lachnospiraceae
F
Others
Metamycoplasmataceae
Rikenellaceae
Unclassified
Tannerellaceae
Erysipelotrichaceae
Lactobacillaceae
Akkermansiaceae
Prevotellaceae
Oscillospiraceae
Muribaculaceae
Bacteroidaceae
Lachnospiraceae

Fig. 7

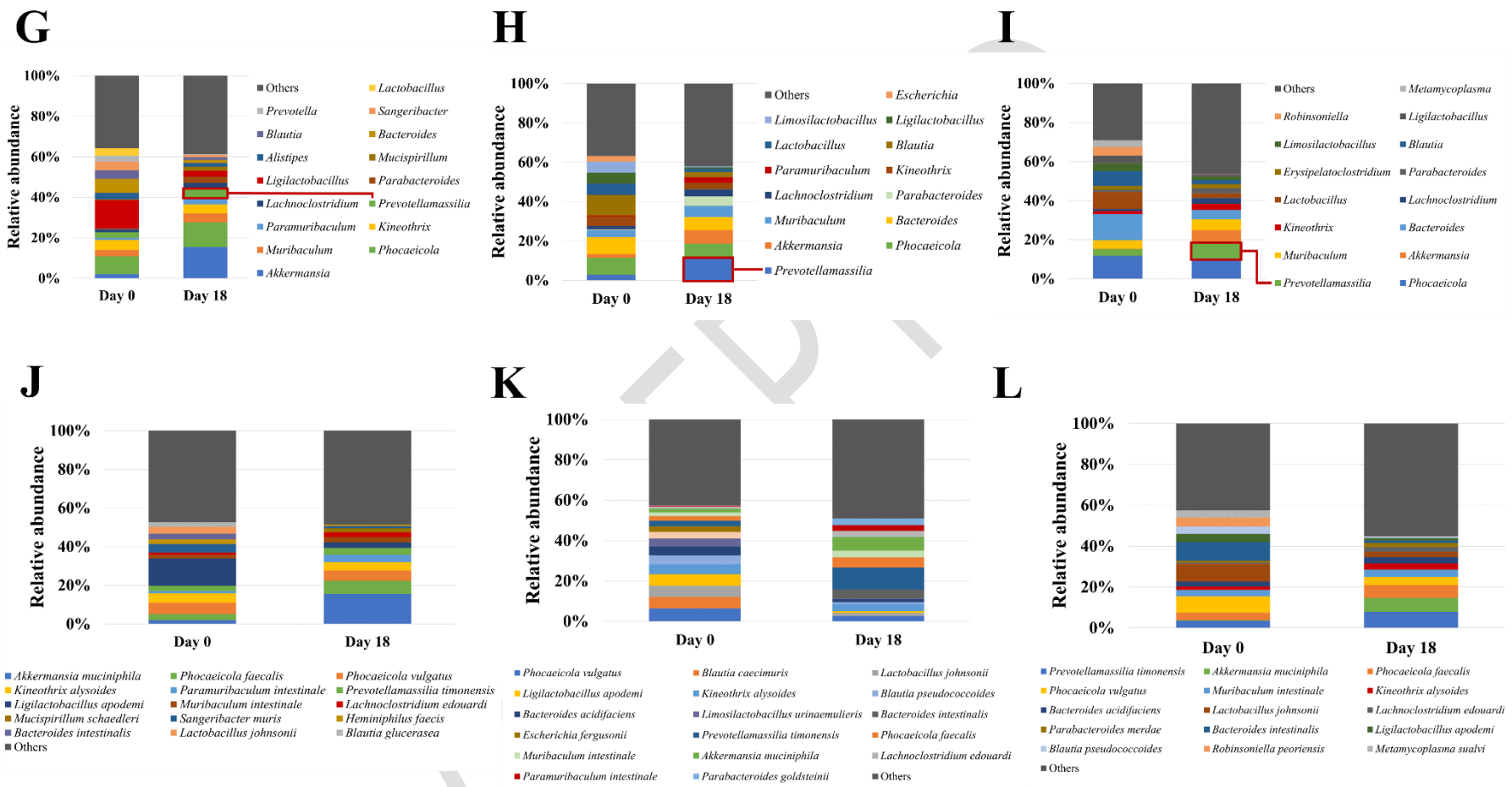
G
H
I
J
K
L
Relative abundance
100%
80%
60%
40%
20%
0%
Day 0
Day 18
Others
Lactobacillus
Prevotella
Sangeribacter
Blautia
Bacteroides
Alistipes
Mucispirillum
Ligilactobacillus
Parabacteroides
Lachnoclostridium
Prevotellamassilia
Paramuribaculum
Kineothrix
Muribaculum
Phocaeicola
Akkermansia
Escherichia
Limosilactobacillus
Ligilactobacillus
Lactobacillus
Blautia
Paramuribaculum
Kineothrix
Lachnoclostridium
Parabacteroides
Muribaculum
Bacteroides
Akkermansia
Phocaeicola
Prevotellamassilia
Metamycoplasma
Robinsoniella
Ligilactobacillus
Limosilactobacillus
Blautia
Erysipelatoclostridium
Parabacteroides
Lactobacillus
Lachnoclostridium
Kineothrix
Bacteroides
Muribaculum
Akkermansia
Prevotellamassilia
Phocaeicola
Akkermansia muciniphila
Phocaeicola faecalis
Phocaeicola vulgatus
Kineothrix alysoides
Paramuribaculum intestinale
Prevotellamassilia timonensis
Ligilactobacillus apodemi
Muribaculum intestinale
Lachnoclostridium edouardi
Mucispirillum schaedleri
Sangeribacter muris
Heminiphilus faecis
Bacteroides intestinalis
Lactobacillus johnsonii
Blautia glucerasea
Others
Phocaeicola vulgatus
Blautia caecimuris
Lactobacillus johnsonii
Ligilactobacillus apodemi
Kineothrix alysoides
Blautia pseudococcoides
Bacteroides acidifaciens
Limosilactobacillus urinaemulieris
Bacteroides intestinalis
Escherichia fergusonii
Prevotellamassilia timonensis
Phocaeicola faecalis
Muribaculum intestinale
Akkermansia muciniphila
Lachnoclostridium edouardi
Paramuribaculum intestinale
Parabacteroides goldsteinii
Others
Prevotellamassilia timonensis
Akkermansia muciniphila
Phocaeicola faecalis
Phocaeicola vulgatus
Muribaculum intestinale
Kineothrix alysoides
Bacteroides acidifaciens
Lactobacillus johnsonii
Lachnoclostridium edouardi
Parabacteroides merdae
Bacteroides intestinalis
Ligilactobacillus apodemi
Blautia pseudococcoides
Robinsoniella peoriensis
Metamycoplasma sualvi
Others

Fig. 8