Different Effects of Sheep- and Duck-Meat Supplemented Diets on Serum Cytokine Levels of Rats

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Summary This study aimed to explore the mechanism underlying the different effects of diet supplemented with sheep- or duck-meat on serum cytokines of rats. Methods: Male Sprague-Dawley rats were randomly divided into three groups fed on sheep meat, duck meat, or soybean, respectively. The profiles of amino acids and fatty acids of the three diets were examined, and the levels of serum cytokines in rats, including interleukin-1β (IL-1β), IL-2, IL-4, IL-6, IL-10 and tumor necrosis factor-α (TNF-α), were detected 30 d after feeding, using radioimmunoassay. The contents of methionine and glycine in the sheep-meat and duck-meat diets were significantly higher than those in the soybean diet. The content of saturated fatty acids in the sheep-meat diet and duck-meat diet was higher than that in soybeans, while the polyunsaturated fatty acids (PUFAs) in the duck-meat diet were highest and those in the sheep-meat diet were lowest. Serum levels of IL-2 and IL-10 in the rats of the sheep-meat and duck-meat groups were significantly higher than those in the rats of the soybean group (p<0.05). IL-10 and TNF-α in the rats of the sheep-meat group were higher than those in the duck-meat group. But the levels of IL-1β and IL-6 were not significantly different among the three groups. Additionally, there were positive correlations between glycine and IL-1β as well as glycine and IL-2, while negative correlation existed between C18:2 and TNF-α. Methionine, glycine and PUFAs in a diet supplemented with sheep- or duck-meat might influence the levels of serum cytokines in rats, suggesting the potential regulatory mechanism of amino acids and fatty acids from diet in immune responses.

Key Words sheep meat, duck meat, serum cytokine, fatty acid, amino acid

It is known that variations in nutrient status can influence the physiological activities of a living body, especially in the immune system (1). Nutritional deficiencies can change immunocompetence and promote the risk of infection, according to epidemiologic and clinical data (2). In addition, recent progress in immunology has demonstrated that the endocrine system and nervous system have strong interrelations with immune systems (3, 4). The acute phase response, as an orchestrated action to tissue injury, infection or inflammation, is involved in the restoration of homeostasis (5). Control of the acute-phase response is mainly mediated by cytokines, which comprise a diverse range of polypeptides and multifunctional molecules as well as numerous important functions, and are required to maintain homeostasis via complex interactions among the endocrine, nervous and immune systems (6). In previous studies, it has been suggested that serum levels of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), and interferon (IFN), can be altered by dietary protein, amino acid and fatty acid intakes (7, 8).

In traditional Chinese medicine, sheep and duck meat have been considered as useful agents for curing disease, as was recorded in Bencaoangangmu (Pents’ao Kang Mu, 1590 AD) in Chinese by Shizhen Li. But in the past, sheep meat, which is characterized by a high content of saturated fatty acids and low content of polyunsaturated fatty acids (PUFAs), was regarded as being disadvantageous for the human diet (9). For duck meat, wild duck meat has been considered as a good source of polyunsaturated fatty acids, particularly those with 20 and 22 carbon atoms (10). However, the specific physiological changes related to the consumption of sheep and duck meat have not been well defined, and the potential mechanisms related with therapeutic action remain unexplored. An understanding of the different effects of sheep and duck meat on physiological activities is necessary.
Therefore, in this study, we examined the amino acid profiles and fatty acid compositions of diets supplemented with sheep- or duck-meat, and analyzed the influences of these diets on serum cytokine levels in young rats, in order to detect the physiological changes of sheep and duck meat on rats and further to explore the underlying mechanism.

MATERIALS AND METHODS

Animal experiments. Twenty-seven healthy male Sprague-Dawley rats (weighing, 160–180 g) were purchased from Shanghai Slaccas Medical Animal Center (Shanghai, China). All rats were accommodated in an environment-controlled room (temperature: 23 ± 3°C, humidity: 75 ± 5%, 12 h dark/12 h light cycle) throughout the experiment, and fed on the same diets supplemented with sheep meat (SHP group, n = 9), duck meat (DUK group, n = 9) or soybean (Soybean group, n = 9). The diet composition of these three groups is listed in Table 1. The sheep meat was supplied by Caoyuanxingfa Group Ltd (Inner Mongolia Province, China), and the duck meat was obtained from Tianhui Food Ltd (Shandong Province, China). The rats had free access to tap water and diet. The body weight and food intakes of the rats in the Soybean, SHP and DUK groups were measured on the 1st day and subsequently on the 10th, 20th and 30th day after feeding. The animal research was undertaken according to the guidelines of the Animal Ethics Committee of Nanjing Agricultural University.

The fresh sheep meat contained 18.0% crude protein, 9% crude fat and 72% water; fresh duck meat contains 15.5% crude protein, 7.35% crude fat and 76% water; fresh soybean includes 46.8% crude protein, 15.5% crude protein, 7.35% crude fat and 72% water; fresh duck meat contains 18.0% crude protein, 9% crude fat and 10% water. A certain amount of fresh meat and soybean were crushed and mixed with corresponding materials. The mixture was pelleted and preformed through air-dry processing at 100°C for 6 h.

Table 1. Body weight and diet consumption of rats in three groups.

|                      | Soybean | Sheep meat | Duck meat |
|----------------------|---------|------------|-----------|
| Initial body weight  | 170 ± 6.5 | 173 ± 6.3  | 179 ± 6.5 |
| Final body weight    | 380 ± 25.5 | 382 ± 30.6 | 368 ± 27.5 |
| Food intake          | 253 ± 22.4 | 258 ± 23.4 | 263 ± 24.8 |

Result are expressed as mean ± SD (n = 9). There is no significant difference (p > 0.05).

The fresh meat and soybean were crushed and mixed with corresponding materials. The mixture was pelleted and preformed through air-dry processing at 100°C for 72 h. The vitamin mixture provided in milligrams per 100 g diet and the detailed information were as follows: ascorbic acid, 31.4; niacin, 5.04; riboflavin, 0.38; thiamin, 0.32; folic acid, 0.06; vitamin B6, 0.25; biotin, 0.03; pantothenic acid, 1.9; and choline, 53.2. Per 100 g the diet included: vitamin A, 1,007 IU; vitamin D, 253 IU; vitamin E, 6.3 IU; vitamin B12, 1.26 μg; and phylloquinone, 63 μg. The mineral mixture content of ions or cations (expressed in milligrams per 100 g diet) and the actual chemical compounds included: 378 Ca (CaHPO4·2H2O) and Ca3(C2H5O7)2·4H2O; 208 P (K2HPO4·2H2O); 7.7 Fe (FeSO4·2H2O); 44 Mg (MgO); 0.38 Cu (CuSO4·5H2O); 2.5 Zn (ZnSO4·7H2O); 0.63 Mn (MnSO4); 840 Cl (CaH12ClNO); 1,050 K (K2HPO4·2H2O); and 245 Na (NaI). Vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets (11).

Amino acid and fatty acid analysis. The amino acid profile of the diet in each group was analyzed using a Hitachi Amino Acid Analyzer 835–50 (Hitachi Co., Tokyo, Japan). The samples were ground and hydrolyzed using 6 N HCl vapor under a vacuum. After hydrolysis, samples were filtered, evaporated, and then dissolved in 0.02 N HCl solution. Amino acids, which were separated by cation-exchange chromatography, were detected spectrophotometrically after post-column reaction with ninhydrin reagent.

The fatty acid compositions were analyzed as described by Bendiksen et al. (12). Briefly, lipid extracts were transmethylated in 3 N methanolic HCl at 80°C over 4 h. Then, the fatty acid methyl esters were separated by gas chromatography using a PerkinElmer Auto System XL gas chromatograph (PerkinElmer, Waltham, MA), which was equipped with a split/splitless injector fitted with a fused silica capillary column (CP Wax 52CB, Chrompak, 25 m × 0.25 mm internal diameter; Agilent Technologies, Santa Clara, CA) and a flame ionization detector. Finally, fatty acids were identified automatically using Turbochrom software (PerkinElmer) by reference to fatty acid ester standards (68D, Nu-ChekPrep., Waterville, MN). Values are presented as a mole percentage of total fatty acids.

Serum sample collection and cytokine assays. Blood samples were collected from rats on the 30th day via orbital venous plexus penetration. The samples were stored at 4°C for 1.5 h and centrifuged at 3,000 × g (4°C, 10 min). The serum samples were then immediately aliquoted and frozen at −70°C.

Serum concentrations of IL-1β, IL-2, IL-4, IL-6, IL-10 and TNF-α were measured using commercial radioimmunoassay kits from Beijing North Institute of Biological Technology (Beijing, China). The coefficients of variance (CV), normal ranges and sensitivities were as follows: IL-1β, CV intra-assay <12%, CV inter-assay <13.0%, normal range 0.1–8.1 ng/mL, sensitivity 0.1 ng/mL; IL-2, CV intra-assay <7%, CV inter-assay <10%, normal range 1–81 ng/mL, sensitivity 0.1 ng/mL; IL-4, CV intra-assay <8%, CV inter-assay <15%, normal range 0.3–24.3 ng/mL, sensitivity 0.165 ng/mL; IL-6, CV intra-assay <10%, CV inter-assay <15.0%, normal range 0.1–3.2 ng/mL, sensitivity 0.1 ng/mL; IL-10, CV intra-assay <5%, CV inter-assay <10%, normal range 3–243 ng/mL, sensitivity 3 ng/mL; TNF-α, CV intra-assay <10%, CV inter-assay <15.0%, normal range 9–590 fmol/mL, sensitivity 6 fmol/mL. All examinations were performed twice and the means of the two measurements were used for subsequent analysis.

Statistical analysis. Statistical analysis was per-
formed using the Statistical Package for Social Sciences version 12.0 for Windows (SPSS Inc., Chicago, IL). Differential analysis was conducted using one-way analysis of variance (ANOVA) and Fisher’s Protected Least Significant Difference (PLSD) multiple-comparison tests. Data are expressed as means ± standard deviations (SD). *p* < 0.05 was considered as significance difference.

**RESULTS**

**Body weight and diet consumption**

The body weight and diet consumption of these three groups are recorded in Table 2. The result showed that there was no significant difference for initial or final body weights or diet consumption among the three groups (Table 1).

**Chemical profiles of the diet**

The diets used in this study were formulated and had similar gross nutritional compositions. These three diets, the Soybean group, SHP group and DUK group, contained similar dry material, crude protein, lipids (ether extract) and energy. As shown in Table 2, the total protein in diet of the Soybean group, SHP group and DUK group were 30.4%, 30.4% and 30.41%, respectively. The ether extract in these three diets were 11.4%, 12.5% and 11.9%, respectively. The total energy of each diet was also similar (1.48 × 10^7 J/kg, 1.42 × 10^7 J/kg and 1.51 × 10^7 J/kg, respectively). In addition, the overall amino acid levels displayed minor quantitative differences (Table 3). Significantly, the diets of the DUK and SHP groups contained approximately twice as much methionine as that of the Soybean group. For alanine and glycine, the content in the diets of the DUK group and SHP group were about 1.5 times of that in the Soybean group.

Although the overall ether extracts (>95% fatty acids) were similar, differential fatty acids were found among the three groups (Table 4). The SHP diet contained approximately 40% saturated fatty acids, which was almost twice of that in the other two groups. The DUK diet contained the lowest level of saturated fatty acids, but had the highest content of total unsaturated fatty acids (approximately 85%) due to a disproportionally high level of polyunsaturated fatty acids (PUFAs) (approximately 60%), while the diets of the Soybean and SHP groups included similar monounsaturated fatty acids (35–40%, almost 1.5 times of the DUK diet). Only 10% of total PUFAs was observed in the SHP diet (Table 4). Meanwhile, the saturated fats myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) were higher in the SHP group than in the DUK group, while the polyunsaturated fatty acids linoleic acid (C18:2) and linolenic acid (C18:3) were lower in the SHP group than in the DUK group.

**Serum cytokine levels**

Changes in the serum cytokines in rats were detected,

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**Table 2. Diet ingredients in three groups (%).**

| Ingredients                  | Soybean diet | Sheep-meat diet | Duck-meat diet |
|------------------------------|--------------|-----------------|----------------|
| Corn                         | 14.5         | 25              | 32             |
| Wheat by-products            | 14.7         | 26.7            | 23.7           |
| Soy bean cake                | 57.5         |                 |                |
| Sheep meat powder            |              |                 | 46             |
| Duck meat powder             |              |                 | 42             |
| Soy oil                      | 11           |                 |                |
| Vitamin mix                  | 1            | 1               | 1              |
| Mineral mix                  | 0.3          | 0.3             | 0.3            |
| NaCl                         | 100          | 100             | 100            |
| Total protein (%)            | 30.4         | 30.4            | 30.4           |
| Ether extract (%)            | 11.4         | 12.5            | 11.9           |
| Total energy (J/kg)          | 1.48 × 10^7  | 1.42 × 10^7     | 1.51 × 10^7    |

1 The levels of protein of sheep meat powder, duck meat powder and soybean cake were 57.9%, 58.1% and 46.8%, respectively.

**Table 3. Amino acid profiles of the diets.**

| Amino acid | Soybean diet | Sheep meat | Duck meat |
|------------|--------------|------------|-----------|
| Threonine  | 1.19         | 1.34       | 1.34      |
| Valine     | 1.31         | 1.59       | 1.58      |
| Methionine | 0.36         | 0.7        | 0.85      |
| Isoleucine | 1.31         | 1.33       | 1.4        |
| Leucine    | 2.25         | 2.54       | 2.57      |
| Phenylalanine | 1.52   | 1.31       | 1.3        |
| Lysine     | 1.78         | 2          | 1.92      |

**Table 4. Fatty acid composition in the diets (%).**

| Essential fatty acid | Soybean diet | Sheep meat | Duck meat |
|----------------------|--------------|------------|-----------|
| C14:0                | —            | 3.85       | 0.1       |
| C16:0                | 17.9         | 22.0       | 10.6      |
| C18:0                | 5.78         | 17.3       | 4.71      |

| Unsaturated fatty acid | Soybean diet | Sheep meat | Duck meat |
|------------------------|--------------|------------|-----------|
| C16:1                  | 1.03         | 1.80       | 0.09      |
| C18:1                  | 33.6         | 36.4       | 22.4      |
| C18:2                  | 36.9         | 9.86       | 53.4      |
| C18:3                  | 1.78         | 1.03       | 6.66      |
| C22:1                  | 1.52         | 0.35       | —         |
| C22:6                  | 1.37         | 0.49       | 0.47      |
| Total                  | 100          | 93.1       | 98.4      |
and the result is shown in Table 5. In the SHP group, significantly higher serum levels of IL-4, IL-10 and TNF-α were observed compared with the DUK group \((p<0.05)\), and significantly higher levels of IL-2 and IL-10 both existed in rats of the DUK and SHP groups in comparison with those of the Soybean group \((p>0.05)\).

Moreover, the correlation between dietary contents and serum cytokines was also analyzed. As shown in Table 6, significant correlations were observed between glycine and IL-1β \((p=0.0145)\), glycine and IL-2 \((p=0.0156)\), C18:2 and TNF-α \((p=0.0371)\), valine and IL-6 \((p=0.045)\), and proline and IL-6 \((p=0.0094)\). No obvious correlation existed in aspartate and IL-6 \((p=0.0549)\).

### DISCUSSIONS

The present study analyzed the components of three diets, sheep meat, duck meat, and soybean, and we found that the levels of methionine, alanine and glycine were significantly higher both in the SHP and DUK group in comparison with the Soybean group. The levels of saturated and unsaturated fatty acids were different among the three groups despite similar total content. Besides, the serum cytokine levels of rats in the three groups were detected, and differential cytokine levels were observed among the three groups.

Amino acids were not only involved in the cell signaling pathway, but also in the regulating gene expression and protein phosphorylation cascade \((13)\). Methionine was necessary for the normal growth and development of mammals \((14)\), and played an important role in immune responses \((15)\). In mammals, methionine metabolism included 2 pathways, the methionine cycle and the transsulfuration sequence \((16)\). Glycine, as a significant component of bile acids secreted into the lumen of the small intestine, was known to be necessary for the digestion of dietary fat and the absorption of long-chain fatty acids, and could improve immunity as well as metabolic disorders \((19)\). Recently, an enteral diet supplemented with 5% glycine had been found that can reduce the mortality of rats challenged with LPS \((20)\). This evidence suggested that the amino acids included in diet play crucial roles in mammals’ metabolic function.

Meanwhile, in this study, the levels of fatty acids were also detected for different diets, and we found that the DUK group had the highest content of polyunsaturated fatty acids, while the SHP group was the lowest. High levels of PUFAAs had been also reported in wild ducks \((21)\). Recently, a higher ratio of PUFAAs to saturated fatty acids and a more favorable balance between n-6 and n-3 PUFAAs had been defined as a healthier meat \((22)\). Long-chain n-3 PUFAAs were potentially potent anti-inflammatory agents because of replacing arachidonic acid as an eicosanoid substrate and inhibiting arachidonic acid metabolism as well as altering the expression of inflammatory genes through transcription factor activation \((23)\). Thus, the DUK diet might be more important to anti-inflammation response than the SHP diet due to the higher level of PUFAAs.

In addition, the serum cytokine levels of rats in the three groups showed that only IL-10 was significantly different among the three groups. Both TNF-α and IL-4 in the SHP group were higher than those in the DUK group obviously, but no significant difference was found between the SHP group and Soybean group nor between the DUK group and the Soybean group. A higher level of IL-2 was observed both in the SHP group and in the DUK group. In fact, IL-10 as a potent anti-inflammatory cytokine was produced by T-cells, B-cells, monocytes and macrophages \((24)\), and played a crucial role in the innate immune system \((25)\), as well as IL-4 \((26)\). IL-10 could potently inhibit the production of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-2 and IL-6 \((27)\). This evidence suggested that IL-10 and IL-4 had an opposite function to IL-1β, IL-2 and IL-6. We supposed that the sheep meat actually influences cytokine production more strongly than the duck meat, and both the sheep meat and duck meat non-selectively

Table 5. Levels of serum cytokines in three dietary groups.

|     | Soybean | Sheep meat | Duck meat | \(p\) |
|-----|---------|------------|-----------|------|
| IL-1β ng/mL | 0.166±0.038 | 0.191±0.030 | 0.178±0.02 | 0.1 |
| IL-2 ng/mL | 2.0±0.62\(^a\) | 5.60±3.2\(^a\) | 5.13±2.06\(^a\) | 0.03 |
| IL-4 ng/mL | 4.41±1.05\(^{ab}\) | 5.72±1.48\(^b\) | 4.29±0.98\(^b\) | 0.04 |
| IL-6 ng/mL | 0.26±0.11 | 0.25±0.07 | 0.26±0.08 | 0.113 |
| IL-10 ng/mL | 102.6±15.1\(^c\) | 159.20±20.8\(^c\) | 129.80±19.8\(^b\) | 0.01 |
| TNF-α fmol/mL | 43.19±9.07\(^{ab}\) | 48.20±7.05\(^a\) | 40.87±6.99\(^b\) | 0.01 |

Data are expressed as mean±standard deviations \((n=10)\). Different superscript letters indicate significant difference at \(p<0.05\).

Table 6. Pearson relationship analysis between dietary contents of amino acids, fatty acids and serum cytokines levels in rats.

| Item   | Correlation coefficient | \(p\) |
|--------|-------------------------|------|
| Glycine–IL-1β | 0.9001 | 0.0145\(^*\) |
| Glycine–IL-2 | 0.8956 | 0.0156\(^*\) |
| C18:2–TNF-α | −0.8509 | 0.0317\(^*\) |

\(^*\) \(p<0.05\) represents a significant difference.
stimulate the immune system, resulting in various cytokine levels.

Furthermore, Pearson relationship analysis in this study showed that glycine was positively related with IL-1β and IL-2, and C18:2 had negative correlation with TNF-α. In previous studies, glycine was also found to be not only dose-dependent in reducing the release of pro-inflammatory TNF-α and IL-1β after exposure to endotoxin, but also capable of accelerating the secretion of anti-inflammatory IL-10 (28). The addition of glycine to lymphocytes could inhibit CD3-stimulated proliferation via an IL-2-independent mechanism (8). Additionally, the amino acids and PUFAs mentioned above were known to play important roles in immune and inflammation responses, so we deduced that glycine and C18:2 might play important roles for changing cytokine levels in rats fed on sheep or duck meat.

In conclusion, we investigated the amino acid and fatty acid compositions of these three diets, sheep meat, duck meat and soybean, and the corresponding levels of serum cytokines in rats were detected. It is possible that higher levels of methionine and glycine in sheep meat and duck meat, as well as higher level of PUFAs in duck meat, were associated with the changes in the serum cytokine levels in rats. However, further studies are needed to elucidate the roles of free amino acids and fatty acids included in the diet on the cytokines levels and even on the immune responses of mammals, as well as their regulatory mechanisms.

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