PE9.14 Use of meat hydrolysate as supplemental feed and its effects on lifestyle-related diseases 98.00

Abdulatef Ahhmed (1&2) abdo772201@yahoo.co.uk, Y Namba(1), T Nakamura(3), M Muguruma (1)
(1)University of Miyazaki, Japan
(2)Gheran Higher Centr for Agricultural Technologies, Japan
(3)Marunaka Foods Inc., Japan

Abstract— **Digested meat was fed to spontaneously hypertensive rats (SHRs), and the effects on lifestyle-related diseases, in terms of reduction of the systolic blood pressure and blood sugar level, were evaluated. The meat was hydrolyzed with pepsin and trypsin, and the hydrolyzates were subjected to gel filtration chromatography. The results indicate that meat hydrolyzates contain some important peptides that could be healthy and nutritious. In addition to their strong antihypertensive activity, meat hydrolyzates also have a diabetes-lowering effect. Thus, these hydrolyzates may have potential applications as functional foods.**

A. M. Ahhmed is with the *Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan,* phone: +81-98558-7203; fax: +81-98558-7203; e-mail: abdo772201@yahoo.co.uk); on leave from *Gheran Higher Centre for Agriculture Technologies, P.O.B, 151-Tripoli, Libya*

Y. Namba is with the Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan

T Nakamura is with Marunaka Foods Inc., Tsukubamirai, Ibaragi, 300-2436, Japan, (marunakasun@miracle.ocn.ne.jp)

M. Muguruma is with the *Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan,*(e-mail: Muguruma@cc.miyazaki-u.ac.jp).

Keywords: meat hydrolysates, life-style related disease, blood pressure, diabetes.

I INTRODUCTION

In all regions of the world, heart failure and cardiovascular diseases are the major causes of death among elderly people. Hypertension and diabetes mellitus are assumed to be the causal agents in many cases of disease and death, and both conditions are generally regarded as lifestyle-related diseases. The World Health Organization and the International Hypertension Society have classified hypertensive blood disease as the sixth major disease in the world. Hypertension is defined as the state in which the systolic blood pressure (SBP) exceeds 140 mmHg or the diastolic pressure exceeds 90 mmHg. In a previous study, we reported that certain recently measured indices indicate that 25% of the Japanese population suffers from high blood pressure [1].

Meat proteins may help in combating lifestyle-related diseases (such as cardiovascular diseases, diabetes, and shrinkage of muscle tissue). Data on the functional properties of meat proteins may be useful for alleviating the effects of lifestyle-related diseases in the general population and elderly persons in particular. The effects of meat proteins are believed to arise from the functional properties of the proteins. It may be possible to use meat proteins as supplemental feed to compensate for low protein intake, particularly in infants and the elderly. The findings of this study may help in reducing the intake of nonnutritious food by the elderly. Meat protein intake can probably be increased to overcome the nutritional deficiency observed in people belonging to these age groups.

II MATERIALS AND METHODS

2.1. Preparation of meat hydrolyzate

Cuts of meat were minced (1 kg was mixed with 1.5 l of d. water), and the mixture was incubated at 70°C for 30 min. Distilled water (0.5 l) was added to the mixture, which was then homogenized in a food processor three times for 1 min each with a 10-s interval. The pH was adjusted to 1.8 using HCl. Subsequently, 0.2 g of pepsin (gastric mucosa origin) (with 1:10000) (Wako Pure Chemical Industries Co., Ltd.) was added, and the mixture was incubated with stirring at 37°C for 2 h. The pH of the sample was again adjusted to 6.8 using NaOH, and the samples were then boiled for 10 min.

The mixture described above was cooled to 40°C, and 0.2 g of trypsin and pancreatin were added (Wako Pure Chemical Industries Co., Ltd.). The mixture was incubated with stirring at 37°C for 2 h and then boiled for another 10 min. The cooked samples were filtered through a cellulose acetate membrane filter (0.45 µm). The filtered samples thus obtained were subjected to HPLC and SDS-PAGE to determine the molecular weights of the peptides in the digested samples.

2.2. Electrophoresis

SDS-PAGE was performed at 20 mA/gel as described earlier in many publications. A gradient slab gel (7.5%–17.5% acrylamide) containing 2-mercaptoethanol was used, and the gels were stained with Coomassie Brilliant Blue.

2.3. Gel filtration

High-molecular-weight hydrolyzates were fractionated by gel filtration chromatography and eluted with a solution of 0.05 M sodium bisphosphate buffer (pH 7.0). The hydrolyzates were applied to a column (Shodex Protein KW 803 column; Showa Denko K.K.) and subjected to further analysis. The column was eluted at a flow rate of 0.5 ml/min, 20μl quantity of infusion with LC-10AD. The eluted fractions were analyzed by passing through a deaerator (DGU-14A; Shimadzu Co., Ltd.), and peptides were detected at a wavelength of 280 nm .

2.4. Determination of the protein concentration

The protein concentration of the meat hydrolyzed by different proteases was determined by a quantitative method (the biuret method). The ultraviolet absorption method was also used in which the measurements were carried out in the absorbance range 215–225 nm.

2.5. Antihypertensive activity after prolonged oral administration

The 18 rats used in this study were maintained in accordance with the guidelines of the Institutional Animal Care and Utilization Committee in agreement with the National Research Council's Guide for the Care and Use of Laboratory Animals. Nine SHRs constituted the control group, while another 9 formed the experimental group (aged 6 wks). All animals were purchased from Elsie Company, Japan. They were kept in a room with a 12-h light-dark cycle (lights on between 07.00 and 19.00). The temperature and humidity were maintained at 23° ± 1°C and 50% ± 10%, respectively. Standard diet (MF; Oriental Yeast Co., Ltd.) and filtered tap water (filtered through an 0.2-μm filter) were available ad libitum. The rats were fed a normal diet for 1 week (at the 6th week of age) and then immediately (in the 7th week of age) received a diet freeze-dry in powder feed MF contains 5% hydrolyzates for another 12 weeks.

2.6. Measurement of parameters of the body condition

Feed intake and body weight

The feed intake of the rats was measured once every 2 days at 9:00 am. This procedure helped in replenishing the diet and simplified the procedure of observing the diet. The diet was replenished every 2 days since it was freely available to the rats. The body weight of rats was also measured once every 3 weeks.

2.7. The SBP of SHRs after oral administration of meat and meat hydrolyzate

After 4 weeks, the experiment was initiated, and the SBP of the experimental SHRs was measured and found to range between 163 and 173 mmHg. The meat hydrolyzate was dissolved in distilled water (10 mg/ml) and orally administered to the SHRs at a dose of 10 mg/kg body weight using a metal syringe. An equal volume of distilled water was administered to the control group. The SBP of the rats was measured at 0, 3, 6, 9, and 24 h after administration of the hydrolyzate using the tail-cuff method with a programmable electro-sphygmomanometer (BP-98A; Softron Co., Ltd., Tokyo, Japan) after warming the rats in a chamber maintained at 38.7°C for 15–20 min.

2.8. Angiotensin II measurements

Sample preparation

The angiotensin II level was measured in the blood of SHRs by the method of [4]. For the insulin/angiotensin II measurements, two buffers were prepared: buffer A was 1% trifluoroacetic acid (TFA), while buffer B was 1% TFA containing 60% acetonitrile. Buffer A (1 ml) was added to 1 ml of obtained blood plasma, and the contents were mixed and centrifuged at 10000 *g* for 20 min at 4°C (LC-200; Tomy Delicate). The supernatant was collected for further experiments (plasma solution, PS). For column conditioning, 1 ml of buffer B was injected in the SEP column (Code RK SEPCOL-1; Phoenix Pharmaceuticals Company), followed by the injection of 3 ml of buffer A (3 times) for column equilibration. After washing and equilibrating the column, an appropriate volume of PS was injected into the SEP column. Subsequently, 3 ml of buffer A was injected in the column 3 times, followed by 3 ml of buffer B, and the final sample containing angiotensin II was collected

in a polypropylene tube. To remove acetonitrile from the final sample, it was dried in an evaporator. The working assay buffer was prepared by adding 50 ml of the assay buffer concentrate to 950 ml of Milli Q water. The evaporated samples (ES) were added to 200 µl of the assay buffer and gently mixed together. For angiotensin II measurements by ELISA, the ES was prepared at different concentrations to evaluate the final level of angiotensin.

ELISA

The ACE inhibitory activity was measured according to the method of [5] with slight modifications. In short, a standard peptide solution of angiotensin II (8 amino acids) was dissolved in 1 ml of assay buffer (1000 ng/ml). The standard solution was further diluted to different concentrations (25 ng/ml, 5 ng/ml, 1 ng/ml, 0.2 ng/ml, and 0.04 ng/ml). An automatic chromatometer (Immuno Mini NJ-2300; Co., Ltd.) was used to measure the absorbance at 450 nm.

2.9. Measurement of the fasting blood sugar

The fasting blood sugar level was measured by fast measurements method as tail vein of the rats was stabbed with a needle of 27G size and after warming the rats in a chamber maintained at 36°C for 15 min. An enzymatic method was used, where the glucose level was measured repeatedly by sampling the blood 3 times on each measurement day. The blood sugar test is a paper test in which immobilized glucose oxidase converts the glucose into hydrogen peroxide and gluconic acid. The measurements were carried out once every 3 weeks.

2.10. Measurement of glycosylated hemoglobin (HbA1c)

Measurements were carried out in fasting animals, and the blood samples were obtained in the same manner as those used for checking the glucose level. HbA1c levels were checked using the DCA2000 HbA1c cartridge (Bayer Medical Company). The HbA1c level is an indicator of the total hemoglobin (Hb) content, and this level is calculated as a percentage of the HbA1c concentration to the total Hb. The HbA1c level was measured by the latex agglutination inhibition reaction method. The HbA1c values were calculated by the following equation:

$$HbA1c\ (\%) = [(HbA1c\ level)/(total\ Hb\ level)] \times 100$$

2.11. Measurement of temporal blood sugar content

Rats received 1.5 g/kg dosage of glucose through oral injection during a period of 12 weeks, the dosage contained D-glucose (0.75 ml). After oral administration, the glucose level was determined at 30, 60, and 90 min. The method used to measure the glucose level was the same as that used to determine the blood sugar level of starved rats, and the measurements were taken for as long as 16 h after the last meal while water was still made available to the rats.

III RESULTS AND DISCUSSION

3. Results and discussion

Mixing the normal diet of rats with a 5% meat and / or meat hydrolyzate resulted in obvious positive effects on some common life-style related diseases. The palatability of the diet to the rats appeared to be normal since the consumption was normal. The body weight increased significantly (Fig.1). However, the positive and control groups showed no significant differences in terms of their body weight. The changes in the SBP of SHRs after oral administration of meat hydrolyzate are shown in (Fig. 2). After administration of the meat hydrolyzate, the SBP decreased by 6 mmHg in 3 h and 13 mmHg in 6 h, indicating that the meat hydrolyzate had a strong blood pressure-reducing effect. The concentration of angiotensin II was measured in the meat and meat hydrolysate groups, and comparison of the results showed that the meat hydrolyzate had a considerable effect on the angiotensin II concentration after rats were fed a diet containing 5% meat hydrolyzate for 2 weeks. The values decreased significantly ($p<0.01$) in comparison with the group that was fed meat diet only (Fig.3). This suggests that the meat hydrolyzate may prevent the conversion of ACE to angiotensin II. The angiotensins circulate in the blood and are "oligopeptides which are important in the regulation of blood pressure (vasoconstriction) and fluid homeostasis via the rennin-angiotenin system. It also is associated with arterial sclerosis and is an aggravation factor for hypertension. Therefore, it is important to suppress any increase in angiotensin levels.

The results indicate that meat hydrolyzate may contains some peptides that function as nutraceuticals and could lower the blood pressure. Administration of meat hydrolyzate led to blood-pressure-lowering effects similar to those observed in previous reports.

We monitored the blood pressure and found that it

could be reduced in both the short-term and long-term perspectives by feeding rats with a diet containing 5% meat hydrolyzate. Figure 4 shows the reduction in the values of the fasting blood sugar level with time (16 h without food). The values increased slightly and then decreased with time. However, the group that had been fed a diet containing 5% meat hydrolyzate exhibited a slightly lower sugar content than that in the control group. This indicates that the meat hydrolyzate has a high peptide content that can reduce the blood sugar. These findings could help in reducing the blood pressure of diabetics.

With time, the HbA1c content of both groups of rats increased. However, the test group, which was fed a diet containing 5% meat hydrolyzate, showed much better results than the control group in terms of the blood sugar content (Fig. 5).

Generally, the HbA1c percentage is correlated with diabetes.

We also examined the blood sugar after a single oral aministration of glocuse by a meat syringe. The values of the test group were lower in comparision with the group that was fed a meat diet only. This reveald that meat hydrolysate could reduce blood sugar within shrot period, otherwise even if rat eat sweety feed the meat hydrolysate can minimize blood sugar level over short time (Fig. 6). An important conclusion of this study is that meat hydrolyzate contains many peptides that could be useful in preventing, or at least reducing, lifestyle-related diseases.

IV CONCLUSION

Since the maximum reduction in blood pressure occurred at 3–6 h after oral administration of the meat hydrolyzate, it appears that this extract may be an important functional food. Moreover, meat hydrolyzate also reduced the glucose level as observed by the decrease in the blood sugar level with time. The results indicate that enzymatic digestion of meat may result in an extract that contains some active peptides with antihypertensive and antidiabetic activities. Meat hydrolyzate may also contain other constituents as a result of which it could be utilized as a functional food and nutraceutical.

REFERENCES

[1] Mugurma, M., Ahhmed, A. M., Katayama, K., Kawahara, S., Maruyama, M., & Nakamura T. (2009). Identification of pro-drug type ACE inhibitory peptide sourced from procine myosin B: Evaluation of its antihypertensive effects in vivo. Food Chemistry, 114, 516-522.

[2] Cushman, D.W. & Cheung, H. S. (1971). Spectrophotometric assay and properties of the angiotensin-I converting enzyme of rabbit lung. Biochemical Pharmacology, 20 1637-1648.

[3] Katayama K., Henny, E. A., Mori, T., Ahhmed, A. M., Kawahara, S. Sugiyama, M., Nakayama, T., Maruyama, M., Muguruma, M. (2008). Journal of Agricultural Food Chemistry, 56, 355-360.

[4] Porstmann, T., & Kiessig, S.T. (1992). Enzyme Immunoassay Techniques,An Overview. Journal of Immunological Methods, 150 5-21.

[5] Engvall, E., Perlmann, P. (1971). Enzyme-linked immunosorbent assay

(ELISA). Quantitive assay of emmunoglobulin G. Immunochemistry, 1 (8), 871-874.

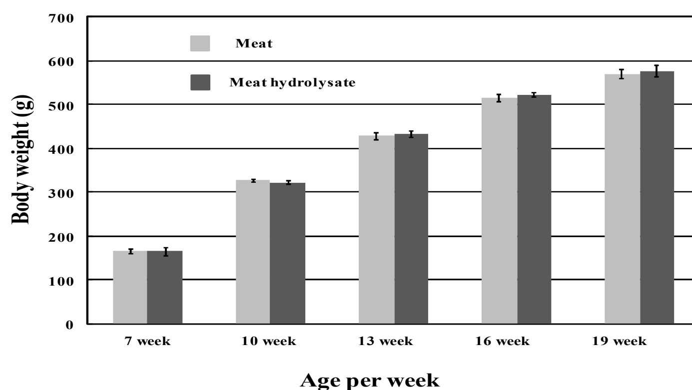


Fig. 1. Changes in the body weight gain of HSR rats as affected by diets include 5 % meat and / or meat hydrolysate over experimental period (3 month).

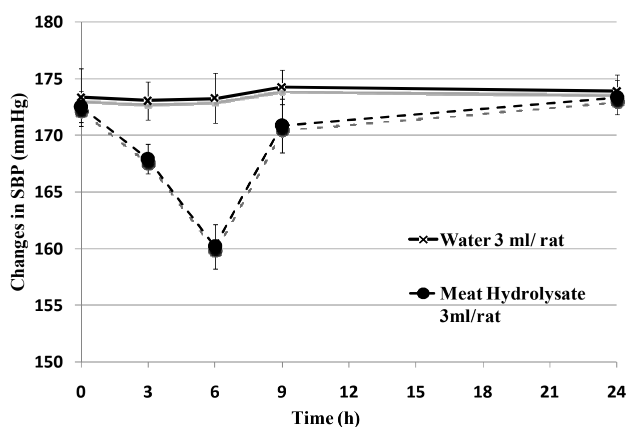
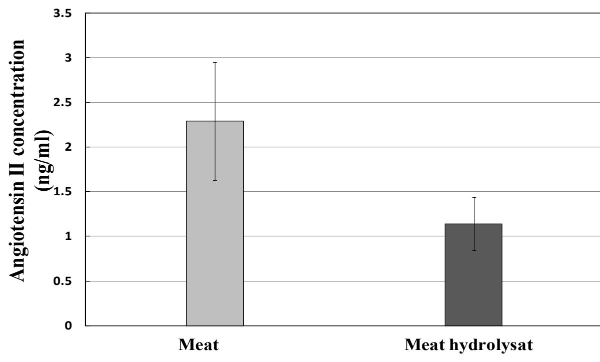
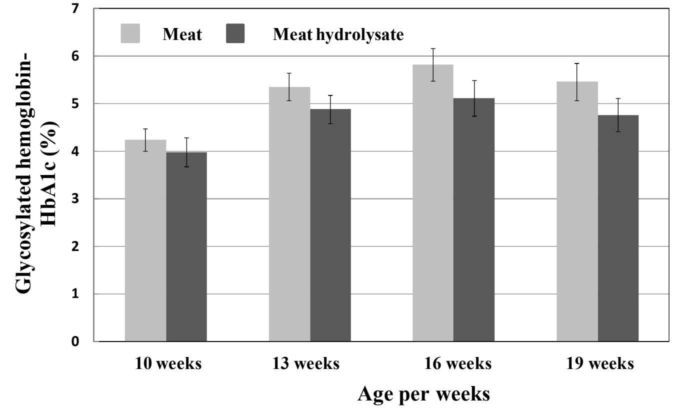
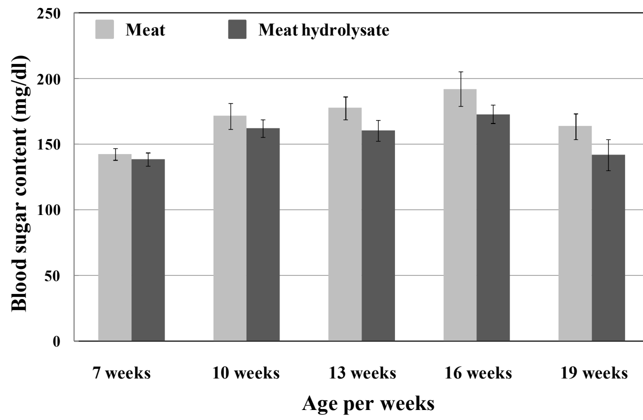
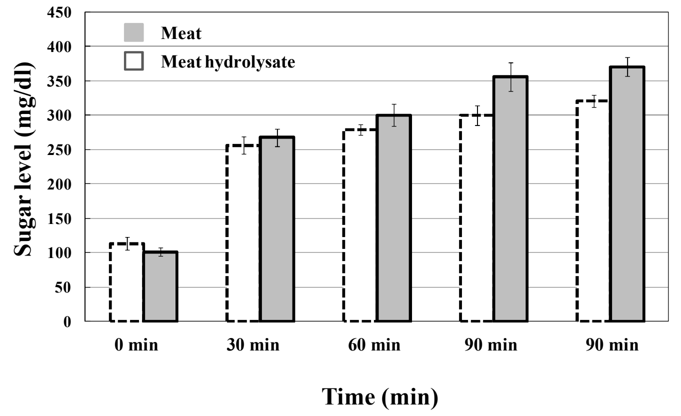


Fig. 2. Effects of single oral administration of meat hydrolysates on the systolic blood pressure of SHRs (10 mg/ml; at a dose of 10 mg/kg body).


Angiotensin II concentration (ng/ml)
3.5
3
2.5
2
1.5
1
0.5
0
Meat
Meat hydrolysat


Glycosylated hemoglobin-HbA1c (%)
Meat
Meat hydrolysate
7
6
5
4
3
2
1
0
10 weeks
13 weeks
16 weeks
19 weeks
Age per weeks


Blood sugar content (mg/dl)
Meat
Meat hydrolysate
250
200
150
100
50
0
7 weeks
10 weeks
13 weeks
16 weeks
19 weeks
Age per weeks


Sugar level (mg/dl)
Meat
Meat hydrolysate
450
400
350
300
250
200
150
100
50
0
0 min
30 min
60 min
90 min
90 min
Time (min)