Effects of Cow Meat and Horse Meat on Mouse Immune Cell Activation Ex Vivo

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***Abstract*—We studied that ex vivo treatment of horse meat (HM) extract enhanced the splenocytes and macrophage proliferation compared to the cow meat (CM). To investigate immunomodulating effect of horse meat and cow meat extracts on murine macrophage and splenocytes, splenocyte was stimulated with ConA or LPS for differenciation to T and B lymphocyte, selectively. The proliferation of splenocytes and macrophage was determined by MTT(3-[4,5-dimethylthiazol-2-y]-2,5- diphenyl terazolium bromide) assay. The result of peritoneal macrophage proliferation, horse meat extract and cow meat extract were increased cell growth 61% and 33% compared to control. And proliferation of splenocytes stimulated with Con A or LPS in the presence of ConA, HM and CM was 54%, 105%, 86%, 123%, 155% and 103% increase when compared to the control.**

To determine the effects of HM and CM on TNFα, IL-15, IL-10, IL-2 and INFγ production, IL-15 production of group treated HM and CM was 456.7pg, 270pg and TNFα production was 16.2pg and 9.8pg, respectively on peritoneal macrophage. And IL-15 and IL-2 production were higher than other cytokine production on T lymphocyte (Fig 3-B). Especially, IL-15 production of group treated HM was higher than group treated ConA as 315pg. On B lymphocyte, IL-10 production level was highest other cytokines and like T lymphocyte, IL-15 production of group treated HM and CM was higher than group treated LPS as 250pg, 160pg, respectively

***Index Terms*—Cow meat (CM), Horse meat (HM), Immune modulation, Macrophage, Splenocyte**

INTRODUCTION

Recently, people are stepping up thier efforts to prevent a disease and keep optimum health in side of preventive medicine. The funtion of foods were been increased not only as source of nutriment but also specific biological function enhancing.

At present, the researches were carrying out a little about horse meat study in the Korea (Yoo, Park, Chung and Kim, 1993; Seong, Lee, Kim, Park, Hah and Ko, 2006). In the Europe, several study on horse meat conduct and they had results about properties of meat (Badiani, Manfredini and Nanni, 1993; Campodoni, Preziuso, Gatta, Colombani and Orlandi, 1994), nutriment (Palenik, Blechova and Palanska, 1980; Badiani, Nanni, Gatta, Tolomelli and Manfredini, 1997), dressed carcass (Moczybroda, 1976), storage (Roth, Brewer, Bechtel, Kline and Mckeith, 1995), and treatment before slaughter (Stull, 2001) etc. The properties of horse meat by their results, the proportion of subcutaneous to abdominal fat on horse meat was higher and the proportion of inter-muscular to intra-muscular fat was lower than cow meat which was similar bodyweight and fattening (Rossier and Berger, 1988). Horse red meat was known low composition of fat and nutritional value of protein was higher than others. When they ate horse red meat 100g, adults were received 40% of daily protein requirement (Bodwell and Anderson, 1986). However, most of research was carried out properties of horse meat and nutritional composition until now.

Therefore, the objective of this study was performed to find physiological activities including cell cytotoxicity and immune activity of horse meat compare to cow meat. Additionally, we are going to suggest that horse meats are available for food industry as various food sources through our study.

II. MATERIALS AND METHODS

Sample Preparation

Horse meat was obtained from National Institute of Animal Science (Suwon, Korea) and cow meat was purchased from Nonghyup (Suwon, Korea). These were chopped into small pieces and then dried with freeze drier. These were extracted by water. The extracts kept at -20℃, filtered through a 0.45um syringe filter before use.

Cells and culture

The mouse monocyte/macrophage cell line, Raw 264.7 cell was purchased from the Korean Cell Line Bank (Seoul, Korea). The cell line was maintained in DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin and was incubated at 37 °C in 5% CO_2.

Animals

C57BL/6J male mice (4 weeks old) were obtained from SLC (Japan), and four mice per group were randomly assigned to filtered cages in an environmentally controlled atmosphere (temperature 22℃; 50% relative humidity), with a 12-h light and 12-h dark cycle. Animals used in this study were male mice aged 8 weeks.

Isolation of inflammatory peritoneal macrophages
Cells were harvested by peritoneal lavage 3 days after intraperitoneal injection of 1 ml of 3% Brewer thioglycollate broth. The cells were washed with PBS twice and resuspended in RPMI1640 supplemented with 10% fetal bovine serum, and 1% penicillin-streptomycin agent and incubated at 37℃ for 2 h. The adherent cells were used. 200㎕ of suspended macrophage, 1×10^5 cells/well, was plated onto a 96-well microplate for 48 h at 5% CO2, 37℃.

Measurement of spleen cell proliferation
The spleens were aseptically removed, and placed in sterile endotoxin-free RPMI 1640 (Gibco, USA) media supplemented, 100 U/㎖ of penicillin, and 100㎍/㎖ of streptomycin (Gibco, USA) Single cell suspensions were prepared by gentle disruption of the spleens between two sterile frosted slide glasses. The splenocytes were then isolated by centrifugation at 1300rpm, and the red blood cells were lysed with 0.83% Tris-NH_4Cl. The splenocytes were washed twice with RPMI 1640, and viability was determined by trypan blue exclusion method. The splenocytes were suspended in RPMI 1640 with 10% heat-inactivated FBS. Then, 200㎕ of suspended splenocytes, at 5×10^5 cells/well, were plated onto 96-well microplates. The microplates were incubated in the presence of T cell mitogen (Concanavalin A, 2.5 ㎍/㎖) and B cell mitogen (Lipopolysaccharide, 10 ㎍/㎖) for 48 h at 5% CO2, 37℃ (Holsapple, McNerney, Tucker and White, 1984).

Cell proliferation assay
Various concentrations of the samples were added to the cells. The cells were then exposed to each test medium for 48 hrs. The MTT solution was added and the cells were incubated for another 4 hr. The growth medium was removed, and DMSO was added to each 96-well and dissolved. The absorbance of the result was determined at 540 nm (Hansen, Nielsen and Berg, 1989).

Determination of cytokines (IL-2, IL-10, IL-15, IFNγ, TNFα) by ELISA
The spleen cells from mice were stimulated with Con A and LPS for 18 h in the presence of HM and CM. Culture supernatants were collected for measurement of cytokine level. Peritoneal macrophages (1×10^5 cells/well) from mice were cultured in the presence of HM and CM for 18h and culture supernatants were collected for determination of cytokine level. The concentration of each cytokine in the culture supernatants was determined by using ELISA commercially available from BD PharMingen (USA). Briefly, microplates was coated with 100㎕ per well of capture Ab, and incubated overnight at 4℃. After washing and blocking with PBS containing 10% FBS, culture supernatants or the standards were added to each well. The plate was incubated for 2 h at RT. The plates were washed and biotinylated detecting mAb was added to each well and incubated at room temperature for 1 h. The plate were washed and further incubated with avidin- peroxidase for 30 min before detection using the TMB solution. Absorbances were measured at 450 nm with an ELISA reader (Molecular Devices, USA). The amounts of cytokine were calculated from the linear portion of the standard curve.

III. Results and discussion
Proliferation in RAW 264.7 cell cultures *in vitro*
In order to determine whether HM and CM could exert a toxic effect on RAW264.7 cells for 48h cultures, we measured the cell viability of the cells. We observed no adverse effect of HM and CM (Fig. 1). Cell proliferation of HM treated group was 146% at 0.1mg protein and 114%, 106% at 0.01mg, 0.001mg protein, respectively. Cell growth of CM treated group was 103%, 106%, 107% at 0.1mg, 0.01mg, 0.001mg protein, respectively. This results show that cell proliferation on HM treated group depend on concentration but cell growth on CM treated group was not significantly difference.

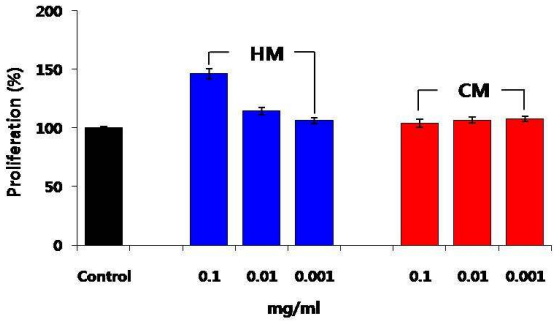

Fig 1. The effect of proliferation of Horse meat(HM) and Cow meat(CM) extracts on Raw 264.7 cell

Macrophage proliferation in peritoneal macrophage cultures
The concentration of treat on the cell of HM and CM would be based on cell proliferation results on RAW264.7 cell line which was 0.1 mg protein as efficacious concentration on the cell proliferation. Immune modulation of HM and CM were evaluated using isolated peritoneal macrophage and splenocytes from mouse. The result showed on the Fig 2.

The result of peritoneal macrophage proliferation, Group treated HM and CM were increased cell growth 61% and 33% compared to control (Fig 2-A).

Lymphocyte proliferation in splenocyte cultures

To determine the effects of HM and CM on the splenocytes proliferation, spleen cells were cultured with Con A or LPS, and MTT assay were conducted at 48h. The effects of HM and CM on the spleen cells proliferation to mitogens are shown in Fig 2-B and C. The proliferation of splenocytes stimulated with Con A in the presence of ConA, HM and CM was 54%, 105% and 86% increase when compared to the control (Fig. 2-B).

Proliferation of splenocytes stimulated with LPS in the presence of LPS, HM and CM, 123%, 155% and 103% increase when compared to the control (Fig. 2-C).

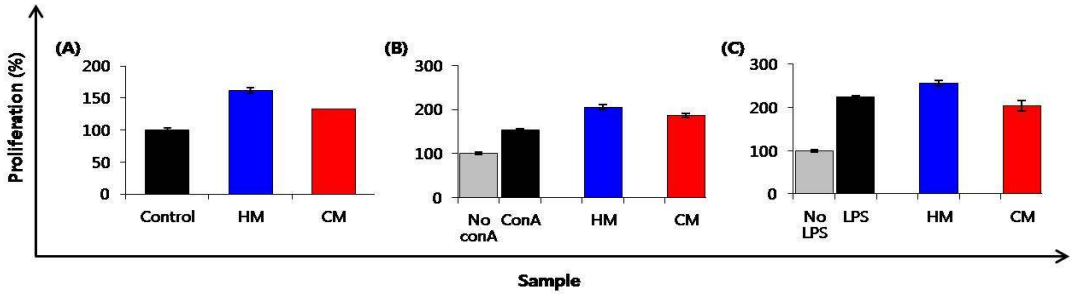

Fig 2. The effect of proliferation of Horse meat(HM) and Cow meat(CM) extracts on peritoneal macrophage and splenocytes from mouse (A) Macrophage (B) T lymphocyte (C) B lymphocyte.

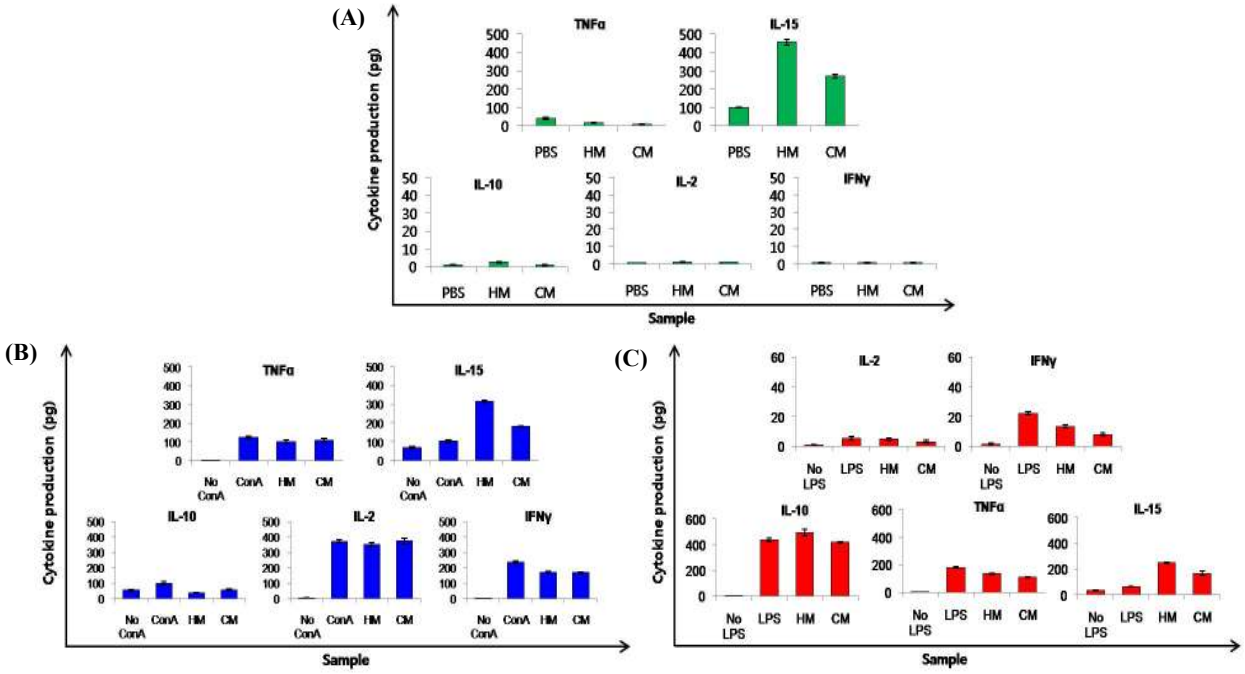

Fig 3.The effect of Horse meat(HM) and Cow meat(CM) on TNFα, IL-15, IL-10, IL-2 and INFγ production from murine pertoneal macrophage (A) and T lymphocyte (B) & B lymphocyte (C).

Cytokines production in peritoneal macrophages

To determine the effects of HM and CM on TNFα, IL-15, IL-10, IL-2 and INFγ production, the peritoneal macrophages were cultured and supernatants were harvested at 18h. Cytokine levels in supernatants were measured using ELISA method. IL-15 and TNFα production were higher than other cytokine production on peritoneal macrophages (Fig 3-A). IL-15 production of group treated HM and CM was 456.7pg, 270pg and TNFα production was 16.2pg and 9.8pg, respectively

Cytokines production in splenocytes

To determine the effects of HM and CM on TNFα, IL-15, IL-10, IL-2 and INFγ production, the splenocytes were cultured with Con A or LPS, and supernatants were harvested at 18h. The cytokines production in supernatants were measured using ELISA method. IL-15 and IL-2 production were higher than other cytokine production on T lymphocyte (Fig 3-B). Especially, IL-15 production of group treated HM was higher than group treated ConA as 315pg IL-2 was produced similar levels both ConA treatment group and HM and CM treatment group. IL-10, TNFα and IL-15 were produced on B lymphocyte (Fig 3-B). In particular, IL-10 production level was highest other cytokines and like T lymphocyte, IL-15 production of group treated HM and CM was higher than group treated LPS as 250pg, 160pg,

respectively.

IV. Conclusion

This study was carried out to find physiological activities including cell proliferation and immune activity of horse meat compare to cow meat. The results follow as:

In Raw264.7 cell proliferation, cell proliferation of group treated HM extract was 146% at 0.1mg protein. And cell growth on group treated CM was not significantly difference.

The result of peritoneal macrophage proliferation, group treated HM and CM were increased cell growth 61% and 33% when compared to control. The proliferation of splenocytes stimulated with Con A in the presence of ConA, HM and CM was 54%, 105% and 86% increase and the splenocytes stimulated with LPS in the presence of LPS, HM and CM, 123%, 155% and 103% increase.

Cytokine levels in supernatants were measured using ELISA method. IL-15 and TNFα production on peritoneal macrophages, IL-15 and IL-2 production on T lymphocyte and IL-10, TNFα and IL-15 production on B lymphocyte were higher than other cytokine production

As the results, we suggest that horse meats are available for food industry as various food sources through our study.

V. References

Badiani, A., Manfredini, M., and Nanni, N. (1993). Qualita della carcassa e della carne di puledri lattoni. *Zoot. Nutr. Anim.* 19, 23-31.

Badiani, A., Nanni, N., Gatta, P. P., Tolomelli, B. and Manfredini, M. (1997). Nutrition profile of horse meat. *J. Food. Comp. Anal.* 10, 254-269

Bodwell, C. E. and Anderson, B. A. (1986). Nutritional composition and value of meat and meat productions. In : Muscle as Food. Bechtel, P. J. (ed), Academic Press, Orlando, USA, 321-369.

Campodoni, G., Preziuso, G., Gatta, D., Colombani, B., and Orlandi, M. (1994). Rilievi in vita e al macello e qualita della carne in puledri derivati Frenches Montagnes. *Zoot. Nutr. Anim.* 20, 35-44.

Hansen, M. B., Nielsen, S. E., and Berg, K. (1989). Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *Journal of Immunological* Methods. 119(2), 203-210.

Holsapple, M. P., McNerney, P. J.. Tucker, A. N. and White, T. K. L. (1984). Effects of N-nitroso-dimetylamine on humoral immunity. *J. Pharmacol. Exp. Ther*. 229(2), 493-500.

Moczybroda, J. (1976). Dressing percentage, characteristics of primal cuts and chemical composition of meat of stallions of different breeds killed after complete reproductive utilization. *Rocz. Nauk Roln. Ser. B*. 98, 45-55.

Palenik, S., Blechova, H., and Palanska, O. (1980) Chemical composition and quality of the meat of cold and warm blooded foals. *Zivoc. Vyroba.* 25, 269-277.

Rossier, E. and Berger, C. (1988). La Viande de Cheval : Des qualities Indiscuteables et Pourtant Meconnues. CERE OPAITEB, Paris, France

Roth, B. M., Brewer, M. S., Bechtel, P. J., Kline, K. H. and Mckeith, F. K. (1995). A research note : sensory, color and composition characteristics of young and mature chevaline. *J. Muscle Food.* 6, 83-89.

Seong, P. N., Lee, C. E., Kim, J. H., Park, B. Y. Hah, K. H., and Ko, M. S. (2006). Effect of replacing pork with horse meat on quality characteristics of emeulsion-type sausage. *Korean J. Anim. Sci. Technol.* 48, 739-746.

Stull, C. L. (2001). Evalution of the proposed fedral slaughter horse transport regulations. *J. Anim. Sci. 79 (E.Suppl.),* E12-E15.

Yoo, I. J., Park, P. S., Chung, C. J. and Kim, K. I. (1993). A study on nutrition value of horse meat. *Korean J. Anim. Sci.* 35, 131-137.