Immunostimulating and Growth-Promoting Effects of Sugar Cane Extract (SCE) in Chickens

Moshira EL-ABASY[1,2], Maki MOTOBU[2], Kameo SHIMURA[2], Ki-Jeong NA[2], Chung-Boo KANG[3], Kenji KOGE[4], Takashi ONODERA[1] and Yoshikazu HIROTA[2]*

[1])*Department of Molecular Immunology, Faculty of Agriculture, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–8657,* [2])*Department of Production Diseases, National Institute of Animal Health, National Agricultural Research Organization, 3–1–5 Kannondai, Tsukuba, Ibaraki 305–0856,* [3])*College of Veterinary Medicine, Gyeongsang National University, 900 Chinju, Kyeongnam 660–701, Republic of Korea and* [4])*Chigasaki Laboratory, Shin Mitsui Sugar Co., Ltd., 1–2–14 Honson, Chigasaki, Kanagawa 253–0042, Japan*

ABSTRACT. Polymorphonuclear cells of the peripheral blood in the chicken significantly increased their phagocytosis when cultured with sugar cane extract (SCE; 250–1,000 μg/ml) for 24 hr. Chickens orally administered SCE (500 mg/kg/day) for 3 or 6 consecutive days at 1 week of age showed significantly higher body weight and gain in body weight/day and a lower food conversion ratio within the growing period of 6 weeks than physiological saline-administered control chickens. Furthermore, oral administration of SCE also resulted in significantly higher immune responses against sheep red blood cells and *Brucella abortus*. These results suggest that SCE has immunostimulating and growth promoting effects in chickens.
KEY WORDS: growth promotion, immunopotentiation, sugar cane extract.

Vaccines and antibiotics have contributed to the control of a lot of different infectious diseases in veterinary and human medicine. Much consumption of many various chemicals and antibiotics has resulted in some problems such as the development of antibiotics-resistant bacteria and pollution of environment. It is imperative to develop the novel production system of economically important food animals based on the consideration of safe food, less polluted environment and recycle of natural resources.

Various kinds of native, synthesized or recombinant biological response modifiers (BRM) have been evaluated on the basis of preventive and therapeutic effects on infectious or non-infectious diseases. Some native BRM [5, 6, 10, 13] with immunostimulating activity such as bacterial derived components and chicken egg white derivatives (EWD) are effective in recovery of immunosuppression. There is almost little information concerning the biological activities of sugar cane, except the finding on activation of classical complement pathway in human serum by its lipopolysaccharide [7, 8]. The purpose of the present study is to define immunological and nutritional features of sugar cane extract (SCE) as one of native BRM.

Original materials including cane juice produced from sugar cane (*Saccharum officinarum* L.) in the raw sugar manufacturing process were subjected to the preparation of SCE. Dried SCE finally prepared by synthetic adsorbent chromatography and cation exchange column chromatograpy consisted of crude protein (16.9%), crude fat (0.5%), ash (36.1%) and nitrogen-free extracts (46.5%).

The original concentration (10 mg/ml) of SCE was prepared in phosphate buffer saline. EWD kindly provided by Eisai Co., Ltd, Japan, were prepared in the same manner described previously [5], and used as a positive stimulator in the phagocytosis assay. At first, to examine the effect of SCE on phagocytosis, polymorphonuclear cells (PMN)-rich fraction from peripheral blood of inbred chickens (MHC; H.B15) was prepared as described previously [10]. The resultant PMN consisted of approximately 95% heterophils and 5% lymphocytes when their Giemsa-stained smear samples were cytologically examined. PMN suspended to a concentration of 1×10^6 cells/ml in Iscove's modified dulbecco's medium (Sigma, MO, U.S.A.) supplemented with 1% normal chicken serum were applied into each well of a 24-well culture plate and cultured in the presence or absence of SCE (250–1,000 μg/ml) or EWD (200–400 μg/ml) at 40°C in a humidified atmosphere of 5% CO_2-incubator. At 23 hr culture, the PMN were supplemented with 100 μl of FITC-conjugated latex beads (2.0 μm; Polyscience Inc., UK), and then additionally cultured for 1 hr. The mean relative proportion of phagocytized cells in the PMN was evaluated by a flow cytometer (Epics XL, Beckman Coulter, U.S.A.). The PMN cultured with medium only were used as control. The phagocytosis assay was done in triplicate. All results were expressed as mean ± standard error. Statistic analysis was performed by the Student's *t* test.

The phagocytic activities of PMN cultured with SCE (250–1,000 μg/ml) significantly increased (p<0.01), as compared to those of control as shown in Fig. 1. The levels in the mean relative proportion of SCE-stimulated PMN phagocytosis were almost the same as those of PMN stimulated with EWD (200–400 μg/ml) which was used as a PMN phagocytosis stimulator.

* CORRESPONDENCE TO: HIROTA, Y., Department of Production Diseases, National Institute of Animal Health, National Agricultural Research Organization, 3–1–5 Kannondai, Tsukuba, Ibaraki 305–0856, Japan.
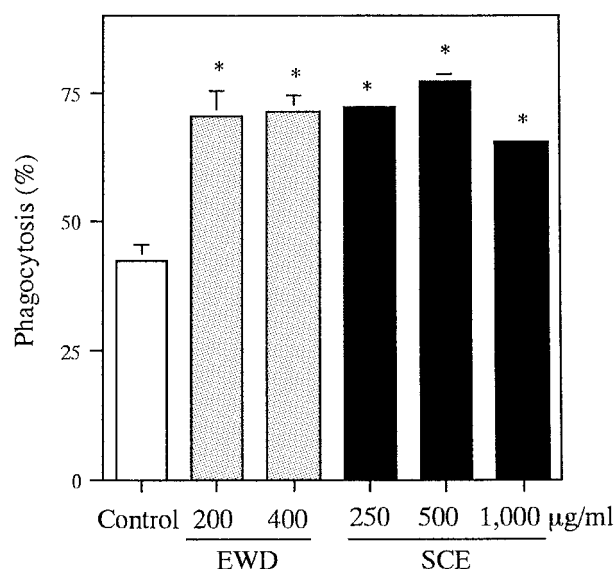
Fig. 1. Phagocytic activity of chicken PMN cultured with different concentrations of SCE and EWD for 24 hr. * $P<0.01$, when compared to control.

Subsequently to examine the effects of SCE on growth and immune responses, SCE was administered into the crop of 1-week-old commercial male chickens (Dekalb) at the dose of 500 mg/kg/day for 3 or 6 consecutive days, which were referred to as SCE (3) and SCE (6), respectively. Chickens administered physiological saline instead of SCE in the same manner was used as control. Each group of SCE (3), SCE (6) and control consisted of 8 chickens. At the age of 3 and 4 weeks, 5 chickens of each group were also immunized intravenously with mixed antigens (0.1 m*l*) of sheep red blood cells (SRBC; 5×10^8 cells) and heat-inactivated *Brucella abortus* (BA; 1×10^9 organisms). Agglutinin titers against SRBC and BA in sera taken at 7 days after each immunization were determined as described earlier [4].

Antibody titers after the treatment of sera with 0.2 M 2-mercaptoethanol (2-ME) were referred to as 2-ME resistant titer. For food conversion ratios, the amount of food added and food lost was measured every day for 6 weeks. Individual body weights and food intake were recorded weekly. The gain in body weight/day and food conversion ratio were determined as described previously [2, 9].

As shown in Fig. 2, chickens orally administered SCE showed greater body weight and gain in body weight/day and lower food conversion ratio (p<0.05) than control chickens when examined for 6 weeks. SCE (3) was more effective than SCE (6) in all growth or nutrition-related parameters to be evaluated.

Effect of SCE on immune responses to SRBC and BA is summarized in Table 1. Chickens orally administered SCE also showed significantly higher immune responses against both antigens (p<0.05) than control chickens. In particular, oral administration of SCE resulted in an increase in 2-ME resistant titer in the antibody responses to the both antigens and in the number of responders in the primary antibody response against BA.

The results of the present study are summarized as follows; (1) enhancement in phagocytosis of PMN cultured with SCE and enhanced antibody production in chickens orally administered SCE and (2) an increase in body weight of growing chickens orally administered SCE. These results suggest that SCE has immunostimulating and growth promoting effects. However, the mechanism by which SCE involved in the development of these effects remains to be open. The supplement of the supernatant from chicken mononuclear cells cultured with SCE for 24 hr into freshly isolated chicken lymphocytes resulted in significantly enhanced numbers of immunoglobulin producing cells (data not shown), suggesting that SCE-driven enhanced phagocytosis is mediated by humoral factors such as cytokines as reported earlier [5, 12]. SCE may act on immune cells to activate PMN and mononuclear cells for production of cytokines and biologically active substances which is involved in immunoenhancement. Some biological activi-
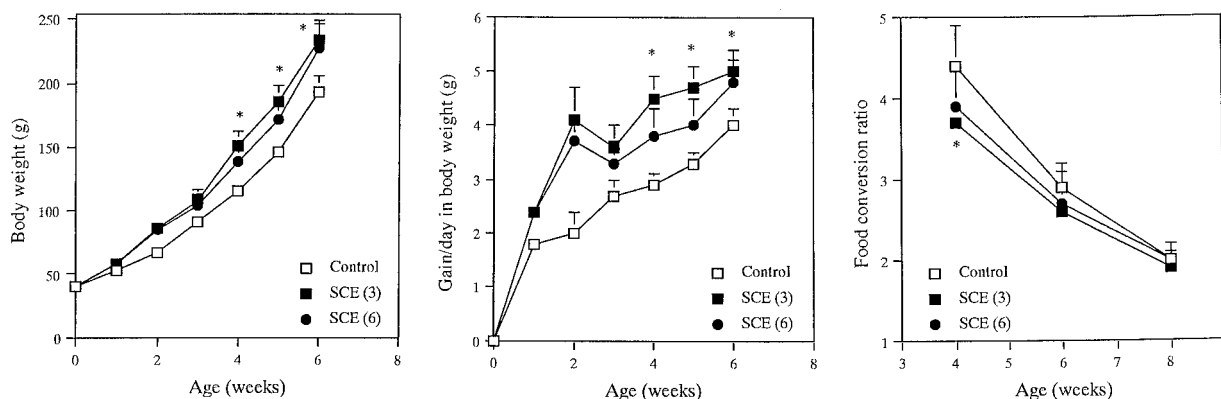

Fig. 2. Effects of oral administration of SCE on body weight, gain in body weight/day and food conversion ratio in chickens. One-week-old chickens were administered SCE for 3 or 6 consecutive days. * $P<0.05$, when compared to control.

Table 1. Antibody responses to SRBC and BA in chickens orally administered SCE

Group	Primary response							
	SRBC				BA			
	No treatment		2-ME treatment		No treatment		2-ME treatment	
	Responders[a]	Titer[b]	Responders	Titer	Responders	Titer	Responders	Titer
Control	5/5	8.5 ± 0.9	4/5	2.2 ± 0.8	5/5	8.2 ± 0.4	2/5	0.5 ± 0.2
SCE (3)	5/5	9.0 ± 0.1	5/5	4.0 ± 0.4*	5/5	9.0 ± 0.1	4/5	2.3 ± 0.4*
SEC (6)	5/5	10.5 ± 0.1*	5/5	5.2 ± 0.7*	5/5	9.5 ± 0.2*	4/5	2.5 ± 0.2*

a) Number of responding chickens per total number of chickens.
b) Mean ± SE of $\log_2$ of the reciprocal titers. * $P<0.05$, compared to control.

ties of immunomodifiers of a native type have been demonstrated as antimicrobial activities of sugar cane against Gram-positive bacteria [1], enhanced human natural killer cell activity of rice bran [3], increased phagocytic and chemotactic activities of canine and feline peripheral blood phagocytes by EWD [5, 12, 13], and enhanced neutrophil- and macrophage-functions in fish by immunoactive peptides [14].

Our results also showed that administration of SCE into the crop of 1-week-old chickens resulted in a higher increase in body weight and gain/day in body weight and lower food conversion ratios than those of control chickens, suggesting the improvement of food utilization and a decrease in the amount of food needed for gain of 1 g of body weight by SCE administration. Farrell *et al.* [2, 9] reported that addition of organic phosphorous and microbial phytase to poultry diet increased the growth rate and gain/day in body weight and decreased the food conversion ratio. Why SCE (3) showed better performance than SCE (6) in growth that is one of economically important parameters could not be explained. Appropriate timing and doses of administrating SCE may be involved in the development of biological activities of SCE, as one of possible explanation. Tschop *et al.* [11] reported that administration of ghrelin, one of neuropeptides involved in the growth and obesity, increased food intake and body weight gain in rats and mice by regulating pituitary growth hormone secretion. More detailed studies concerning the expression of messenger RNA cytokines and hormones related to immunostimulation and growth in chickens orally administered SCE are needed.

Taken together, SCE has physiological properties enhancing the phagocytosis, immune responses and growth rate in chickens.

REFERENCES

1. Abdel-Nasser, M., Safwat, M. S. and Ali, M. Z. 1983. *Zentralbl. Mikrobiol.* **138**: 63–69.
2. Farrell, D. J. and Martin, E. A. 1998. *Brit. Poultry Sci.* **39**: 601–611.
3. Ghoneum, M. and Jewett, A. 2000. *Cancer Detect. Prevent.* **24**: 314–324.
4. Hirota, Y., Suzuki, T. and Bito, Y. 1980. *Immunology* **39**: 29–36.
5. Hirota, Y., Yang, M.-P., Ohta, Y., Araki, S., Matsumoto, Y., Kang, C.-B., Mohamed, A., Yoshihara, K., Furusawa, S., Suzuki, K., Onodera, T., Akiyama, K. and Sugii, S. 1994. pp. 57–63. *In*: Vaccines in Agriculture: Immunological Application to Animal Health and Production (Wood, P., Willadsen, P., Vercoe, J., Hoskinson, R. and Demeyer, D. eds.), CSIRO Press, Melbourne, Australia.
6. Hirota, Y., Yang, M-P., Araki, S., Yoshihara, K., Furusawa, S., Yasuda, M., Mohamed, A., Matsumoto, Y. and Onodera, T. 1995. *J. Vet. Med. Sci.* **57**: 825–829.
7. Li, X. Y. and Vogt, W. 1982. *Immunopharmacol.* **5**: 31–38.
8. Li, X. Y., Nolt, R. and Vogt, W. 1983. *Immunobiol.* **164**: 110–114.
9. Martin, E. A., Nolan, J. V., Nitsan, Z. and Farrell, D. J. 1988. *Brit. Poultry Sci.* **39**: 612–621.
10. Maslog, FS., Motobu, M., Hayashida, K., Yoshihara, K. Morozumi, T., Matsumura, M. and Hirota, Y. 1999. *J. Vet. Sci.* **61**: 283–285.
11. Tschop, M., Smiley, D. L. and Heiman, M. L. 2000. *Nature (Lond.)* **407**: 908–913.
12. Yang, M.-P. and Kim, K.-H. 1999. *Korean J. Vet. Clin. Med.* **16**: 31–36.
13. Yang, M.-P., Eoum, H., Na, K.-J., Araki, S., El-Abasy, M., Motobu, M. and Hirota, Y. 2001. *J. Vet. Med. Sci.* **63**: 269–274.
14. Yoshida, T., Sakai, M., Titao, S. M., Araki, S., Saitoh, R., Ineno, T. and Inglis, V. 1993. *Aquaculture* **109**: 207–214.