Effect of Dietary Administration of Bananas on Immunocytes in F1 Hybrid Calves

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ABSTRACT. The effect of dietary administration of bananas on immunocytes in calves was investigated. Twenty F1 hybrid calves were used in this study (treated group n=10, control group n=10). Banana (2 g/kg BW) was administered to the calves for 5 days. Leukocyte subsets were examined on days 0, 5, 10, and 15. The numbers CD3+, CD3+CD45R+, and CD3+TcR+ cells significantly increased between day 0 and day 5 in the treated group (P<0.01), and were significantly higher on day 5 in the treated group relative to the control group (P<0.05). These data showed that feeding banana to calves increased T-lymphocytes, suggesting it might be possible to enhance protective functions against infections.

KEY WORDS: Banana, F1 hybrid calf, immunity.

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It is known that diarrhea and other infectious diseases tend to spread easily in these calves because their immune functions are immature, as compared with the immune functions in adult cattle [6]. Moreover, it has been reported that activation of T-lymphocytes by phytohemagglutinin (PHA) is inadequate and that immune function is depressed in juvenile Japanese Black calves [7]. Transportation stress or other factors have been suggested as reasons for the spread of infectious disease in calves [8, 17]. However, except for studies of the effect of vaccines in preventing disease [4, 9], there have been few reports on immunological strategies for preventing diarrhea in calves. Agents such as active egg white product (AEWP) [12], IFNα [14, 18], levamisole hydrochloride [12, 23] are currently being used to activate immunity. However, none of these products has been clearly shown as effective in preventing the spread of infectious disease at the production farm level.

In recent years, the role of food products as immune response modifiers has been recognized, and it is now clear that vegetables [21], fruits [22] and other plant-based food products have immunomodulatory effects. Bananas have been reported to have a powerful stimulatory effect on the immune system in mice [11, 22], and it is thought that administration of bananas might also activate immunity in cows.

The purpose of the present study was to evaluate the effect of dietary administration of bananas in activating immunity in calves. Bananas were added to the feed of F1 hybrid calves, and the effect on immunocyte populations was analyzed.

The study period was 16 days, from June 18, 2004 through July 3, 2004. The subjects were 20 clinically healthy, one-month-old, male F1 (Japanese black × Holstein) hybrid calves delivered to a rearing farm in the local district. The calves were divided into a banana administration group (banana group) and a control group that contained 10 animals each. The sire of all calves was SHIGEKATSU. There was no illness during the study period.

Beginning the day following their arrival at the farm, 2 g (as-fed)/kg (body weight) of banana was combined with powdered skim milk in a mixer and administered to the calves orally in feeding buckets for 5 days. The control group received only skim milk. Skim milk was fed twice daily, 250 g each time. Composition of the diets and the mean body weight in each group are shown in Table 1.

Blood samples were collected on the day the calves arrived at the farm (day 0), and on days 5, 10, and 15. Samples were taken from the jugular vein and placed in vacuum blood collection tubes containing EDTA-2K. Leukocytes were evaluated using an automatic blood cell counter, and leukocyte surface antigens were analyzed by flow cytometry (FCM).

FCM: Two ml of the sampled blood was mixed with 2 ml of 0.83% ammonium chloride lysing solution and then washed in phosphate-buffered saline (PBS). The leukocytes were separated out and analyzed by flow cytometry. Analysis of leukocyte surface antigens followed the method of Wyatt [19]. Cow leukocyte surface antigen analysis was

| Table 1. Composition of diets and the mean body weight of each group |
|-----------------------|-----------------------|
| Ingredient (g)        | Banana group | Control group |
| Skim milk(+)          | 500          | 500           |
| Calf starter(+)       | 500          | 500           |
| Timothy hay           | free         | free          |
| Banana(+)             | 2/kg BW      |               |
| Body weight (kg)      |              |               |
| Start                 | 56.1 ± 6.6   | 54.7 ± 6.1    |
| End                   | 66.4 ± 5.7   | 64.5 ± 5.1    |
| a) TDN 110%, CP 23%,  b) TDN 75%, CP 16%,  c) TDN 87%, CP 5.5% TDN, total digestible nutrient, CP, crude protein. |

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performed using anti-cow CD3 antibody, anti-cow CD4 antibody, anti-cow CD8 antibody, anti-cow CD14 antibody, anti-cow CD45R antibody, anti-cow TcR-N12 antibody, and anti-cow MHC class-II antibody (Table 2) as the primary antibodies. Cell surface markers were visualized with anti-mouse FITC-labeled antibody (ICN, Ohio, U.S.A.) and anti-mouse PE-labeled antibody (ICN, Ohio, U.S.A.) as the secondary antibodies. Positive cells were counted using a flow cytometer (FACScan, Becton Dickinson, U.S.A.). Cytogram results enabled us to distinguish between low side scatter monocytes/lymphocytes and other lymphocytes, and those classified as monocytes or lymphocytes were considered mononuclear cells. Cells positive for each surface marker were calculated on the basis of the positive reaction rates for each marker and the actual leukocyte counts. Monocyte numbers were determined on the basis of the positive reaction rates from the cytograms and the actual leukocyte counts.

**Statistical analysis:** Differences within each group on day 0 compared with the values for later days and the differences between groups for each day were evaluated using the Student's t-test. Multiple comparisons of changes in the values for each group were evaluated using the Friedman test. Values of P<0.05 were regarded as significant.

FCM results showed that the numbers of CD3+ cells significantly increased between day 0 and day 5 in the banana group (P<0.01, Fig. 1A). They were significantly higher on day 5 in the banana group relative to the control group (P<0.05). The numbers of CD3+CD45R- cells showed a significant increase between day 0 to day 5 in the banana group (P<0.01, Fig. 1B). These cells were significantly higher on day 5 in the banana group relative to the control group (P<0.01). Similarly, the numbers of CD3+TeR- cells significantly increased between day 0 and day 5 in the banana group (P<0.01, Fig. 1C). They were significantly higher in the treated group than in the control group on day 5 (P<0.05). No significant differences were observed for the numbers of CD3+CD4+ cells, CD4- cells, CD8+ cells, CD14+ cells, or MHC class II+CD14+ cells.

The results of the present study showed that oral administration of banana to F1 hybrid calves induced an increase in the number of CD3+ cells, which are ordinary T-lymphocytes. They also demonstrated that these amplified T-lymphocytes consisted of CD3+CD45- cells, which are memory T-lymphocytes, and CD3+TeR+ cells, which are CD4+ cells, CD8+ cells, CD14+ cells, or MHC class II+CD14+ cells.

Memory T-lymphocytes are an activated form of T-lymphocytes that have the capacity to produce large amounts of cytokines [2]. Peumans et al. [15] reported that banana lectin was a powerful murine T-cell mitogen, and Koshte et al. [10] reported that banana lectin-I stimulated T-cell proliferation. Based on these reports, it would appear that banana lectin might have been involved in the increase in memory T-lymphocytes that was observed in the calves in the present study after administration of banana as a feed sup-

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**Table 2. Antibodies used in the immunostaining of peripheral blood mononuclear leukocytes**

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Clone</th>
<th>Isotype</th>
<th>Specificity</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>MM1A</td>
<td>IgG1</td>
<td>Pan-T-cell</td>
<td>VMRD</td>
</tr>
<tr>
<td>CD4</td>
<td>CACT83B</td>
<td>IgG1</td>
<td>Helper/inducer</td>
<td>VMRD</td>
</tr>
<tr>
<td>CD8</td>
<td>CACT80C</td>
<td>IgG1</td>
<td>Cytotoxic-suppressor</td>
<td>VMRD</td>
</tr>
<tr>
<td>CD14</td>
<td>MY4</td>
<td>IgG2b</td>
<td>Monocyte</td>
<td>Coulter</td>
</tr>
<tr>
<td>CD45R</td>
<td>GC6A</td>
<td>IgM</td>
<td>Memory/naive T-cell</td>
<td>VMRD</td>
</tr>
<tr>
<td>TcR-N12</td>
<td>CACT61A</td>
<td>IgM</td>
<td>γδT-cell</td>
<td>VMRD</td>
</tr>
<tr>
<td>MHC class-II</td>
<td>TH14B</td>
<td>IgG1</td>
<td>B-cell/Monocyte</td>
<td>VMRD</td>
</tr>
</tbody>
</table>

a) VMRD = VMRD, Inc. (Pullman, WA, U.S.A.).
b) Coulter = Coulter Immunology. (Hialeah, Florida, U.S.A.)
CD3'TcR' cells are λδ-type T-lymphocytes that are commonly observed in the juvenile period [1], and it is known that levels of these lymphocytes are low from birth in infirm calves with low resistance to disease [13]. Fikri et al. [5] reported that λδ-type T-lymphocytes produce IFNa, which promotes the cellular immune response. It is suggested that the increase in the CD3'TcR' cells seen in the banana group is participating in the cellular immune response. However, further research is needed to determine the mechanism of such an effect.

Yamazaki et al. [22] reported in a murine model that banana was as effective as immunostimulants in priming tumor necrosis factor (TNF). Maeda et al. [11] reported in mice that addition of banana juice in vitro to the intracelical macrophage growth system produced a concentration-dependent rise in macrophages, and that intravenous administration of banana juice induced priming of TNF activity, also in a dose-dependent manner. These results suggest that the activation of immunity by bananas does not act selectively on T-lymphocytes.

Banana contains a large amount of carbohydrates, potassium, B vitamins, and vitamin C [3]. However, it is unknown which ingredient in the banana acts on immunity activation. Therefore, further studies are needed to understand the active ingredient of the banana.

It was clear from the results of this study that dietary administration of banana boosted cellular immunity in F1 hybrid calves by increasing the number of memory T-lymphocytes and λδ-type T-lymphocytes. These data showed that feeding banana to calves increased T-lymphocytes, suggesting that it might be possible to enhance protective functions against infections.

REFERENCES