

## CHAPTER 5

### DISCUSSION

Natural antioxidants, including the carotenoids and vitamin A, E, C, are important to animal health. They function as antioxidants to remove harmful free radicals produced through normal cellular activity and from environmental stress, thereby maintaining the structural integrity of immune cells. In addition, they may regulate cellular events. Therefore, carotenoids and vitamin A, E, C are important for animal health by enhancing immunity (Chew, 1996). Gamma oryzanol is a natural antioxidant (Duve and White, 1991) which is composed high level (2.468 %DM) in purple glutinous rice bran oil (Pongpiachan *et al.*, 2004). But immune response property has not been proved yet. Therefore, experiment I aimed to prove the immune response property of  $\gamma$ -oryzanol via the responsibility of male mice immunity. Thereinafter,  $\gamma$ -oryzanol in natural status of purple glutinous rice bran was practiced for animal health. Then experiment II was designed to find out the satiable diet level of purple glutinous rice bran for positive immune response. The average  $\gamma$ -oryzanol in four varieties of purple glutinous rice bran was 2.468 %, it was used to formulate mice diets because there was an insufficient amount of any single purple glutinous rice bran to make up a proper mouse ration for the experiment. This experiment used BSA for immunization because it is non toxic for mice and has a high molecular weight. So it was not necessary is conjugate with other protein before immunization. Additionally, it easy to use and cheaper than other antigens.

#### 5.1 Discussion on productive performances

The mice in 800 mg/kg pure  $\gamma$ -oryzanol supplemented group tended to have a higher average ADG and TWG than the control group. However, the mice in 1,340 mg/kg pure  $\gamma$ -oryzanol supplemented group tended to have a lower average ADG and TWG than the other groups, as same as in experiment II. Besides, the mice in 800 mg/kg pure  $\gamma$ -oryzanol supplemented

group and the mice which had been fed with 8 % purple glutinous rice bran tended to have a lower average feed conversion ratio than those in other groups. Productive performances were slightly different between groups. Moreover, the mice which had been fed with 8 % purple glutinous rice bran tended to have a higher average ADG and TWG than those the control group and the mice supplemented with 1,340 mg/kg pure  $\gamma$ -oryzanol. The mice supplemented with 1,340 mg/kg pure  $\gamma$ -oryzanol tended to have a lower average ADG and TWG than other groups because, the mice in this group received high concentration of antioxidant. Therefore, the antioxidants/oxidants ratio is imbalance. Consequently, the remaining antioxidants interrupted other reactions in the body (Packer and Yoshikawa, 1999) and another reason was that there were more antioxidants in the body than needed. Hence, they stimulated the immune system, which resulted in the requirement for higher proteins, particularly lysine, which is an essential amino acid in the building of immunoglobulins. As the result, it caused a decrease in the delivery of nutrients to other parts of the body. Therefore, it will affect the productive performances (Humphrey *et al.*, 2004). The absorption of pure  $\gamma$ -oryzanol is higher than the natural  $\gamma$ -oryzanol. Nevertheless, using 8 % purple glutinous rice bran in mice ration had a concentration of  $\gamma$ -oryzanol as much as 1,800 mg/kg diet, but the absorption of  $\gamma$ -oryzanol in nature depends on digestibility of feedstuff. This average that the mice which had been fed with 6 % and 8 % purple glutinous rice bran did not have the ability to absorb  $\gamma$ -oryzanol in the form of purple glutinous rice bran up to 100%. Thus, the absorbed  $\gamma$ -oryzanol will not reach the quantity of 1,340 mg/kg diet. Consequently, it will not be over antioxidants consumption.

## 5.2 Discussion on BSA IgA response

The mice which had been supplemented 800 mg/kg pure  $\gamma$ -oryzanol had tendency to a higher average  $\log_2$  BSA IgA titer than the mice which had been supplemented 1,340 mg/kg pure  $\gamma$ -oryzanol, especially compared with the mice in the control group. This was nearly the study of McCorrkle *et al.*, (1980) adult birds fed vitamin C had a higher antibody titers against *Brucella abortus*. This is similar to the average area under BSA IgA titer the mice supplemented with  $\gamma$ -oryzanol 800 mg/kg diet tended to have a higher average than those other groups. This result was similar to experiment II. It was shown that the mice that had been fed with 6 % purple glutinous rice bran had average  $\log_2$  BSA IgA titer better than the mice that had been

supplemented with 1,340 mg/kg pure  $\gamma$ -oryzanol. This was close to the mice that had been fed with 6 % purple glutinous rice bran in ration tended to have a higher average area under BSA IgA titer curve than those in other groups. Though, the  $\gamma$ -oryzanol concentration of 6 % purple glutinous rice bran ration has an approximated value of 1,340 mg/kg, but the responding value of the IgA response is different. This is owing to the  $\gamma$ -oryzanol in pure form had a greater absorption rate than the  $\gamma$ -oryzanol in nature. Though the use of 6 % purple glutinous rice bran in mice diet give the concentration of  $\gamma$ -oryzanol as 1,340 mg/kg diet, but the absorption of the  $\gamma$ -oryzanol in nature depends on digestibility of feedstuff. Thus, it was hypothesized that 6 % purple glutinous rice bran ration had the same value which was close to 800 mg/kg pure  $\gamma$ -oryzanol. That would make the characteristic of IgA response similar. Moreover, the increment of the  $\log_2$ BSA IgA titer would appear to be higher after each boosted time, also it was found that after the second immunization the  $\log_2$  BSA IgA titer became increasingly greater if the boost was repeated.

### 5.3 Discussion on BSA IgM response

The mice which had been supplemented 1,340 mg/kg pure  $\gamma$ -oryzanol tended to have a lower average  $\log_2$  BSA IgM titer than those other group. This is similar to value of average area under BSA IgM titer curve. Especially, compared with the mice in the control group, it was noticeable that the mice in the supplemented groups tended to have a lower average  $\log_2$  BSA IgM titer than control group. This result was similar to experiment II, it was found that the mice in the negative control had average  $\log_2$  BSA IgM titer better than the mice which had been supplemented 1,340 mg/kg pure  $\gamma$ -oryzanol (positive control group) and the mice which had been fed with 6 % and 8 % purple glutinous rice bran. This finding was similar to the mice in negative control had average area under BSA IgM titer curve than those other groups. From this experiment, it could not be explained why average  $\log_2$  BSA IgM titer of the mice in control group had increased. But it decreased in the mice in the supplemented group. According to this experiment, when observing the  $\log_2$  BSA IgM titer, it was found that the highest occurred on day 10. After that the  $\log_2$  BSA IgM titer decreased after the second immunization, because the body had produce IgG to replace IgM, in the experiment the results showed that the  $\log_2$  BSA IgM titer, which reduced rapidly on day 14 of experiment, and the value went down gradually even though

the mice were boosted, except the mice in the control group when the  $\log_2$  BSA IgM titer was higher on day 10, and increased again on day 21 of the experiment.

#### 5.4 Discussion on BSA IgG response

The average  $\log_2$  BSA IgG titer showed great increase in the mice which had been supplemented 800 mg/kg pure  $\gamma$ -oryzanol. Therefore, the mice which had been supplemented 1,340 mg/kg pure  $\gamma$ -oryzanol had a tendency to a higher IgG response than those in the control group, but the difference found would be clearly distinguished when compared with average  $\log_2$  BSA IgG titer between the mice which had been supplemented 800 mg/kg pure  $\gamma$ -oryzanol and control group. This finding is similar to Tengerdy *et al.*, (1973) who found that the mice supplemented 60-180 mg/kg vitamin E increased anti body titer following sheep red blood cell challenge. It has been reported that high doses of vitamin E improved anti body response to *Escherichia coli* challenge (Bains, 1994). Jurin and Tannock, (1972) and Cohen and Cohen, (1973) reported that blood antibody titers were stimulated in vitamin A supplemented mice immunized with antigens. It has been reported that mice supplemented with retinoic acid had secondary IgG responses to ovalbumin (Barnett, 1983). This result was similar to experiment II, when it was found that the mice which had been fed with 6 % purple glutinous rice bran had average  $\log_2$  BSA IgG titer better than the mice in the negative control group and the mice which had been supplemented 1,340 mg/kg pure  $\gamma$ -oryzanol (positive control group). The average  $\log_2$  BSA IgG titer, was seen clearly when comparing the mice in the control group and those in the supplemented with  $\gamma$ -oryzanol on those levels 800 and 1340 mg/kg. This result was close to the mice which had been fed with 6 % purple glutinous rice bran tended to have a higher average area under BSA IgG titer curve than those in other groups. Therefore, on the last day of the experiment I, it was found that the mice in group II had a decreased average  $\log_2$  BSA IgG titer on day 42 which was the last day of the experiment, because the mice bit each other causing stress. After a careful consideration of both experiments, it was found that the mice which had been fed with 6 % purple glutinous rice bran had an average  $\log_2$  BSA IgG titer better than those in the control group, and the mice which had been supplemented 1,340 mg/kg pure  $\gamma$ -oryzanol (positive control group). Moreover, the mice which had been fed with 8 % purple glutinous rice bran tended to have a higher average area under BSA IgG titer curve than the mice which had been fed with 6 %



purple glutinous rice bran but the value was slightly different. Due to the supplement of the pure  $\gamma$ -oryzanol at a level of 800 mg/kg diet was the suitable level, but the supplement with  $\gamma$ -oryzanol 1,340 mg/kg was too concentrated, which resulted in the ratio of antioxidants/oxidants became imbalanced. In due course, it would make remaining antioxidants interrupt with other reactions in the body, to receive the pure  $\gamma$ -oryzanol, which make the rate of absorption higher than receiving  $\gamma$ -oryzanol in the natural form, although the use of 6 % purple glutinous rice bran in mice diet would have the concentration of  $\gamma$ -oryzanol 1,340 mg/kg diet, but the absorption of the  $\gamma$ -oryzanol in natural form depended on the digestion of the feedstuff. Thus, the hypothesis would be stated as 6 % purple glutinous rice bran ration would have an absorption value close to 800 mg/kg pure  $\gamma$ -oryzanol and 8 % purple glutinous rice bran ration would have value close to the absorption of 1,340 mg/kg pure  $\gamma$ -oryzanol. Thus, the IgG response characteristics are similar, and it is noticeable that the mice which had been supplemented pure  $\gamma$ -oryzanol and the mice which had been fed with purple glutinous rice bran had a tendency to a higher IgG response than control group but they had a tendency to a lower IgM response than those in the control group. According to this experiment, it could not give an explanation on the mechanism and the relation of increase and decrease in term of IgG and IgM.

### 5.5 Conclusions

According to both experimentations, it was shown that mice with additional pure  $\gamma$ -oryzanol treatment and mice treated with purple glutinous rice bran had a tendency to a higher IgA and IgG response than those in the control group. Therefore, it was found that the mice in the control group had a tendency to a higher IgM response than the mice in the pure  $\gamma$ -oryzanol supplemented groups and the mice which had been fed with purple glutinous rice bran in diets. The mice which had been supplemented 800 mg/kg pure  $\gamma$ -oryzanol and pure  $\gamma$ -oryzanol and the mice which had been fed with 6 % purple glutinous rice bran tended to have better IgA and IgG response than those in other groups. The results are shown that, the study successfully to pass the objective of this research. It was found that the optimum concentration of  $\gamma$ -oryzanol and purple glutinous rice bran in ration were 800 mg/kg and 6 % respectively. Pure  $\gamma$ -oryzanol and purple glutinous rice bran contains a property for stimulating the immune response in mice, which increases IgA and IgG in the plasma. Also it could be said that it could produce good health in

animals. Thus, animals treated with pure  $\gamma$ -oryzanol or treated with purple glutinous rice bran should be strong and healthy since they have the ability to resist diseases. As a result, it saves expenses on drugs to protect health. Also it decreases drugs and chemical residue in animal carcasses, which might be beneficial for the health of the consumers; it could reduce epidemics of animal diseases as well.

### 5.6 Further investigation

Further research it is need to prove the effect of  $\gamma$ -oryzanol on mice immune response. Especially, the interaction between  $\gamma$ -oryzanol and anthocyanin in purple glutinous rice bran on IgA and IgG response. The mice which had been fed 6 % and 8 % purple glutinous rice bran tended to have a higher IgA and IgG response to BSA-antigen than those of the mice supplement with 1,340 mg/kg pure  $\gamma$ -oryzanol. This research may cause additive effects between  $\gamma$ -oryzanol and anthocyanin in purple glutinous rice bran. Therefore in further study needs to be undertaken to investigate the relationship between  $\gamma$ -oryzanol and anthocyanin and establish the levels of anthocyanin intake to optimize immune response, and also determined to verify this result and explain it in a wide extent.