CHAPTER 4

RESULTS

4.1 Determination of γ-oryzanol concentration in purple glutinous rice bran

This part aimed to analyse γ-oryzanol in four varieties of purple glutinous rice bran; Kum Nan, Kum Doi Saket, Kum 87061 and Kum Omkoi, in order to formulate a mouse ration for the next experimentation. It was found that the γ-oryzanol in four varieties of purple glutinous rice bran; Kum Nan, Kum Doi Saket, Kum 87061 and Kum Omkoi: 2.854, 2.848, 2.287, and 1.882 %DM respectively. Four sources of glutinous rice bran were mixed to gather equally and the content of γ-oryzanol was 2.4677 %DM, because the amount of each source was insufficient for experimental rations.

4.2 Effect of dietary pure γ-oryzanol treatments on productive performances (Experiment I)

4.2.1 Effect of dietary pure γ-oryzanol treatments on average daily feed intake (ADFI)

Male mice ADFI for 4 groups: group I (0 mg/kg pure γ-oryzanol), group II (280 mg/kg pure γ-oryzanol), group III (800 mg/kg pure γ-oryzanol) and group IV (1,340 mg/kg pure γ-oryzanol) and then, were supplemented by pure γ-oryzanol, at 0, 280, 800, and 1,340 mg/kg respectively. The results showed that mice in group I had the highest tendency of ADFI, which was 3.949 grams per day. The lower were group II, 3.895 and group IV, 3.857 grams per day respectively, and the lowest was group III 3.828 grams per day. However, there was only a little difference from the mice in group I. Experimental results are shown in Table 8 and Figure 33.

4.2.2 Effect of dietary pure γ-oryzanol treatments on average daily gain and total weight gain (ADG and TWG)

Referred to 4.2.1, the results showed that there were no significant differences in ADG between the control group and supplemented groups ($p>0.05$). Nonetheless, the mice in group III

had a tendency to give higher ADG than group IV and group I, as the following ADG; 0.249, 0.199 and 0.183 gram per day respectively. The tendency of lowest value of average daily gain was found in group II. Its value was 0.166 gram per day. The total weight gain (TWG) were the same as ADG, there were no significant differences between the control group and supplemented groups ($p>0.05$). Nevertheless, the mice in group III had a tendency to a higher TWG than group IV and group I: 10.714, 8.571 and 7.857 gram per day respectively. The lowest TWG was found in group II, 7.143 gram per day. Though, its value was slightly different from other groups ADG and TWG are shown in Table 8 and Figure 33.

4.2.3 Effect of dietary pure γ-oryzanol treatments on average feed conversion ratio (AFCR)

Referred to 4.2.1, it was found that the mice in group III had a tendency to a lower AFCR than group IV and group I: 15.361, 19.347 and 21.601 respectively. The highest AFCR was found in group II, 23.449. AFCR are shown in Table 8 and Figure 33.

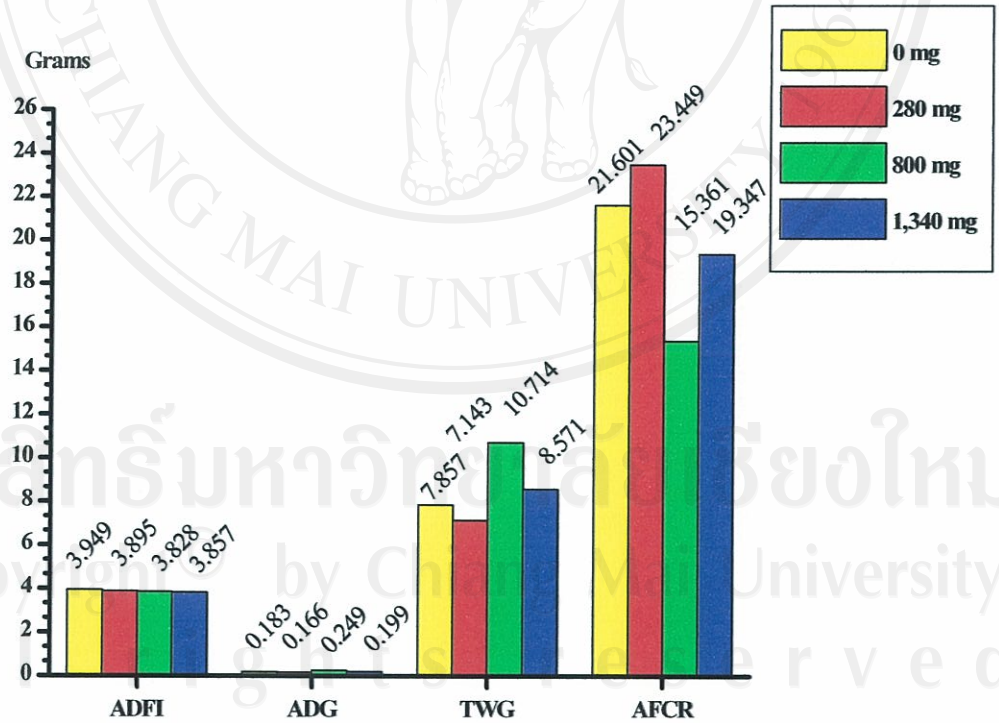

Figure 33. Effect of dietary pure γ-oryzanol on productive performances in 32 male mice (data from table 8), average daily feed intake (ADFI), average daily gain (ADG), total weight gain (TWG), average feed conversion ratio (AFCR).

Table 8. Effect of dietary pure γ-oryzanol treatments on productive performances

Pure γ-oryzanol supplement (mg/kg)	Productive performances (mean ± S.E.)			
	ADFI (gm/hd/day)	ADG (gm/hd/day)	TWG (gm/hd)	AFCR
0	3.9486 ±.1005	0.1827 ±.0346	7.8571 ±1.4869	21.6010
280	3.8953 ±.1036	0.1661 ±.0235	7.1429 ±1.0101	23.4499
800	3.8275 ±.0851	0.2492 ±.0395	10.7142 ±1.7003	15.3612
1340	3.8566 ±.1096	0.1993 ±.0332	8.5714 ±1.4285	19.3473

Average daily feed intake (ADFI), average daily gain (ADG), total weight gain (TWG), average feed conversion ratio (AFCR)

4.3 Effect of dietary pure γ-oryzanol on the immune response in male mice

4.3.1 Effect of dietary pure γ-oryzanol treatments on BSA IgA titer

The dietary effect of γ-oryzanol on the immune response of the male mice was investigated. The average of the $\log_2$ BSA IgA titer was used as an indicator. The mice were divided into 4 groups as before, the results showed no significant differences among treatments on day 1 ($p>0.05$). Nevertheless, on day 10 of the experiment, there was significant difference on the average of the $\log_2$ BSA IgA titer of group III higher than other groups, 0.0178 ± 0.0086 ($p<0.05$). There was no significant difference on the average of the $\log_2$ BSA IgA titer among treatments on day 14 ($p>0.05$). On day 21 of the experimentation, there was significant difference on the average of the $\log_2$ BSA IgA titer of group II, III and IV higher than the control group: 0.1410 ± 0.0305, 0.1807 ± 0.0196, 0.1437 ± 0.0285 and 0.0141 ± 0.0068 ($p<0.05$), respectively. On day 28 there was no significant difference on the average of the $\log_2$ BSA IgA titer. On day 42, which was the last day of the experiment, it was found that the mice in group III

showed significant difference $\log_2$ BSA IgA titer higher than other group, 0.5894 ± 0.0738 ($p<0.05$). In summary, it was found that the mice in supplemented groups had the greater level of the $\log_2$ BSA IgA titer than the control group. The results are shown in Table 9 and Figure 34.

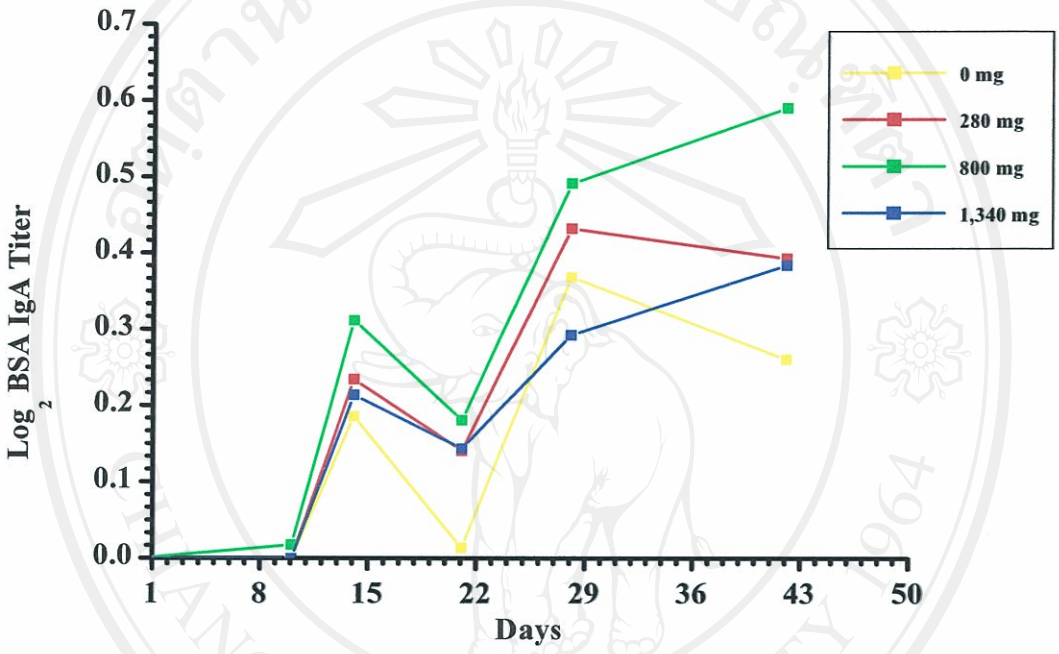

Figure 34. Effect of dietary pure γ-oryzanol treatments on Log_2 BSA IgA titer in 32 mice following BSA immunization on day 0, 14 and 28, (data from table 9).

Table 9. Effect of dietary pure γ-oryzanol treatment on Log_2 BSA IgA titer

γ-oryzanol supplement (mg/kg)	Log_2 OD (mean ± S.E.)					
	Day 1	Day 10	Day 14	Day 21	Day 28	Day 42
0	0.0000 ±.0000	0.0000[b] ±.0000	0.1860 ±.0489	0.0141[b] ±.0068	0.3684 ±.0884	0.2601[b] ±.0248
280	0.0000 ±.0000	0.0000[b] ±.0000	0.2344 ±.0733	0.1410[a] ±.0305	0.4319 ±.0965	0.3924[b] ±.0469
800	0.0000 ±.0000	0.0178[a] ±.0086	0.3117 ±.0509	0.1807[a] ±.0196	0.4910 ±.0887	0.5894[a] ±.0738
1340	0.0000 ±.0000	0.0000[b] ±.0000	0.2138 ±.0846	0.1437[a] ±.0285	0.2923 ±.0898	0.3836[b] ±.0609

Means within column with different superscripts are significantly different ($p<0.05$).

4.3.2 Effect of dietary pure γ-oryzanol treatments on area under BSA IgA titer curve

The time series of IgA BSA titer obtained from each mouse were graphically plotted and the area under BSA titer curve were calculated by Autocad software (2000i).

The average area under BSA IgA titer the mice supplemented with γ-oryzanol 800 mg/kg diet had a tendency to be higher than those of other groups. However, a significant difference was found only in the comparison of the control group, 1.059 ± 0.1510, 0.804 ± 0.1889, 0.747 ± 0.1822 and 0.581 ± 0.1351 square units respectively ($p<0.05$). The lowest average area under BSA IgA titer curve was found in group I (control group), 0.581 ± 0.1350 square units. The areas under BSA IgA titer curve are shown in Table 10 and Figure 35.

Table 10. Effect of dietary pure γ-oryzanol treatments on area under BSA IgA titer curve

Groups	Gamma oryzanol supplements mg/kg	Area under BSA IgA titer curve Mean ± S.E. Square unit
I	0	0.581 ± 0.1350^{b}
II	280	0.804 ± 0.1889^{ab}
III	800	1.059 ± 0.1510^{a}
IV	1,340	0.747 ± 0.1822^{ab}

Means within column with different superscripts are significantly different ($p<0.05$).

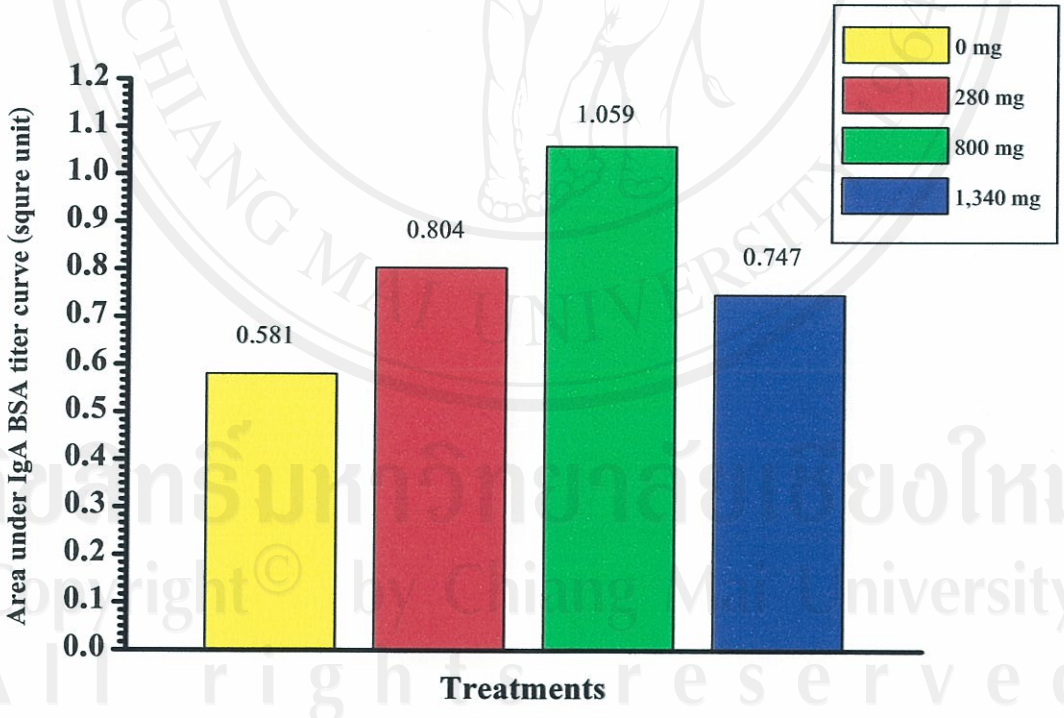

Figure 35. Effect of dietary pure γ-oryzanol treatments on area under BSA IgA titer curve in male mice following BSA immunization on day 1, 14 and 28, BSA IgA titer were tested for 42 days, (data from table 10).

4.3.3 Effect of dietary pure γ-oryzanol treatments on BSA IgM titer

Referred to 4.3.1, the results showed no significant differences among treatments on day 1 ($p>0.05$). Nevertheless, on day 10 of the experiment, there was significant difference on the average of the $\log_2$ BSA IgM titer of control group higher than group II, III and IV: 0.2339 ± 0.0158, 0.1923 ± 0.0106, 0.1887 ± 0.0189 and 0.0969 ± 0.0087 ($p<0.05$) respectively. There was no significant difference on the average of the $\log_2$ BSA IgM titer between the control and supplemented group on day 14 ($p>0.05$). But it was found that the mice in group II and III had significantly different $\log_2$ BSA IgM titer than the mice in group IV: 0.0840 ± 0.0194, 0.0751 ± 0.0204 and 0.0100 ± 0.0045 ($p<0.05$) respectively. On day 21 of the experimentation, there was a significant difference on the average of the $\log_2$ BSA IgM titer of control group higher than group II, III and IV: 0.1890 ± 0.0134, 0.0166 ± 0.0050, 0.0841 ± 0.0143 and 0.0362 ± 0.0070 ($p<0.05$) respectively. On day 28 there was no significant difference on the average of the $\log_2$ BSA IgM titer among treatments. On day 42, which was the last day of the experiment, it was found that the mice in group III and IV had a significantly different $\log_2$ BSA IgM titer higher than other group: 0.0870 ± 0.0138 and 0.1076 ± 0.0129 ($p<0.05$) respectively. In summary, it was found that the mice in control group had a higher of $\log_2$ BSA IgM titer than the supplemented groups. The results are shown in Table 11 and Figure 36.

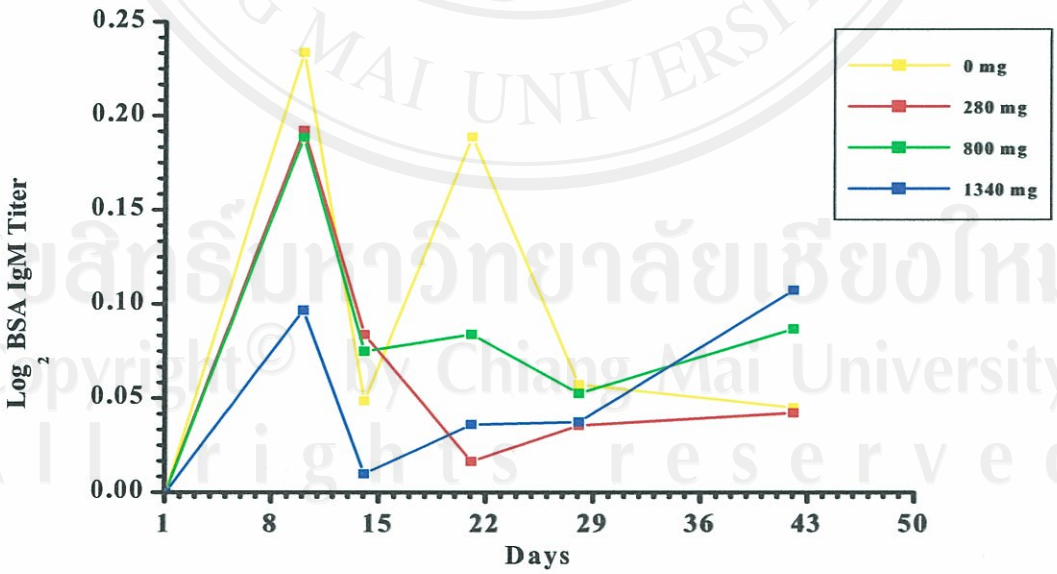

Figure 36. Effect of dietary pure γ-oryzanol treatments on Log_2 BSA IgM titer in 32 mice following BSA immunization on day 0, 14 and 28, (data from table 11).

Table 11. Effect of dietary pure γ-oryzanol treatments on Log_2 BSA IgM titer

γ-oryzanol supplement (mg/kg)	Log_2 OD (mean ± S.E.)					
	Day 1	Day 10	Day 14	Day 21	Day 28	Day 42
0	0.0000 ±.0000	0.2339^{a} ±.0158	0.0488^{ab} ±.0088	0.1890^{a} ±.0134	0.0575 ±.0112	0.0452^{b} ±.0026
280	0.0000 ±.0000	0.1923^{b} ±.0106	0.0840^{a} ±.0194	0.0166^{c} ±.0050	0.0357 ±.0033	0.0424^{b} ±.0031
800	0.0000 ±.0000	0.1887^{b} ±.0189	0.0751^{a} ±.0204	0.0841^{b} ±.0143	0.0527 ±.0091	0.0870^{a} ±.0138
1340	0.0000 ±.0000	0.0969^{c} ±.0087	0.0100^{b} ±.0045	0.0362^{c} ±.0070	0.0375 ±.0048	0.1076^{a} ±.0129

Means within column with different superscripts are significantly different ($p<0.05$).

4.3.4 Effect of dietary pure γ-oryzanol treatments on area under BSA IgM titer curve

The time series of BSA IgM titer obtained from each mouse were graphically plotted and area under BSA titer curve were calculated by Autocad software (2000i).

The mice in control group had a significantly different average area under BSA IgM titer than those groups II, III and IV: 0.418 ± 0.0188, 0.270 ± 0.0147, 0.319 ± 0.0227 and 0.168 ± 0.0143 square units, ($p<0.05$) respectively. Moreover, a significant difference was found in the mice supplemented with γ-oryzanol 280 and 800 mg/kg diet comparison with the mice supplemented with γ-oryzanol 1,340 mg/kg diet 0.270 ± 0.0147, 0.319 ± 0.0227 and 0.168 ± 0.0143 square units, ($p<0.05$) respectively. The lowest average area under BSA IgM titer curve was found in group IV (1,340 mg/kg γ-oryzanol supplemented). The areas under BSA IgM titer curve are shown in Table 12 and Figure 37.

Table 12. Effect of dietary pure γ-oryzanol treatments on area under BSA IgM titer curve

Groups	Gamma oryzanol supplements mg/kg	Area under BSA IgM titer curve Mean ± S.E. Square unit
I	0	0.418 ± 0.0188^{a}
II	280	0.270 ± 0.0147^{b}
III	800	0.319 ± 0.0227^{b}
IV	1,340	0.168 ± 0.0143^{c}

Means within column with different superscripts are significantly different ($p<0.05$).

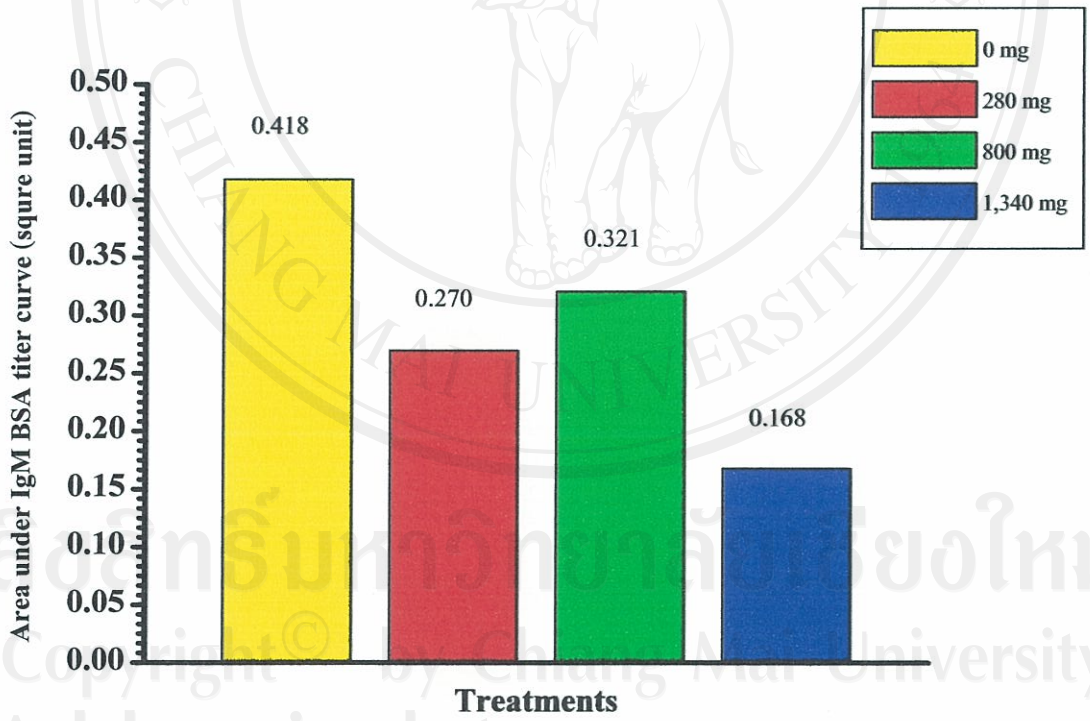

Figure 37. Effect of dietary pure γ-oryzanol treatments on area under BSA IgM titer curve in male mice following BSA immunization on day 1, 14 and 28, BSA IgM titer were tested for 42 days, (data from table 12).

4.3.5 Effect of dietary pure γ-oryzanol treatments on BSA IgG titer

Referred to 4.3.1, the results showed no significant differences among treatments on day 1 ($p>0.05$). Nonetheless, on day 10 of the experiment, the mice in the control group had $\log_2$ IgG titer significantly higher than the mice in group III and group IV: 0.0111 ± 0.0012, 0.0042 ± 0.0007 and 0.0039 ± 0.0012 ($p<0.05$) respectively. However, there was no significant difference on the average of the $\log_2$ BSA IgG titer between the control group and group II. On day 14 of the experimentation, there was no significant difference on the average of the $\log_2$ BSA IgG titer between the control group and the supplemented groups ($p>0.05$). But it was found that the mice in group III had a significantly different $\log_2$ BSA IgG titer than the mice in group IV: 0.0437 ± 0.0028 and 0.0311 ± 0.0036 ($p<0.05$) respectively. On day 21 of the experimentation, there was significant difference on the average of the $\log_2$ BSA IgG titer of group IV higher than control group: 0.1322 ± 0.0166 and 0.0910 ± 0.0044 ($p<0.05$) respectively. On day 28, it was found that the mice in group II had a significantly different $\log_2$ BSA IgG titer than the mice in the control group: 0.1791 ± 0.0229 and 0.1262 ± 0.0141 ($p<0.05$) respectively. On day 42, which was the last day of the experiment, it was found that the mice in group III had a significantly different $\log_2$ BSA IgG titer over the mice in the control group: 0.1924 ± 0.0247 and 0.1348 ± 0.0111 ($p<0.05$) respectively. However, no significant difference was found in the supplemented groups. In summary, it was found that the mice in supplemented groups had the highest level of the $\log_2$ BSA IgG titer than the control group. The results are shown in Table 13 and Figure 38.

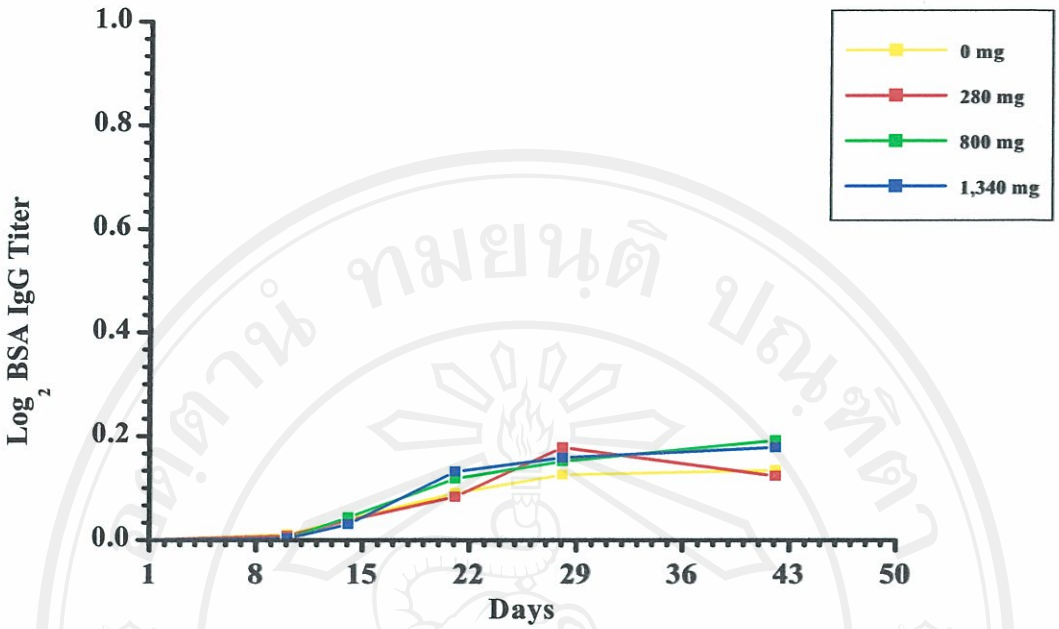

Figure 38. Effect of dietary pure γ-oryzanol treatments on Log_2 BSA IgG titer in 32 mice following BSA immunization on day 0, 14 and 28, (data from table 13).

Table 13. Effect of dietary pure γ-oryzanol treatments on Log_2 BSA IgG titer

γ-oryzanol supplement (mg/kg)	Log_2 OD (mean ± S.E.)					
	Day 1	Day 10	Day 14	Day 21	Day 28	Day 42
0	0.0000 ±.0000	0.0111^{a} ±.0012	0.0407^{ab} ±.0057	0.0910^{b} ±.0044	0.1262^{b} ±.0141	0.1348^{bc} ±.0111
280	0.0000 ±.0000	0.0085^{a} ±.0008	0.0386^{ab} ±.0031	0.0837^{b} ±.0064	0.1791^{a} ±.0229	0.1236^{c} ±.0216
800	0.0000 ±.0000	0.0042^{b} ±.0007	0.0437^{a} ±.0028	0.1190^{ab} ±.0166	0.1520^{ab} ±.0097	0.1924^{a} ±.0247
1340	0.0000 ±.0000	0.0039^{b} ±.0012	0.0311^{b} ±.0036	0.1322^{a} ±.1659	0.1589^{ab} ±.0168	0.1791^{ab} ±.0112

Means within column with different superscripts are significantly different ($p<0.05$).

4.3.6 Effect of dietary pure γ-oryzanol treatments on area under BSA IgG titer curve

The time series of BSA IgG titer obtained from each mouse were graphically plotted and area under BSA titer curve were calculated by Autocad software (2000i).

The results showed that there were no significant differences among group ($p>0.05$). Nonetheless, the mice in group III had a tendency to a higher area under BSA IgG titer curve than group II and group IV: 1.056 ± 0.1334, and 1.160 ± 0.1062 square units respectively. The lowest area under BSA IgG titer curve was found in group I (control group), 0.894 ± 0.6126 square units. The areas under BSA IgG titer curve are shown in Table 14 and Figure 39.

Table 14. Effect of dietary pure γ-oryzanol treatments on area under BSA IgG titer curve

Groups	Gamma oryzanol supplements mg/kg	Area under BSA IgG titer curve Mean ± S.E. Square unit
I	0	0.894 ± 0.6126
II	280	1.056 ± 0.1334
III	800	1.167 ± 0.0932
IV	1,340	1.160 ± 0.1062

Means within column with different superscripts are significantly different ($p<0.05$).

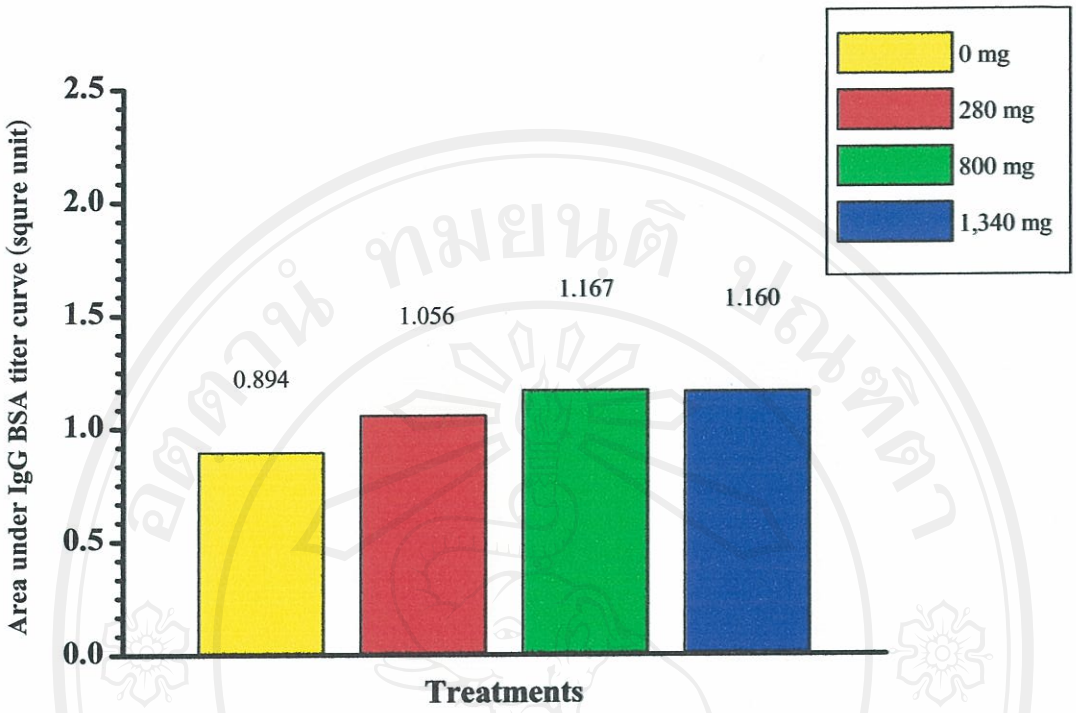

Figure 39. Effect of dietary pure γ-oryzanol treatments on area under BSA IgG titer curve in male mice following BSA immunization on day 1, 14 and 28, BSA IgG titer were tested for 42 days, (data from table 14).

4.4 Effect of dietary purple glutinous rice bran on productive performances (Experiment II)

4.4.1 Effect of dietary purple glutinous rice bran on average daily feed intake (ADFI)

The study aimed to investigate the effect of purple glutinous rice bran (PGRB) on productive performances of the male mice. The ADFI was used as an indicator. The mice were divided into 4 groups: three groups (I, II and III) aimed to investigate the effect of each level (0, 6 and 8 per cent in diets) of purple glutinous rice bran and the fourth group (IV) was supplemented with pure γ-oryzanol (GON) 1,340 mg per kg to mouse basal diet γ-oryzanol in purple glutinous rice bran levels in this experiment were calculated equal to some levels of pure γ-oryzanol supplemented in experiment I. The results showed that the mice in group I had the highest tendency ADFI at 3.648 grams per day. The second below to the mice in group II and Group III, their ADFI was 3.6047 grams per day and 3.6192 grams per day and the mice in group IV had the lowest ADFI at 3.4609 grams per day. However, there was only a slight difference from the mice in group I. Experimental results are shown in Table 15 and Figure 40.

4.4.2 Effect of dietary purple glutinous rice bran on average daily gain and total weight gain (ADG and TWG)

Referred to 4.4.1, the results showed that there was no significant differences among groups ($p>0.05$). Nevertheless, the mice in group III had a tendency to a higher ADG than those in other groups, 0.262 gram per day. The total weight gain (TWG) was the same as ADG, though its value was not different from other groups. Nonetheless, the mice in group III had a tendency to a higher TWG than those in other groups, 11.250 grams. Although its value was slightly different from other groups. The ADG and TWG are shown in Table 15 and Figure 40.

4.4.3 Effect of dietary purple glutinous rice bran on average feed conversion ratio (AFCR)

Referred to 4.4.1, it was found that the mice in group III had a tendency to a lower AFCR than group IV and group I: 13.833, 18.083 and 14.642 respectively. The highest AFCR was found in group IV, 18.083. AFCR are shown in Table 15 and Figure 40.

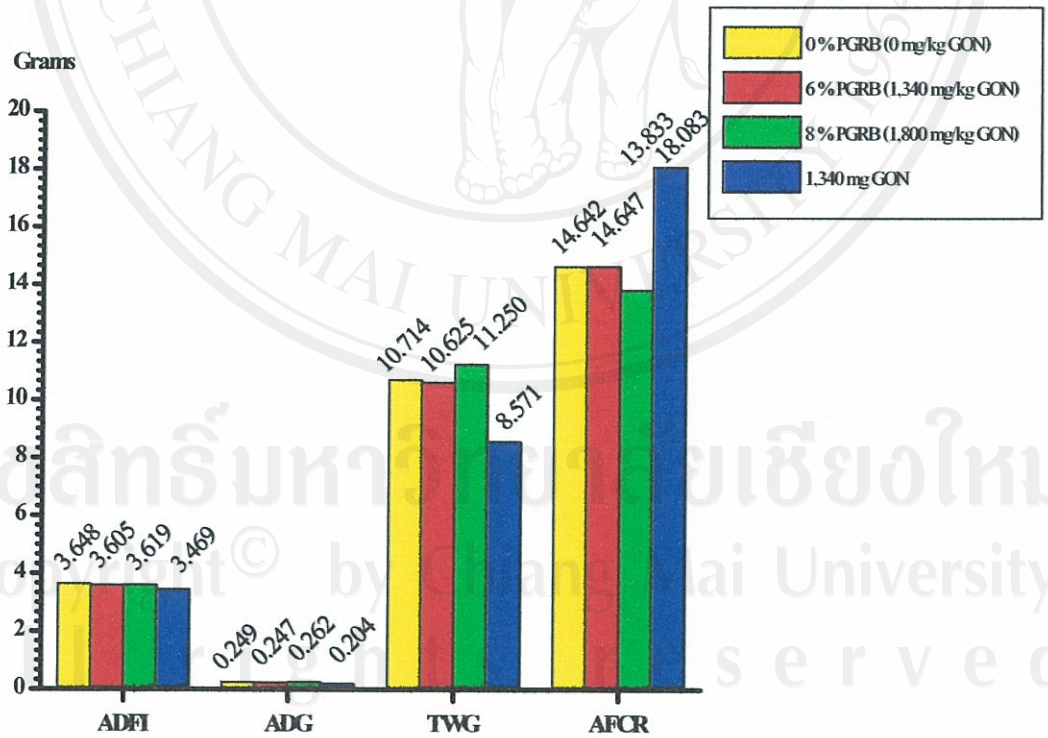

Figure 40. Effect of dietary PGRB on productive performances (data from table 15), purple glutinous rice bran (PGRB), γ-oryzanol (GON), average daily feed intake (ADFI), average daily gain (ADG), total weight gain (TWG), average feed conversion ratio (AFCR).

Table 15. Effect of dietary purple glutinous rice bran on productive performances

PGRB (%)	γ-oryzanol in ration (mg/kg)	Productive performances (mean ± S.E.)			
		ADFI (gm/hd/day)	ADG (gm/hd/day)	TWG (gm/hd)	AFCR
0	0	3.6483 ±.0806	0.2492 ±.0303	10.7143 ±1.3041	14.6416
6	1,340	3.6047 ±.0556	0.2471 ±.0145	10.6250 ±.6250	14.6471
8	1,800	3.6192 ±.0586	0.2616 ±.0291	11.2500 ±1.2500	13.8333
1,340 mg/kg GON	1,340	3.469 ±.0747	0.2035 ±.0538	8.5714 ±.9221	18.0834

Average daily feed intake (ADFI), average daily gain (ADG), total weight gain (TWG), average feed conversion ratio (AFCR), purple glutinous rice bran (PGRB)

4.5 Effect of dietary purple glutinous rice bran on the immune response in male mice

4.5.1 Effect of dietary of purple glutinous rice bran on BSA IgA titer

The study aimed to investigate the effect of purple glutinous rice bran (PGRB) on the immune response of the male mice. The averages of the $\log_2$ BSA IgA titer were used as an indicator. The mice were divided into 4 groups, three groups (I, II and III) aimed to investigate the effect of each level (0, 6 and 8 per cent in diets) of purple glutinous rice bran and the fourth group (IV) was supplemented with pure γ-oryzanol (GON) 1,340 mg per kg to mouse basal diet, γ-oryzanol in purple glutinous rice bran levels in this experiment were calculated equal to some levels of pure γ-oryzanol supplementation in experiment I. The results showed no significant differences among treatments on day 1, 10, 14, 21, 28 and 42 ($p>0.05$), However, on day 28 of the experiment, it was found that the mice in group IV had a tendency to a higher $\log_2$ BSA IgA titer than the group I, as the following value 0.1561 ± 0.0423 and 0.1030 ± 0.0078 ($p>0.05$) respectively. In summary, it was found that the mice in the positive control group (group IV) had

the highest level of the $\log_2$ BSA IgA titer than the negative control group (group I) and other groups on day 28. The results are shown in Table 16 and Figure 41.

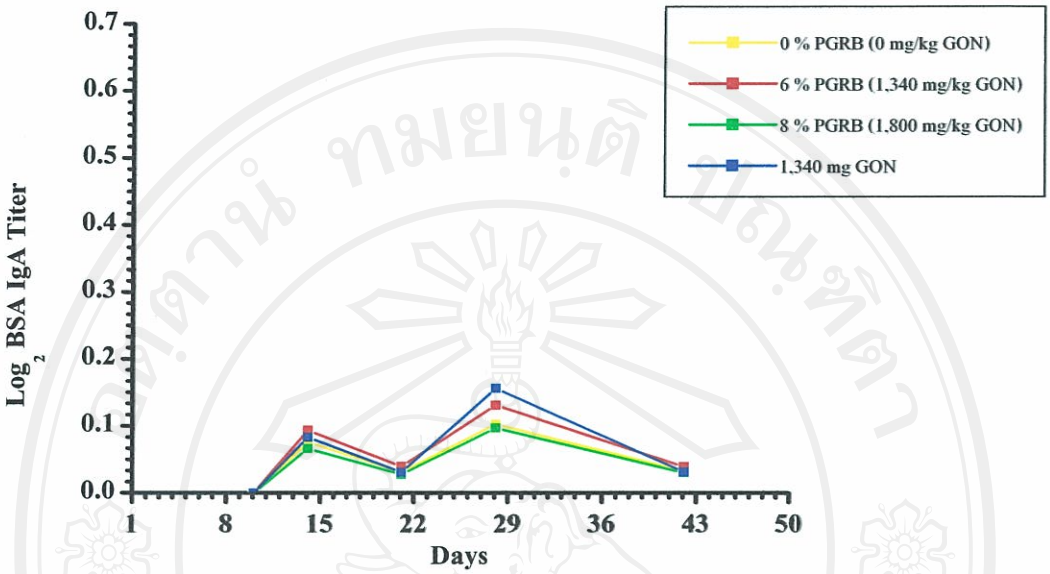

Figure 41. Effect of dietary purple glutinous rice bran on BSA IgA titer, (data from table 16). purple glutinous rice bran (PGRB), γ-oryzanol (GON).

Table 16. Effect of dietary purple glutinous rice bran on BSA IgA titer

Dietary treatments	Log_2 OD (mean ± S.E)					
	Day 1	Day 10	Day 14	Day 21	Day 28	Day 42
0% PGRB	0.0000 ±.0000	0.0000 ±.0000	0.0763 ±.0103	0.0315 ±.0047	0.1030 ±.0078	0.0327 ±.0058
6% PGRB	0.0000 ±.0000	0.0000 ±.0000	0.0936 ±.0126	0.0398 ±.0073	0.1311 ±.0188	0.0391 ±.0072
8% PGRB	0.0000 ±.0000	0.0000 ±.0000	0.0665 ±.0117	0.028 ±.0048	0.0970 ±.0423	0.0303 ±.0050
1340 mg/kg γ-oryzanol	0.0000 ±.0000	0.0000 ±.0000	0.0834 ±.0137	0.0310 ±.0048	0.1561 ±.0423	0.0314 ±.0047

Purple glutinous rice bran (PGRB)

4.5.2 Effect of dietary of purple glutinous rice bran on area under BSA IgA titer curve

The time series of BSA IgA titer obtained from each mouse were graphically plotted and area under BSA titer curve were calculated by Autocad software (2000i).

The mice which had been fed with 6 % purple glutinous rice bran in ration had a tendency to a higher average area under BSA IgA titer curve than those other groups. However, a significant difference was found only in the comparison of group II and group III, 0.206 ± 0.0234 and 0.147 ± 0.0207 square units respectively ($p<0.05$). The lowest average area under BSA IgA titer curve was found in group III. Nevertheless, there was only a little difference from the mice in group I. The areas under BSA IgA titer curve are shown in Table 17 and Figure 42.

Table 17. Effect of dietary purple glutinous rice bran on area under BSA IgA titer curve

Groups	Dietary treatments	Area under BSA IgA titer curve Mean ± S.E. Square unit
I	0% PGRB (0 mg/kg GON)	0.160 ± 0.0130^{ab}
II	6% PGRB (1,340 mg/kg GON)	0.206 ± 0.0234^{a}
III	8% PGRB (1,800 mg/kg GON)	0.147 ± 0.0207^{b}
IV	1340 mg/kg γ-oryzanol	0.171 ± 0.0117^{ab}

Means within column with different superscripts are significantly different ($p<0.05$).

Purple glutinous rice bran (PGRB), γ-oryzanol (GON)

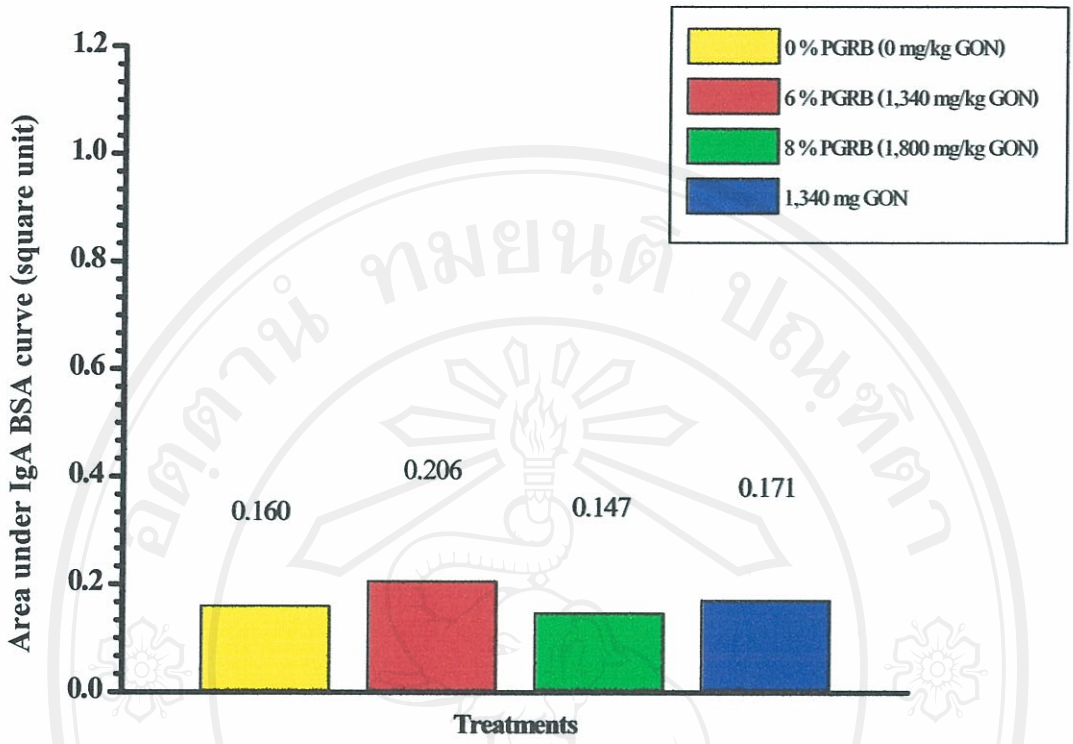

Figure 42. Effect of dietary purple glutinous rice bran on area under BSA IgA titer curve in male mice following BSA immunization on day 1, 14 and 28, BSA IgA titer were tested for 42 days (data from table 17), purple glutinous rice bran (PGRB), γ-oryzanol (GON).

4.5.3 Effect of dietary of purple glutinous rice bran on BSA IgM titer

Referred to 4.5.1, the results showed that no significant differences among treatments on day 1 ($p>0.05$). Nevertheless, on day 10 of the experiment, there was significant difference on the average of the $\log_2$ BSA IgM titer of group I higher than group II, III and IV: 0.1277 ± 0.0104, 0.0873 ± 0.0127, 0.0643 ± 0.0066 and 0.0868 ± 0.0081 ($p<0.05$) respectively. On day 14 of the experimentation, there was significant difference on the average of the $\log_2$ BSA IgM titer of group I and II higher than group IV: 0.0324 ± 0.0067, 0.0301 ± 0.0061 and 0.0089 ± 0.0025 ($p<0.05$) respectively, but no significant difference in group I, II and III ($p>0.05$). On day 21 it was found that the mice in group I and II had a significantly different $\log_2$ BSA IgM titer than the mice in group III and IV: 0.1185 ± 0.0102, 0.1108 ± 0.0183, 0.0488 ± 0.0081 and 0.0668 ± 0.0100 ($p<0.05$) respectively. On day 28 there was significant difference on the average of the $\log_2$ BSA IgM titer of group I higher than group II, III and IV: 0.0704 ± 0.0089, 0.0410 ± 0.0061,

0.0162 ± 0.0037 and 0.0261 ± 0.0052 ($p<0.05$) respectively. On day 42, which was the last day of the experiment, it was found that the mice in group II had significant difference $\log_2$ BSA IgM titer than the mice in other groups: 0.0543 ± 0.025 ($p<0.05$). In summary, it was found that the mice in group I and II had a tendency higher level of the $\log_2$ BSA IgM titer than other groups and the mice in group IV had a tendency lower level of $\log_2$ BSA IgM titer than other groups . The results are shown in Table 18 and Figure 43.

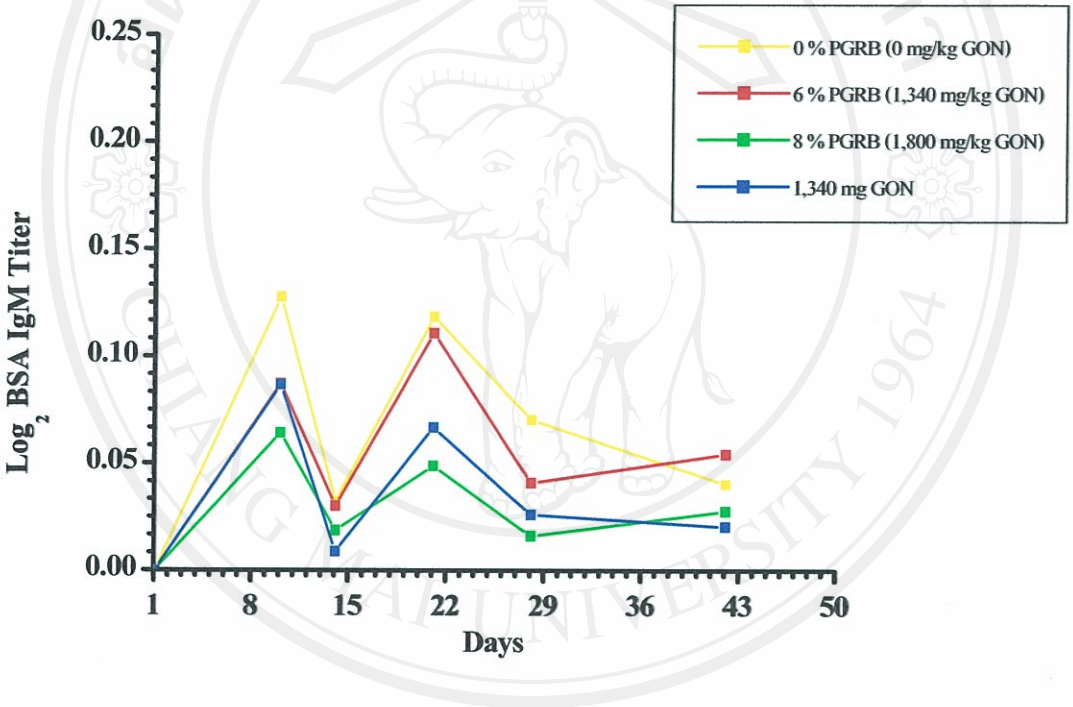

Figure 43. Effect of dietary purple glutinous rice bran on BSA IgM titer (data from table 18) purple glutinous rice bran (PGRB), γ-oryzanol (GON).

Table 18. Effect of dietary purple glutinous rice bran on BSA IgM titer

Dietary treatments	Log_2 OD (mean ± S.E)					
	Day 1	Day 10	Day 14	Day 21	Day 28	Day 42
0% PGRB	0.0000	0.1277^{a}	0.0324^{a}	0.1185^{a}	0.0704^{a}	0.0403^{b}
	±.0000	±.0104	±.0067	±.0102	±.0089	±.0105
6% PGRB	0.0000	0.0873^{b}	0.0301^{a}	0.1108^{a}	0.0410^{b}	0.0543^{a}
	±.0000	±.0127	±.0061	±.0183	±.0061	±.0086
8% PGRB	0.0000	0.0643^{b}	0.0187^{ab}	0.0488^{b}	0.0162^{c}	0.0276^{b}
	±.0000	±.0066	±.0050	±.0081	±.0037	±.0067
1340 mg/kg	0.0000	0.0868^{b}	0.0089^{b}	0.0668^{b}	0.0261^{c}	0.0204^{b}
γ-oryzanol	±.0000	±.0081	±.0025	±.0100	±.0052	±.0059

Means within column with different superscripts are significantly different ($p<0.05$).

Purple glutinous rice bran (PGRB)

4.5.4 Effect of dietary purple glutinous rice bran on area under BSA IgM titer curve

The time series of BSA IgM titer obtained from each mouse were graphically plotted and area under BSA titer curve were calculated by Autocad software (2000i).

The effect of diet of purple glutinous rice bran on the immune response of male mice was investigated. The average of area under BSA IgM titer curve was used as an indicator. The result showed significant difference among treatments, there was significant difference on the average of area under BSA IgM titer curve of the negative control group higher than group II, III and IV: 0.268 ± 0.2275, 0.217 ± 0.1639, 0.115 ± 0.0927 and 0.143 ± 0.1253 square units, ($p<0.05$) respectively. The highest value found in group I: 0.268 ± 0.2275 square units and the lowest value found in group III: 0.115 ± 0.0927. The areas under BSA IgM titer curve are shown in Table 19 and Figure 44.

Table 19. Effect of dietary purple glutinous rice bran on area under BSA IgM titer curve

Groups	Dietary treatments	Area under BSA IgM titer curve Mean ± S.E. Square unit
I	0% PGRB (0 mg/kg GON)	0.268 ± 0.2275^{a}
II	6% PGRB (1,340 mg/kg GON)	0.217 ± 0.1639^{b}
III	8% PGRB (1,800 mg/kg GON)	0.115 ± 0.0927^{c}
IV	1340 mg/kg γ-oryzanol	0.143 ± 0.1253^{c}

Means within column with different superscripts are significantly different ($p<0.05$).

Purple glutinous rice bran (PGRB), γ-oryzanol (GON)

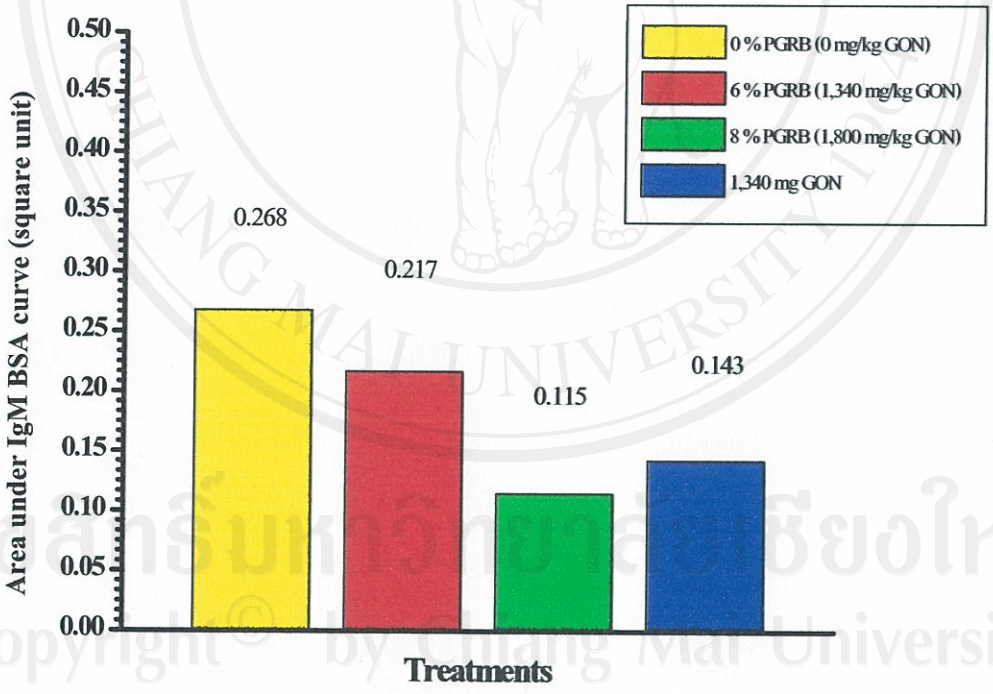

Figure 44. Effect of dietary purple glutinous rice bran on area under BSA IgM titer curve in male mice following BSA immunization on day 1, 14 and 28, BSA IgM titer were tested for 42 days (data from table 19), purple glutinous rice bran (PGRB), γ-oryzanol (GON).

4.5.5 Effect of dietary purple glutinous rice bran on BSA IgG titer

Referred to 4.5.1, the results showed that no significant differences among treatments on day 1 ($p>0.05$). Nonetheless, on day 10 of the experiment, the mice in the IV had a $\log_2$ IgG titer significantly higher than the mice in group I: 0.1524 ± 0.0010 and 0.1041 ± 0.0127 ($p<0.05$) respectively. However, there was no significant difference on the average of the $\log_2$ BSA IgG titer between group IV, II and III. On day 14 of the experimentation, there was no significant difference on the average of the $\log_2$ BSA IgG titer among groups ($p>0.05$). On day 21 of the experimentation, there was significant difference on the average of the $\log_2$ BSA IgG titer of group II and III higher than group I and IV: 0.5835 ± 0.0342, 0.5710 ± 0.0293, 0.3997 ± 0.0301 and 0.4660 ± 0.0068 ($p<0.05$) respectively, but there was no significant difference on the average of the $\log_2$ BSA IgG titer between the group I and IV. On day 28 of the experimentation, there was significant difference on the average of the $\log_2$ BSA IgG titer of group II and III higher than group I and IV: 1.0899 ± 0.0369, 1.1787 ± 0.0378, 0.9053 ± 0.0609 and 0.9485 ± 0.0396 ($p<0.05$) respectively, but there was no significant difference on the average of the $\log_2$ BSA IgG titer between the group I and IV. On day 42, which was the last day of the experiment, it was found that the mice in group III had a significantly different $\log_2$ BSA IgG titer than the mice in group I: and IV, 1.226 ± 0.0656, 1.0587 ± 0.0540 and 1.1003 ± 0.0074 ($p<0.05$) respectively. However, we found no significant difference was found in supplemented groups. In summary, it was found that the mice in the supplemented groups had a higher level of the $\log_2$ BSA IgG titer than negative control group. The results are shown in Table 20 and Figure 45.

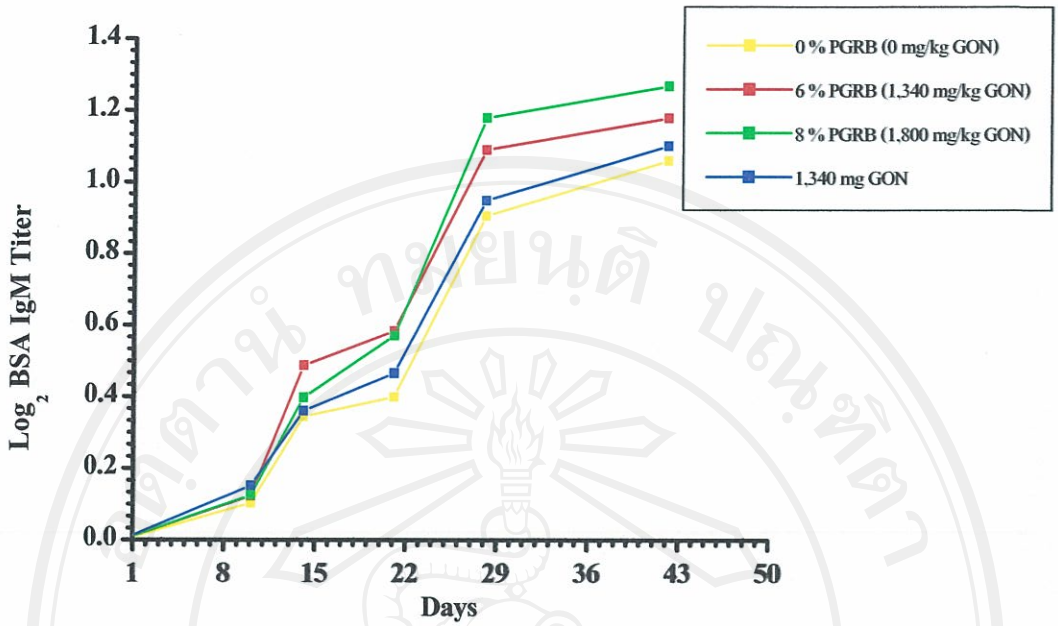

Figure 45. Effect of dietary purple glutinous rice bran on BSA IgG titer, (data from table 20) purple glutinous rice bran (PGRB), γ-oryzanol (GON).

Table 20. Effect of dietary purple glutinous rice bran on BSA IgG titer

Dietary treatments	Log_2 OD (mean ± S.E)					
	Day 1	Day 10	Day 14	Day 21	Day 28	Day 42
0% PGRB	0.0000 ±.0000	0.1041^{b} ±.0127	0.3447 ±.0377	0.3997^{b} ±.0301	0.9053^{b} ±.0609	1.0587^{c} ±.0540
6% PGRB	0.0000 ±.0000	0.1244^{ab} ±.0152	0.4876 ±.1057	0.5835^{a} ±.0342	1.0899^{a} ±.0369	1.1778^{abc} ±.0588
8% PGRB	0.0000 ±.0000	0.1254^{ab} ±.0167	0.3982 ±.0744	0.5710^{a} ±.0293	1.1787^{a} ±.0378	1.2667^{a} ±.0656
1340 mg/kg γ-oryzanol	0.0000 ±.0000	0.1524^{a} ±.0010	0.3605 ±.0133	0.4660^{b} ±.0068	0.9485^{b} ±.0369	1.1003^{bc} ±.0074

Means within column with different superscripts are significantly different ($p<0.05$).

Purple glutinous rice bran (PGRB)

4.5.6 Effect of dietary purple glutinous rice bran on area under BSA IgG titer curve

The time series of IgG BSA titer obtained from each mouse were graphically plotted and area under BSA titer curve were calculated by Autocad software (2000i).

The results showed that the mice in group III (the mice which had been fed with 8 % purple glutinous rice bran) had a tendency to a higher area under BSA IgG titer curve than other group. However, it was a slightly different from the mice in group II. Nevertheless, the mice in group III had a significantly different area under BSA IgG titer curve than the mice in negative control, 2.470 ± 0.1710 and 1.887 ± 0.1184 ($p<0.05$) respectively. The lowest area under BSA IgG titer curve was found in group I (negative control group), 1.887 ± 0.1184 square units. The areas under BSA IgG titer curve are shown in Table 21 and Figure 46.

Table 21. Effect of dietary purple glutinous rice bran on area under BSA IgG titer curve

Groups	Dietary treatments	Area under BSA IgG titer curve Mean ± S.E. Square unit
I	0% PGRB (0 mg/kg GON)	1.887 ± 0.1184^{c}
II	6% PGRB (1,340 mg/kg GON)	2.318 ± 0.1391^{ab}
III	8% PGRB (1,800 mg/kg GON)	2.470 ± 0.1710^{a}
IV	1340 mg/kg γ-oryzanol	1.948 ± 0.0509^{bc}

Means within column with different superscripts are significantly different ($p<0.05$).
Purple glutinous rice bran (PGRB), γ-oryzanol (GON)

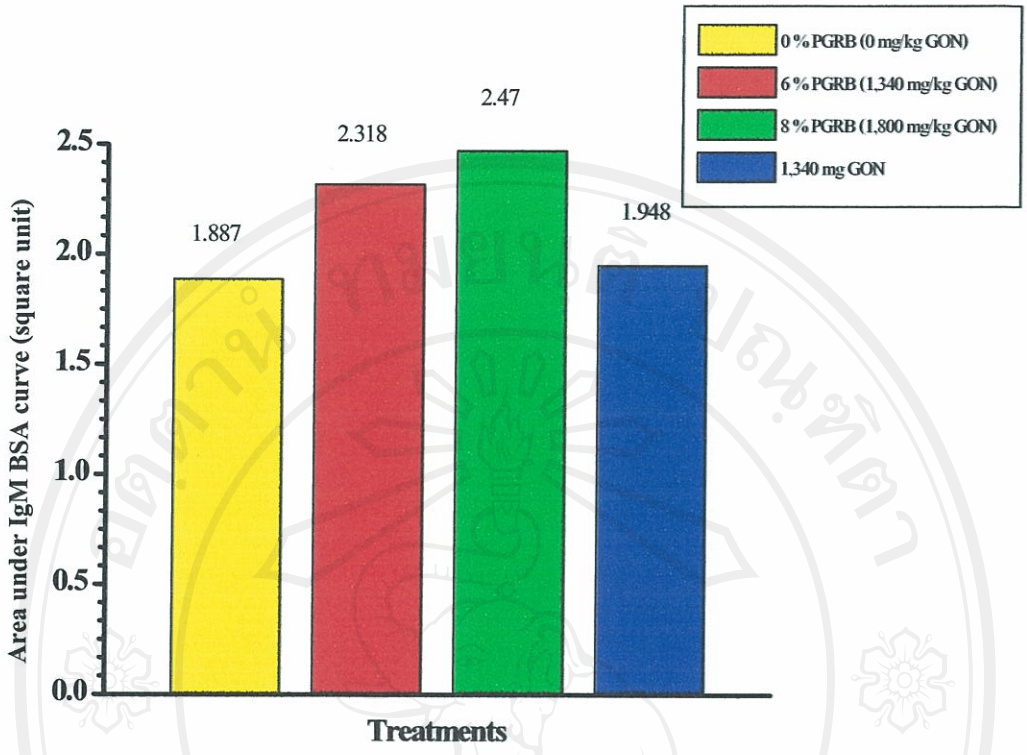

Figure 46. Effect of dietary purple glutinous rice bran on area under BSA IgG titer curve in male mice following BSA immunization on day 1, 14 and 28, BSA IgG titer were tested for 42 days (data from table 21), purple glutinous rice bran (PGRB), γ-oryzanol (GON).

4.7 Intra and inter coefficient assay

The evaluation of the intra and inter coefficient assay of Horseradish peroxidase goat anit-mouse IgA, IgM and IgG, there were found at 8.3 %, 6.7 %, IgM 2.8 %, 5.5 % and IgG 4.2 % 8.4 % respectively. It was shown that when evaluated the same sample on the same analytical plate, using the Horseradish peroxidase goat anit-mouse IgA, IgM and IgG, the analytical values were found to be more or less the values of 8.3 %, 2.8 %and 4.2 % respectively. If the sample was evaluated on the different plates, the results would be more or less different in term of values 6.7 %, 5.5 % and 8.4 % respectively.