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METHODOLOGICAL DISCUSSION ON THE RESULT OF
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By

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Recently in Japan various methods of feeding trials are being attempted to determine the effect of antibiotics, A. P. F., vitamin B₁₂ etc. on domestic animals although no general agreement has been reached. It is not always possible to confirm their effect.

With concern to the above mentioned, one of the writers (Kametaka) published a paper entitled "Methodology of the Feeding Trial¹⁾," and herein is presented the results obtained in the pig-growing trial with the application of the paired-feeding method²⁾.

Experimental procedure

1) Pigs: Eight *Middle Yorkshire* pigs of the 13 born at the Kawatabi Farm of our faculty, were selected as materials. Four pigs, male and female, 74 days old, were paired on weight, sex, condition and probable outcome. One of each pair was fed with the check ration, while its pair mate was fed with the test ration containing a vitamin B₁₂ supplement.

2) Feeds: The trial aimed to determine the effect of a special fermented fodder on the growth of pigs. It was found by using *Euglena*, that this special fodder contained vitamin B₁₂ from 15 to 25 γ per 100 g.

Basal mixture consisted of defatted rice bran, wheat flour middlings, wheat bran, soybean oil meal and fish meal. Besides this, fresh red clover, calcium carbonate and common salt were mixed daily, respectively in the proportions of 8, 1.5 and 0.5 per cent of this mixture. These feeds were analysed chemically, as shown in Table 1.

The proportions of soybean oil meal and fish meal were mixed variously to change the percentage of protein content in the ration as the pigs increased

Table 1. Chemical composition of feeds used (%).

Feeds	Dry substance	Crude protein	Crude fat	Crude fiber	Ash	Nitrogen free extract	True protein
Defatted rice bran	89.79	18.09	6.03	10.17	17.10	38.40	16.42
Wheat flour middlings	86.78	10.28	2.21	24.10	8.19	42.00	9.91
Wheat bran	89.86	14.18	4.06	9.13	6.17	56.32	13.53
Soybean oil meal	89.77	40.27	8.77	5.13	6.18	28.79	38.91
Fish meal	89.49	60.49	9.09	0.81	14.07	5.03	56.81
Fermented fodder	89.49	7.19	1.51	31.19	13.83	35.77	3.24
Red clover	20.00	2.37	0.70	7.33	1.07	7.31	1.32

their body weight.

The percentages fed are given in Table 2. The content of crude protein in the ration given (Table 2) is calculated from the results of chemical analysis.

Table 2. Variation in the proportion of the ration at different live weights.

Feeds	Proportion in which feeds were mixed for pigs weighing					
	42kg or less		42 to 60kg		Over 60kg	
	Test ration	Check ration	Test ration	Check ration	Test ration	Check ration
	%	%	%	%	%	%
Defatted rice bran	15	20	10	10	26	11
Wheat flour middlings	30	40	40	60	50	65
Soybean oil meal	20	10	10	5	4	2
Fish meal	5	10	5	10	5	10
Fermented fodder	10		10		10	
Wheat bran	20	20	25	15	7	6
Total	100	100	100	100	100	100
Crude protein	20.5	20.6	17.3	18.1	15.0	15.4

3) Feeding and caring: The pigs were fed twice daily in a wood trough which was provided for each individual crate. The feed allowances were weighed to one-twentieth of a kilogram before feeding. An amount of water equal to three or four times that of the feed was poured on it to prevent waste. Feed refusals were taken away, dried and weighted.

The feed allowances were decided according to the body weight, the pigs being fed 1/18, 1/20 and 1/25 of their weight up to 32 kg., 45 kg. and 60 kg. respectively, and 1/30 of its weight when exceeding 60 kg. Individual weights of the pigs were taken at weekly intervals and the feed allowances for the following week were adjusted in accordance with the weight gains made during the week.

Each pig was kept on the ground floor of their individual crates, one half of which rice-straw was strewn. The experiment commenced on June 29, 1951 and continued until the smallest of the light pigs had attained a final weight of 50 kg.

Results and Discussion

The results of the experiment are summarized in Tables 3, and 4. The growth curve as the average body weight of the four pigs in the test and the check group is given in Fig 1.

Table 3 shows that each pig of the test group consumes more feed than that of the check group and gains more weight.

Fig. 1 shows that the average body weight of the four pigs in the test group always exceeded that of the check group. The crude fiber content of each ration must be considered with regard to weight increase. For example, the ration

Table 3. Weight, gains, and feed consumption of four pairs of pigs, one of each pair being on the test and the other on the check ration.

Item	Pair 1		Pair 2		Pair 3		Pair 4		Average	
	test pig No. 17(♂)	check pig No. 18(♂)	test pig No. 20(♂)	check pig No. 21(♂)	test pig No. 12(♀)	check pig No. 14(♀)	test pig No. 15(♀)	check pig No. 16(♀)	test pig	check pig
Final weight kg.	53.3	50.0	55.0	54.2	57.3	51.0	67.0	54.3	58.15	52.38
Initial weight kg.	11.6	11.9	13.0	13.4	13.0	11.7	13.6	13.5	12.8	12.6
Total gain kg.	41.7	38.1	42.0	40.8	44.3	39.3	53.4	40.8	45.35	39.78
Period in test days.	155	155	155	155	155	155	155	155	155	155
Gain ratio %	360	320	323	204	341	336	393	302	354	316
Average daily gain kg.	0.27	0.25	0.27	0.26	0.29	0.25	0.34	0.26	0.29	0.26
Total feed eaten kg.	218.74	204.84	235.70	233.54	233.51	213.50	265.72	223.51	238.42	218.85
Average ration kg.	1.41	1.32	1.52	1.51	1.51	1.38	1.71	1.44	1.54	1.41
Feed consumed per kg of gain kg.	5.22	5.38	5.61	5.72	5.27	5.27	4.94	5.48	5.26	5.50
Gain kg per kg of feed eaten kg.	0.19	0.19	0.18	0.17	0.19	0.18	0.20	0.18	0.19	0.18
Gain kg per kg of crude protein eaten kg.	0.99	0.96	0.95	0.90	0.97	0.91	1.14	0.94	1.01	0.93

Table 4. Weekly weights (kg.) of four pairs of pigs, one of each pair being on the check and the other on the fermented fodder (test) ration.

Week	Pair 1		Pair 2		Pair 3		Pair 4		Average	
	Test pig 17 (♂)	Check pig 18 (♂)	Test pig 20 (♂)	Check pig 21 (♂)	Test pig 12 (♀)	Check pig 14 (♀)	Test pig 15 (♀)	Check pig 16 (♀)	Test pig	Check pig
0	11.6	11.9	13.0	13.4	13.0	11.7	13.6	13.5	12.8	12.6
1	12.6	11.7	14.8	11.0	14.9	12.8	14.8	13.9	14.1	12.3
2	13.0	12.2	14.9	14.0	14.7	13.5	15.0	14.3	14.4	13.5
3	15.6	14.6	17.1	16.8	16.7	15.5	17.6	16.2	16.8	15.8
4	16.4	15.1	18.1	18.5	18.0	16.1	19.2	17.6	17.9	16.8
5	17.9	16.8	20.5	21.0	20.0	18.0	22.5	20.0	20.2	19.0
6	20.0	13.2	21.5	22.5	22.5	20.0	25.0	21.5	22.3	19.3
7	21.3	19.0	23.3	24.5	25.5	21.5	27.7	23.5	24.4	22.1
8	24.0	20.0	25.0	27.5	27.0	22.5	31.0	23.8	26.8	23.5
9	25.5	21.3	27.5	27.5	30.0	24.2	34.0	25.4	29.3	24.6
10	28.0	24.2	29.5	29.9	32.0	26.5	37.5	26.0	31.8	26.7
11	30.5	25.5	32.0	32.0	36.0	28.0	41.0	28.5	34.9	28.5
12	31.5	27.5	34.7	32.4	37.0	30.0	43.0	30.4	36.6	30.1
13	33.0	30.5	36.0	36.0	40.0	32.5	46.5	34.5	38.9	33.1
14	37.1	33.1	39.9	35.6	43.2	35.1	50.2	36.6	42.6	35.1
15	39.9	36.2	43.0	38.9	45.0	38.0	52.3	40.7	45.1	38.5
16	40.3	38.9	43.2	41.0	45.4	38.9	54.1	41.2	45.8	40.0
17	43.0	37.9	45.4	44.5	47.5	42.7	58.2	45.0	48.5	42.5
18	43.9	40.6	45.4	45.6	49.1	42.8	61.0	46.0	49.9	43.8
19	46.2	42.9	47.1	48.2	50.0	45.1	61.5	48.6	51.2	46.2
20	47.0	44.9	48.0	48.0	53.0	46.2	64.4	50.0	53.1	47.3
21	50.0	47.5	51.7	51.5	56.0	49.0	66.0	53.5	55.9	50.4
22	53.3	50.0	55.0	54.2	57.3	51.0	67.0	54.3	58.2	52.4

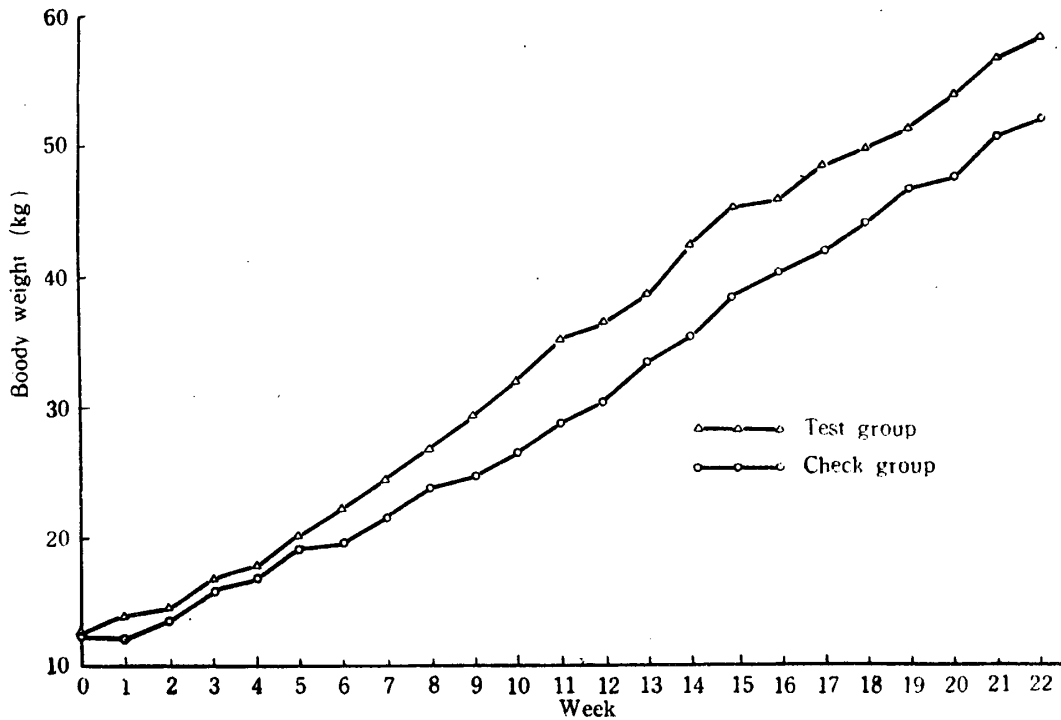
in the check group, fed to pigs weighing less than 42 kg. contained 14 per cent of crude fiber, while that of the test group contained 13 per cent. Accordingly, it may be admitted qualitatively that this fermented fodder is effective to the growth of pigs.

The above stated was quantitatively verified by statistical treatment of the data with the F-test by Snedecor's method, with the t-test for paired differences³⁾ and with another t-test for standard error of each group by Cochran and Cox's method.

Judging from the values shown in Table 5, it is clear that the difference between the two groups is significant at the 12th week with the F-value and at the 2nd week with the t_1 value.

It is not significant every week with the t_2 value, and only two F values and four t_1 values are significant during the period of 22 weeks.

Fig. 1 Growth curve, as indicated by the average body weight of four pigs.



The average gain per week per kg. of feed eaten in the two groups is calculated and shown in Fig 2.

Figure 2 suggests that the gains in weight are not uniform. When weight gains of each pig are shown with curves, they are found to be variable and complicated. In general, however, it seems that a small gain in weight is followed by a large one, thus showing rather regular unevenness in the curve. Particularly, it is found that the unevenness of the weight gain decreases with growth, showing a sort of decay curve.

The mean values (Fig. 1), of the body weight increase of the four pigs in the two groups show a large difference at the 12th week, and are significant statistically. Judging from the average curves shown in Fig. 1, thereafter to the end of the trial, the difference between the two mean values seems to be almost the same, whereas it is insignificant as shown in Table 5.

From the above stated and from the weekly variation of body weight increase shown in Fig. 2, it appears that the growth variation of the individual pig is very great, that is, the ratio increase of body weight in one week compared with that of the previous one is variable. In general, therefore, both variance and error become large even though no significant difference is found.

Though it is possible with the above approval to discuss the difference between the two groups every week, it can not be claimed that the growth in the test

Table 5. Values obtained with the F-test and t-test, in which t_1 shows the value for paired differences and t_2 the value for standard error of each group.

Week	F	t	
		t_1	t_2
0	— (0.07)	— (0.46)	— (0.31)
1	3.48	2.34	1.78
2	1.86	8.31*	1.38
3	2.34	4.21*	1.56
4	1.34	1.19	1.15
5	— (0.91)	3.06	— (0.97)
6	1.58	1.87	1.22
7	1.57	1.83	1.27
8	2.25	1.60	1.60
9	4.29	2.59	2.07
10	4.50	2.06	2.24
11	5.56	2.44	2.36
12	6.07*	2.38	2.43
13	3.46	2.32	1.88
14	6.61*	3.42*	2.56
15	5.57	3.63*	2.36
16	3.58	2.20	1.90
17	2.60	2.32	1.63
18	2.24	1.87	1.49
19	1.75	1.67	1.32
20	1.98	1.82	1.40
21	2.10	2.03	1.43
22	3.15	2.23	1.78

* Significant—exceeds the 5 per cent point.

ration was significantly superior to the check throughout the trial of 22 weeks.

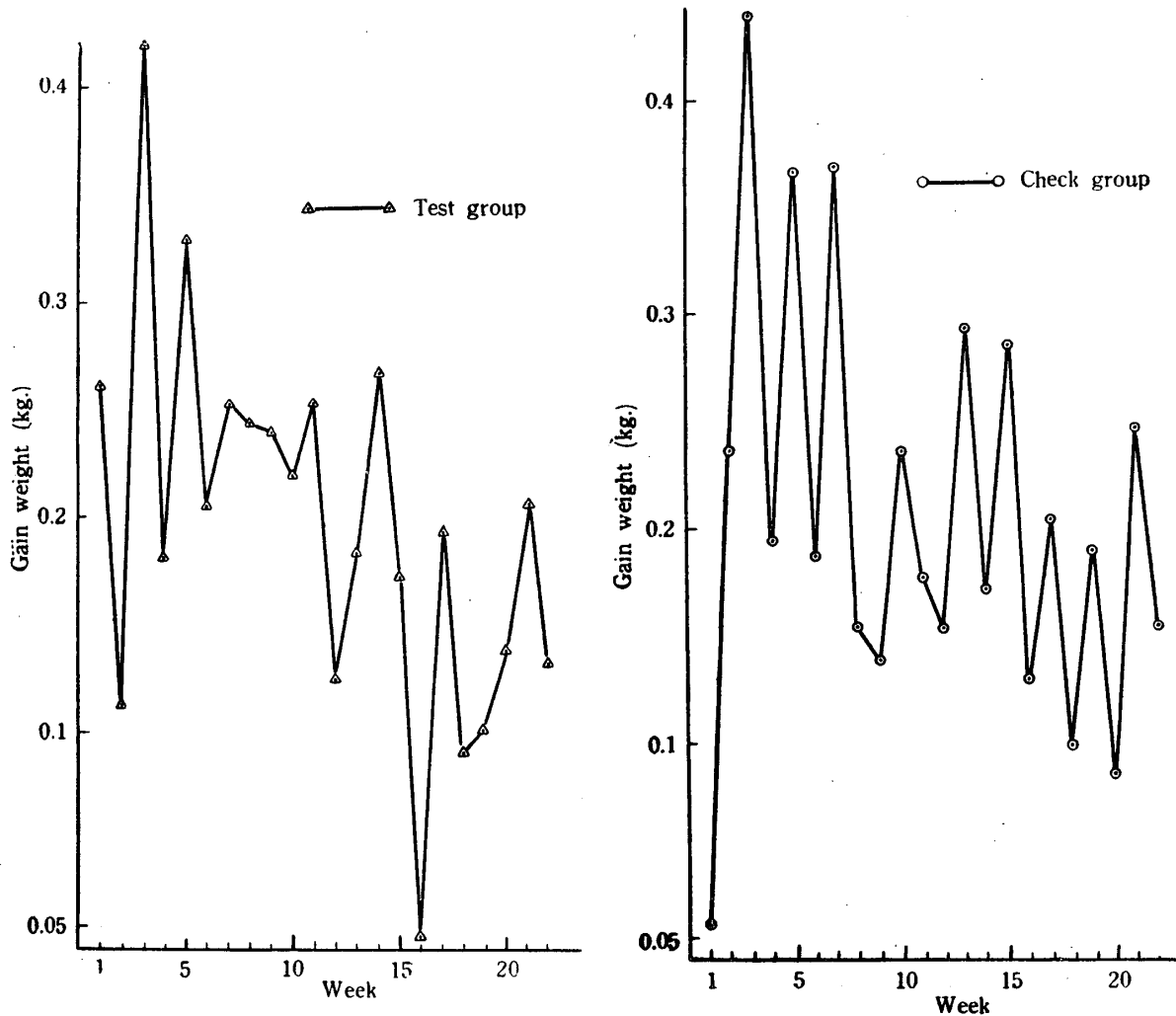
With the premise of the correlation between the body weight gain and the passing of weeks, the value of F, according to the result obtained by the analysis method of covariance⁴⁾ is 102.9, which is highly significant.

Source of variation	D. f.	Sum of squares and products			Errors of estimate		
		Sy ²	Sxy	Sx ²	Sum of squares	D. f.	Mean square
Total	43	7900.32	1771	3675.2	273.56	42	
Groups	1	195.62	0	0			
Error	42	7704.70	1771	3675.2	77.94	41	1.90
Difference for testing adjusted means					195.58	1	195.58**

$$F=195.58/1.90=102.9$$

**Highly significant-exceeds the 1 per cent point.

Fig. 2 Average weekly gain of four pigs per kg. of feed eaten.



This approval is, however, open to question whether it is correct to treat these estimated values in such manner, in other words, whether these estimated values in the biological test are independent of each other.

It is certain that each body increases its weight with time and thus its gain is continuous, so the above statistical analysis method may be unsuitable in this case.

To compare the average gain curve in the test ration with that in the check, a statistical analysis is made, by which it is known whether there is any difference in time between the two types of progress.

The result of the analysis of stochastic series⁵⁾ is shown in Table 6.

It is suggested in this table that the more increases P—the numbers of weekly value of body weight which are picked up in this analysis, the more decreases

$n-p+1$ —the degrees of freedom towards the assumption of variation and consequently that it becomes insufficient to decide on the significance of difference, depending upon the decrease of the preciseness.

Table 6. Analysis of stochastic processes

Every week (No. of week)	p	$n-p+1$	$F^{(1)}$
7 (7, 14, 21)	3	4	8.36*
6 (6, 12, 18, 22)	4	3	10.41*
5 (5, 10, 15, 20)	4	3	9.99*
4 (4, 8, 12, 16, 20)	5	2	1.82
3 (3, 6, 9, 12, 15, 18)	6	1	4.41
11 (11, 22)	2	5	3.54

* Significant-exceeds the 5 per cent point.

$$1) \dots F = \frac{n-p+1}{p} T^2 \text{ (6)}$$

where n means the degrees of freedom (N_1+N_2-2) and p the number of point in time series.

Thus, the problem in such a feeding trial is how to treat the experimental data obtained. With regard to solving this problem, it may be not necessarily be said that the difference is definitely found by the increase of estimated value. In our experiment it seems satisfactory for discussing the significance of difference by using three or four of the 22 weekly values for the analysis.

Against that, it is shown in Table 6 that the difference between the two rations is insignificant when using only two weekly values out of the total 22 in the analysis. It is doubtful whether only two weekly values can represent the total experimental data, and accordingly it may be necessary that four instead of two weekly values should be taken in the analysis.

Even though three or four weekly values of the total 22 are employed in the analysis, the questionarises as to what week during the trial should the estimated value be collected.

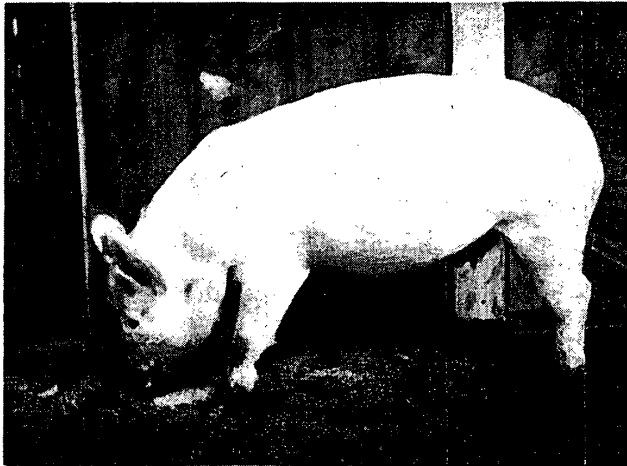
The data shown in Table 6, were collected at regular intervals as indicates therein.

Whether the results of analysis will differ according to the method of data collection has not been confirmed.

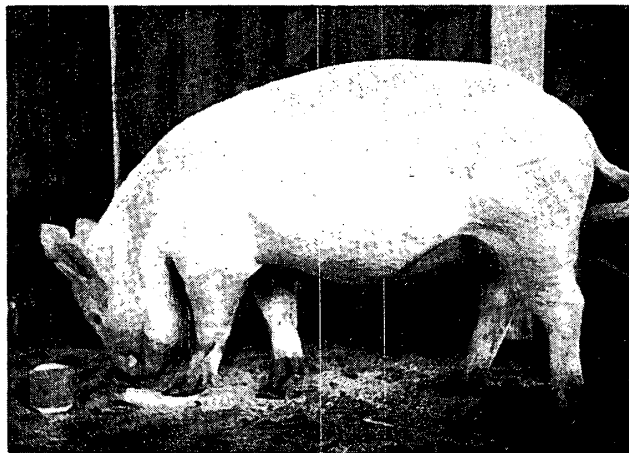
From the obtained result, the number of times necessary for estimation of body weight during the trial to find the significance of difference between the

2 groups can be known.

A photograph of the pair No. 4, AOB A 26, of which one female pig No. 15 is fed with the test ration and the male No. 16 is fed with the check ration, were taken at the end of the trial. They are shown in the following picture.



No. 15 test ration fed



No. 16 check ration fed

Summary

Herein is reported a methodological discussion of the results obtained by the pig-growing trial with the application of the paired-feeding method.

The average body weight gain of four pigs in two groups every week during the trial is subject to the F- and t-test to find the significance of difference between the two groups. A special analysis was made in which the two types of time series in progress are compared with each other as the total curve.

It is concluded that it may be necessary in our trial, using four pairs, to estimate the body weight four times during the 22 weeks at regular intervals, to discuss and find the significance of difference.

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