THE INFLUENCE OF DIETARY CAROTENE LEVELS ON SEMEN PHOSPHATASES IN FARM ANIMALS

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INTRODUCTION

Physiological significance of phosphatases has attracted considerable attention during the last decade. Elevated serum phosphatase is generally accepted as indicating bone disease or liver damage (40). Although phosphatases are of paramount importance in nutrition they are not fully appreciated as entities in nutrition. They are undoubtedly the more important of the enzymes nutritionally, physiologically and pathologically. Semen is a rich source of this enzyme, and several investigators have demonstrated the influence of diet on serum phosphatases. But practically no work has been done to determine the possible role of diet on phosphatases of semen.

From the standpoint of normal reproduction, vitamin A occupies an important place in the diet. It has been proved beyond doubt that an inadequate amount of vitamin A adversely affects fertility of farm animals.

The present investigation was undertaken to determine the influence of dietary carotene, the precursor of vitamin A, on acid and alkaline phosphatases of semen.

REVIEW OF LITERATURE

The acid and alkaline phosphatases belong to a larger group of enzymes known as phosphatases which catalyze the transformations of organic phosphates (Doyle et al., 11). In bull semen the occurrence of acid and alkaline phosphatases was reported by Reid et al. (42) and Haq and Mullen (22). Semen owes its powerful phosphatase activity mainly to seminal plasma which carries several different dephosphorylating enzymes derived from male accessory organs of reproduction. In addition to the two aforementioned enzymes, seminal plasma contains 5 nucleotidases, a pyrophosphatase and several adenosine triphosphatases as observed by Mann (37, p. 117). An observation that the phosphatase activity of male urine is usually higher than that of women led Kutscher and Wolbergs (28) to examine the phosphatase in semen and in the prostate gland. They soon found that semen and prostate are among the richest sources of acid phosphatase in the human body.

Acid phosphatase is an important secondary male sex characteristic. Investigations by Gutman and Gutman (17) have shown that the level of enzyme in the human prostate is low in childhood but increases rapidly at puberty; thus the activity expressed in King and Armstrong units per gram prostate tissue was 1½ units at four years of age,

73 units at puberty and 522 to 2284 units in adult men. A similar relation to age was observed in monkeys and dogs; in both these species administration of androgenic hormones to immature males stimulates considerably the output of the enzyme from the prostate gland (Gutman and Gutman, 18; Huggins and Russel, 26). A certain correlation appears to exist in adult men between the level of acid phosphatase in semen and androgenic activity (Gutman and Gutman, 19; Engberg et al., 12). An important addition to our knowledge of the physiological function of acid phosphatase in seminal plasma has been the discovery made by Lundquist (34) that freshly ejaculated human semen contains phosphorylcholine which on ejaculation is rapidly dephosphorylated by the acid phosphatase to free choline and orthophosphate. Of considerable interest also is the finding that the acid phosphatase exhibits in vitro a distinct transferase activity (Green and Meyerhof, 15).

Alkaline phosphatase like the "acid" enzyme is widely distributed in male accessory organs. Human semen with its conspicuously high level of acid phosphatase has a low concentration of alkaline phosphatase. Bull semen on the other hand has only slight acid phosphatase activity but contains more of the alkaline phosphatase (Haq and Mullen, 22; Reid, Ward and Salsbury, 42). This difference between

human and bovine semen is not altogether unexpected, since the bulk of bull seminal plasma is derived not from the prostate but from the seminal vesicle. Alkaline phosphatase has an optimum activity at about pH 9, and is capable of hydrolysing among others 1-phospho-fructose, 6-phospho-fructose and 1:6-diphosphofructose. The researchers indicate that this activity may represent an essential step in the process of fructose formation and secretion by accessory organs (Mann and Lutwak Mann, 37).

Diet is reported to affect phosphatase levels (Tyler, 53; Lawrie and Yudkin, 31; Allison, 2). Lawrie and Yudkin (31) studied the effect of varying proportions of dietary protein, fat, and carbohydrate on alkaline phosphatase of the small intestine of the rat and found that the diet containing 55 or 70% sucrose produced significantly lower amounts of enzyme than the diet in which sucrose was replaced by fat or protein. Ross and Batt (45) in their investigation revealed alkaline phosphatase in rat liver to be sensitive to variations in proportion of casein and dextrose in the diet. Tuba et al. (54) showed that in weanling rats fed a diet containing 0, 5, 10, 30 or 91% casein, serum alkaline phosphatase showed significant correlation with fat intake and not with casein consumption.

Weil and Russel (56) in their experiment with rats demonstrated that when food was free of alcohol-ether soluble fraction plasma phosphatase was lowered. Allison (2) demonstrated diminished alkaline phosphatase activity in all tissues, and bone lesions which are indistinguishable from those of rickets.

Shivachman and Gould (48) reported lowering alkaline phosphatase in guinea pigs on scorbutogenic diet. Ludwig (33) revealed that alkaline phosphatase was reduced in the epiphyseal junction of the knee joint of young rats fed a vitamin A deficient diet. Feeding high vitamin levels produced an increase alkaline phosphatase in bones where epiphyseal junction had previously been ossified.

Rosenthal et al. (43) observed in protein depleted rats fasted four days that liver arginase increased an average of 58% and liver alkaline phosphatase decreased 58%. Miller (38) in his investigation with fasted rats demonstrated a loss of rat liver catalase, alkaline phosphatase and xanthine dehydrogenase.

Many factors are identified as affecting enzyme activity (Bodansky, 6; Sadasivan, 46). Sadasivan (47) is of the opinion that the activity of alkaline phosphatase is influenced by zinc and magnesium ions. Bodansky (5) reports an increase in enzyme activity by certain alpha

amino acids.

Considerable variation was found in both alkaline and acid phosphatase levels of semen of individual bulls, and semen of various bulls, according to Reid et al. (42). No definite pattern of phosphatase concentration accompanied the sequence of ejaculates. In most cases the levels of alkaline phosphatase were maintained more uniformly throughout a series of ejaculates. Haq and Mullen (22), in their investigation of fertile, infertile bulls and bulls with testicular hypo-plasia, observed that phosphatase activity goes down with testicular disease.

MATERIALS AND METHODS

The investigation reported herein includes data from eight dairy bulls and eighteen New Zealand rabbits maintained by the Dairy and Animal Husbandry Department, Oregon State College. In bulls, semen was collected once weekly according to the method described by Lambert and McKenzie (29). The semen thus collected was transferred immediately to a water bath at 5°C. and stored in the refrigerator until dilution. Homogenized and pasteurized milk heated to 95°C. for ten minutes and cooled, was used for dilution of semen. The milk thus heated showed no phosphatase activity which was further confirmed by phosphatase determinations at regular intervals. The eight bulls under experiment include three pairs of identical twins and two unrelated animals. Of the three pairs of identical twins on experiment, two were on carotene schedule and one remaining pair on normal ration supplemented by molasses. The breed and feeding schedules are given in Table 1.

The bull semen collected and stored as described earlier was diluted with the prepared milk to facilitate enzyme determination. To 9.5 ml of processed milk was 0.5 ml of semen to give a dilution of 1:20. One tenth milliliter of the semen so diluted was used for enzyme

TABLE 1

Daily carotene feeding schedule during experimental period

Animal	Breed	Vitamin A intake	Pounds of grain	Pounds of hay	Remarks
BS-1)	Brown	On normal feeding			) Identical
BS-2)	Swiss	supplemented by molasses			) twins
TW-1)	Guernsey	442320 I.U.	5	12	) Identical
TW-2)	Holstein x	42560 I.U.	5	12	) twins
TV-1)	Brown	484120 I.U.	6	14	) Identical
TV-2)	Swiss	45980 I.U.	6	14	) twins
266-B-1	Jersey	353400 I.U.	5	7	)
G-12-B-1	Holstein	36632 I.U.	5	7	) Unrelated

determination to be described later. The dilution rate was arrived at by various dilution trials to suit the range of spectrometric measurements.

The eighteen New Zealand rabbits were assigned to three groups by random numbers and semen collected twice weekly according to the method described by Macirone and Walton (35). The experiment was conducted for about six months, of which the first three months constitute a pre-experimental period and the remaining three months the experiment. During the three months pre-experimental period, the rabbits were fed pelleted feed and during the three months on experiment they were fed a specially designed carotene-free basal ration supplemented with the required carotene level. The composition of the basal ration is given in Table 2.

TABLE 2

Composition of basal ration fed to rabbits during experimental period

Ingredients	Per cent of mixture	Remarks
Rolled barley	25	Vitamin D 100 USP units/lb of feed
Ground oats	40	
Wheat mill run	10	Vitamin C 100 I.U/lb of feed
Linseed meal	22	
Ground limestone	2	
Iodized salt	1	

Vitamin D was provided by irradiated yeast which supplies 9000 USP units per pound of feed; vitamin E in the ration was supplied by "Myvamix" yielding 20,000 IU per pound. The above feed constituents were thoroughly mixed and ground in a feed mixture and grinder. The ground feed was divided into three equal portions to which was added carotene to give the resultant mixture carotene levels of 0, 10, and 20 mg per pound of feed. This served as stock ration for the entire experimental feeding period. The animals were tatooed in the left ear for easy identification and housed in separate cages. They were fed and watered daily, and accurate records of daily feed consumption were kept for individual rabbits. The animals were

closely watched for possible lesions due to vitamin A deficiency. A weekly weight record was maintained throughout the experimental feeding period. Sexual response was noted individually by recording the time taken for first ejaculation. As stated earlier semen collected twice weekly was immediately transferred to a water bath and stored in a refrigerator until dilution. The semen was then diluted with physiologic saline. To 11.8 ml of chilled saline was added 0.2 ml of semen to give a final dilution of 1:60. After thorough mixing, 1 ml of saline diluted semen was used for enzyme determination. "The incubation procedure of the Bodansky method is used for 'alkaline' phosphatase determinations with modifications to permit the use of the Fiske and SubbaRow (see p. 630) for the determination of phosphate liberated; for acid phosphatase the conditions prescribed by Shinowara, Jones, and Reinhart are followed likewise modified to permit the use of the Fiske and SubbaRow phosphate method." (Quotation from Hawk et al., 23, 636 p.) Sodium glycerophosphate buffered with sodium barbital was the substrate used. A standard curve was determined with various wave lengths using a spectrometer. From the above standard curve determinations, wave length of 650 millimicrons was chosen as best suited for measuring enzyme activity. The

result was expressed in Bodansky units, one Bodansky unit being two King and Armstrong units. The Bodansky unit is the number of mg of inorganic phosphate liberated from a substrate incubated at 37°C. for 1 hour. A King and Armstrong unit is mg per cent of phenol liberated. The enzyme activity was calculated according to the method described earlier. In the case of bull semen, the enzyme determinations were carried out using 0.1 ml of diluted semen instead of 1 ml and such calculations were corrected accordingly. In order to ascertain the possible effect milk has on phosphatase activity, simultaneous determinations were carried out with milk and saline diluted semen.

RESULTS

At least ten determinations were carried out from semen collected from each experimental bull at intervals of one week. Mean alkaline and acid phosphatase activity in Bodansky units for the entire period is given in Table 3.

TABLE 3

Mean alkaline and acid phosphatase levels of dairy bull semen in Bodansky units/100 ml semen

Animal	Treatment	Alkaline phosphatase activity	Acid phosphatase activity	Remarks
BS-1	Control	1248	270	On normal ration
BS-2	Control	1127	261	
TW-1	High carotene	477	206	
TW-2	Low carotene	364	149	
TV-1	High carotene	332	180	
TV-2	Low carotene	265	107	
266B-1	High carotene	502	276	
G-13-B-1	Low carotene	156	161	

The above data indicate that acid and alkaline phosphatase activity of dairy bull semen appears to be higher in animals fed high carotene ration than that of low carotene fed bulls. In the case of control bulls no noticeable differences were observed. The data collected were

subjected to statistical treatment and t values are given in Table 4.

TABLE 4

t values for acid and alkaline phosphatase levels in dairy bull semen

Animal	Treatment	Alkaline phosphatase		Acid phosphatase	
		t	df	t	df
BS-1 - BS-2	Control	1.99	16	1.796	16
TW-1 - TW-2	Treated	3.676**	16	1.555	16
TV-1 - TV-2	Treated	1.962	18	2.800*	16
266B-1 - G-13-B-1	Treated	6.500**	16	3.088**	16

* Significant

** Highly significant

The analysis indicates that bulls 266-B-1 and G-13-B-1 showed a significant difference at 1% level, in alkaline and acid phosphatase activity between high and low carotene fed animals. The activity was higher in high carotene fed bulls. A highly significant difference at 1% level was also observed between semen of TW-1 and TW-2 bulls in alkaline phosphatase activity. In case of bulls TV-1 and TV-2 a significant difference was shown between them at 5% level in acid phosphatase. However, alkaline phosphatase level for the high carotene fed bulls was not

nearly as high as the control bulls, indicating a significant effect of other diet ingredients, possibly carbohydrate on semen alkaline phosphatase. But control bulls failed to show any significant difference in alkaline or acid phosphatase activity. The bulls BS-1 - BS-2, TW-1 - TW-2, and TV-1 - TV-2 were identical twins. Alkaline and acid phosphatase in semen showed considerable variation between experimental and control bulls. Wide variation also exists between carotene fed bulls and between ejaculates of the same bull. Alkaline phosphatase showed more repeatability than acid phosphatase. The variations observed were more pronounced when determinations were undertaken with saline diluted semen. When simultaneous determinations were carried out, a tremendous discrepancy was noticed between milk and saline diluted semen. The milk diluted semen gave as much as twofold activity when compared to saline diluted semen. The increased activity could be attributed to magnesium, zinc or other trace minerals or amino acids present in milk which are reported to accelerate the activity of the enzyme.

From the eighteen New Zealand rabbits on experiment, the semen was collected twice weekly and eighteen such determinations were made for each rabbit prior to experimental feeding and during carotene feeding period. The mean enzyme activity in Bodansky units is given in Table 5.

TABLE 5

Mean phosphatase activity of rabbit semen prior to experimental feeding and during carotene feeding period (Bodansky units)

Treatment	Alkaline phosphatase activity		Acid phosphatase activity	
	Prior to experimental feeding period	During the feeding period	Prior to experimental feeding period	During the feeding period
Devoid of carotene	530	557	14.9	7.8
Low carotene	489	442	12.8	4.4
High carotene	503	492	12.7	5.2

The above data show no appreciable difference between the pre-experimental period and the experimental feeding in alkaline phosphatase of semen. A difference significant at the 1% level was observed between groups during carotene feeding period and none prior to the experiment. This shows that carotene feeding did bring about a difference between groups which was absent prior to experimental period. In all groups except the group fed ration devoid of carotene, there was a reduction in alkaline phosphatase activity. This clearly indicates a difference in response while on experiment. The data obtained prior to and during the experiment for each group were subjected to t test and analysis of variance (Table 6).

TABLE 6

Alkaline phosphatase activity in rabbit semen pre-experiment and on experiment subjected to analysis of variance and t test

Treatment	t	df	Analysis of variance Prior to experimental period	df	Analysis of variance During the carotene feeding	df
Carotene-free	0.780	5	F	2 and 272	F	2 and 266
Low carotene	0.501	4	0.718		5.342**	
High carotene	0.385	5				

** Highly significant

This analysis indicates that when the rabbits were on carotene-free, low, or high carotene levels there was no difference between the pre-experiment and experiment groups. During the experiment, rabbit semen showed a significant difference at 1% level between groups in alkaline phosphatase of semen and no difference prior to the experimental period.

Table 5 shows that mean acid phosphatase activity prior to experimental and carotene feeding period appear noticeably different. There was a drastic reduction in acid phosphatase activity of semen of rabbits fed three levels of carotene. But differences were not discernable

between groups on pre-experiment and experiment. The data collected were subjected to analysis of variance and t test and results are given in Table 7.

TABLE 7

Acid phosphatase activity of rabbit semen subjected to analysis of variance and to t test for the pre-experimental and experimental feeding period

Treatment	t	df	Analysis of variance Prior to experimental feeding period	df	Analysis of variance During carotene feeding period	df
Devoid of carotene	3.341*	5	F	2 and 234	F	2 and 234
Low carotene	4.988**	4	1.725		1.848	
High carotene	3.488*	5				

* Significant
** Highly significant

This analysis showed differences within the same group before and during the experiment, but no differences between groups. It is interesting to note that there was a reduction of both alkaline and acid phosphatase activity while on carotene feeding.

A close inspection of the data showed a very interesting phenomenon. There was a steep decrease in enzyme activity towards the close of the experiment. To take

advantage of this information in terms of decreased enzyme activity, the last five observations from all experimental animals were tabulated for prior to and during experimental period.

The purpose was to bring to light any differences brought about at the last phase of the experiment which could be possibly masked when all observations are taken together. Table 8 gives the mean enzyme activity of rabbit semen. The last five pre-experiment and experiment period observations constitute the data.

TABLE 8

Mean alkaline and acid phosphatase activity of five last determinations prior to and during experiment in Bodansky units/100 ml semen

Treatment	Alkaline phosphatase activity		Acid phosphatase activity	
	Prior to experimental period	During experimental period	Prior to experimental period	During experimental period
Devoid of carotene	697	417	16.0	5.2
Low carotene	586	385	10.2	2.9
High carotene	598	405	11.5	3.9

The table clearly shows there was a pronounced difference in alkaline phosphatase activity in rabbit semen between pre-experiment and experimental carotene feeding.

The last five observations of each trial were subjected to statistical treatment and results are given in Table 9.

TABLE 9

Last five observations of alkaline phosphatase activity for pre-experimental and experimental period subjected to statistical treatment

Treatment	t		Analysis of variance pretreatment period		variance during treatment period	
		df	F	df	F	df
Devoid of carotene	3.205*	5	1.278	2 and 82	0.138	2 and 82
Low carotene	2.999*	4				
High carotene	3.493*	5				

* Significant at 5%

The analysis showed a significant difference at 5% level for the same group during pre-experimental and experimental period in alkaline phosphatase activity. This is true in all three groups.

There were no significant differences observed between groups either in pretreatment or during experimental period. As in the previous case there was a reduction of alkaline phosphatase activity while on carotene feeding period. The mean of five last acid phosphatase determinations of pre-experimental and during experimental feeding

given in Table 8 shows a steep decrease in enzyme activity while on carotene feeding period.

The data thus obtained were subjected to appropriate statistical analysis and results are given in Table 10.

TABLE 10

Last five acid phosphatase determinations made at pre-experimental and experimental period subjected to statistical analysis

Treatment	t		Analysis of variance pre-experimental period		Analysis of variance during experimental period	
		df	F	df	F	df
Devoid of carotene	3.535*	5	0.800	2 and 82	0.963	2 and 82
Low carotene	3.277*	4				
High carotene	4.208**	5				

* Significant at 5%
** Significant at 1%

The analysis shows a difference significant at 5% level in no carotene and low carotene fed rabbits between pre-experimental feeding and during carotene feeding period in acid phosphatase of semen. But in the case of rabbits fed high carotene ration, acid phosphatase of semen showed a highly significant difference between pre-experimental and during experimental period. No significant differences were noticed between groups either in

pre-experimental or during experimental carotene feeding in acid phosphatase activity. As noted earlier, there was a tremendous decrease in acid phosphatase activity during the carotene feeding period.

DISCUSSION

It is appropriate to discuss the data presented in the light of earlier work done in the field. While the work in the field is scanty especially pertaining to semen phosphatases, there are a few parallel works which are closely related to the topic under discussion. Ludwig (33) in his investigation observed that alkaline phosphatase was reduced in the epiphyseal junction of the knee joint of young rats fed vitamin A deficient diets for six weeks. Hypervitaminosis produced an increase in enzyme activity in bones whose epiphyseal junctions had previously been ossified and fractured. Other bones showed a reduced phosphatase content. Although the work does not pertain to alkaline phosphatase in semen the general pattern agreed with the result obtained in the present investigation especially with dairy bulls. Reid *et al.* (42) found considerable variation in both alkaline and acid phosphatase levels of individual bulls and ejaculates of various bulls. No definite pattern of phosphatase concentration accompanied the sequence of ejaculates. In most cases the level of alkaline phosphatase was maintained more uniformly throughout a series of ejaculates than acid phosphatase. There was no significant relationship of the

semen alkaline phosphatase to sperm concentration, initial motility, pH and change of pH. A striking dietary influence upon alkaline and acid phosphatase of semen was observed. The ten bulls used in the study constituted two feed groups, of which Group I received a simple unsupplemented concentrate mixture and Group II a complex concentrate mixture supplemented with minerals and vitamins. Both groups received the same average grade of hay. The mean enzyme activity for Group I was 307.3 units for alkaline phosphatase and 141.9 units for acid phosphatase, the units used being King and Armstrong units/100 ml semen.

In the case of animals fed a "complex concentrate mixture" supplemented with minerals and vitamins, the mean enzyme activity was 477.6 units for alkaline phosphatase and 198.8 units for acid phosphatase expressed in King and Armstrong units. The above figures indicate a noticeable difference between groups fed a simple ration and a complex concentrate mixture supplemented with minerals and vitamins. The enzyme activity was much higher in Group II fed a complex concentrate. In addition, the Group I bulls were fed a diet lacking in vitamins A and D, whereas the Group II bulls had adequate vitamins A and D in the diet. This disparity in vitamins A and D in the diet of Group II bulls could possibly be interpreted as the cause for

increased enzyme activity of semen. It is quite possible the factor responsible for increased enzyme activity is vitamin A, as is found in the present investigation.

Although the contention formulated is not quite well substantiated with facts, the work of Reid et al. (42) certainly agrees with the present investigation to the extent that diet does have influence on acid and alkaline phosphatase of semen. Haq and Mullen (22) in their investigation involving normal and sterile bulls and bulls with testicular hypoplasia noticed that alkaline phosphatase and particularly acid phosphatase go down with testicular disease but also run parallel to sperm density and percentage of abnormal sperm. This could be offered as an explanation for reduced alkaline and acid phosphatase, further confirmed by the work of Siddhijai (49) that semen quality of "high carotene" bulls was higher than that of "low carotene" bulls.

Weil and Russel (56) in their investigation on plasma phosphatase activity in relation to fat metabolism in rats observed that when food was free of the alcohol/ether soluble fraction, plasma phosphatase was found lowered. He noticed that the fraction responsible for increased plasma phosphatase is of lipid nature. As vitamin A is associated with fat it is fairly logical to presume that

the fraction responsible for lowered plasma phosphatase is vitamin A. The reasoning, though farfetched, agrees with the present investigation.

Now turning attention to the present, experimental findings indicate that acid and alkaline phosphatase activity of dairy bull semen has an appreciably higher activity in favor of "high carotene" fed animals. The contention is well borne out by the fact that two pairs of bulls on high and low carotene schedules showed a highly significant difference between them in semen alkaline phosphatase, while acid phosphatase differed significantly in two other pairs. The control bulls did not differ in alkaline or acid phosphatase activity. This gives a strong indication in favor of the hypothesis that carotene has a definite influence on semen phosphatases. The high level of alkaline phosphatase activity in the case of the control dairy bulls could be attributed to other dietary ingredients, possibly carbohydrates. It has to be borne in mind that high intake of carbohydrates in the form of molasses will tend to increase alkaline phosphatase levels (41).

In the case of the experiment with rabbits no appreciable difference in alkaline phosphatase shows between pre-experiment and the experiment period. However, a difference significant at 1% was observed between groups

during the carotene feeding period, compared to no difference in the period prior to the experimental period. This shows that carotene feeding did bring about a difference between groups. Further, in all groups except the carotene free group there was a reduction in alkaline phosphatase, which clearly indicates a difference in response while on experimental feeding. This could be partially explained by the fact that there was drastic reduction in food intake and consequently animals lost weight which could mask the effect of carotene altogether. This may explain disparity in the results obtained. Table 11 gives the relationship between weight loss of rabbits and the decreased alkaline enzyme activity. The data given in Table 11 show that loss of weight is directly proportional to reduced alkaline enzyme activity. Although the per cent of weight loss of individual rabbits is not the same as per cent of loss of alkaline enzyme activity, the means for the groups are amazingly similar in all three. This appears to indicate that for a certain per cent loss of body weight there is a similar per cent decrease in alkaline enzyme activity.

TABLE 11

Relationship between weight loss and loss of alkaline enzyme activity

Animal	Group	Per cent loss of wt. at the end of experiment	Mean per cent loss of wt. for the group	Per cent decrease in enzyme activity at the close	Mean per cent decrease for the group
2	No carotene	11		10	
4	"	22		16	
7	"	30	22.3	6	19.3
11	"	26		41	
12	"	29		25	
28	"	16		18	
3	Low carotene	18		45	
5	"	27		20	
9	"	18	19.8	40	21.8
24	"	20		--	
18	"	15		4	
6	High carotene	17		33	
10	"	29		24	
15	"	22	21	15	21.3
16	"	21		--	
17	"	19		27	
23	"	18		28	

The difference in results obtained with dairy bulls and rabbits could be due to the fact that the experiment was not carried out long enough to produce any gross vitamin A deficiency in the rabbits. Further, all rabbits do have a storage of vitamin A in liver and as such a certain time may have to elapse before any deficiency symptoms could possibly be observed. Hence, taking all

observations from the day the experiment began will mask any possible differences brought about at the last phase of the experiment. To remedy the situation only the last five observations were statistically analyzed and these showed a significant difference in alkaline phosphatase activity at the 5% level which substantiates the contention previously expressed.

The acid phosphatase activity of rabbit semen after treatment agrees with the result obtained with dairy bulls, although the differences observed between different carotene levels were on the negative side. In other words, there was a reduction in acid phosphatase activity of semen of rabbits fed on any of the three levels of carotene. This decrease in acid phosphatase activity could be attributed to the weight loss as was also shown in the case of alkaline phosphatase activity.

Alkaline phosphatase is a hydrolytic enzyme producing phosphoric acid and alcohol from the phosphate ester. Axelrod and Meyerhoff and Green (9, p. 60) have independently demonstrated that phosphatase may catalyse the transfer of phosphate residue from one organic molecule to another. The extremely high concentration of phosphatase in semen supports the fact that the enzyme is somehow related to sperm metabolism. The kidney, according

to Danielli (9, p. 69), appears to be the regulating mechanism of phosphatase in blood. It is probable that kidney function might somehow be hampered by vitamin A deficiency, which in turn affects enzyme activity in the blood and subsequently affecting the phosphatase activity of the semen.

SUMMARY AND CONCLUSIONS

The influence of dietary carotene levels on acid and alkaline phosphatases of semen of eight dairy bulls and eighteen New Zealand rabbits was considered.

1) High carotene rations produced more alkaline and acid phosphatase activity in the semen of dairy bulls than those on low carotene intake.

2) One pair of identical twin bulls not on this carotene feeding regime but on usual barn ration failed to show any significant difference in acid or alkaline phosphatase activity in the semen.

3) It appears that for each per cent loss of body weight there is a similar per cent decrease in alkaline enzyme activity.

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A P P E N D I X

Figure 1. Alkaline phosphatase activity in semen of dairy bulls

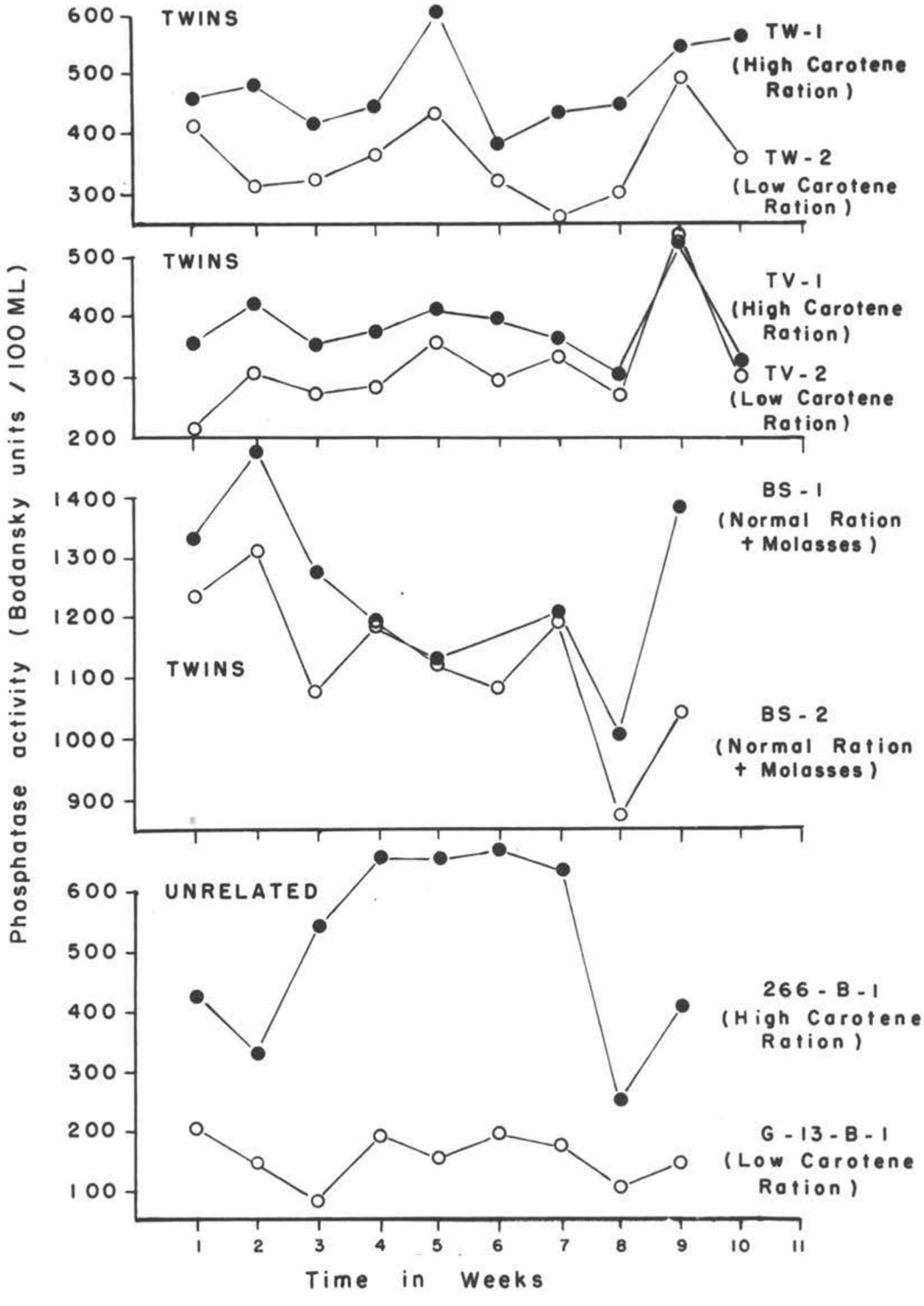

Figure 2. Acid phosphatase activity in semen of dairy bulls

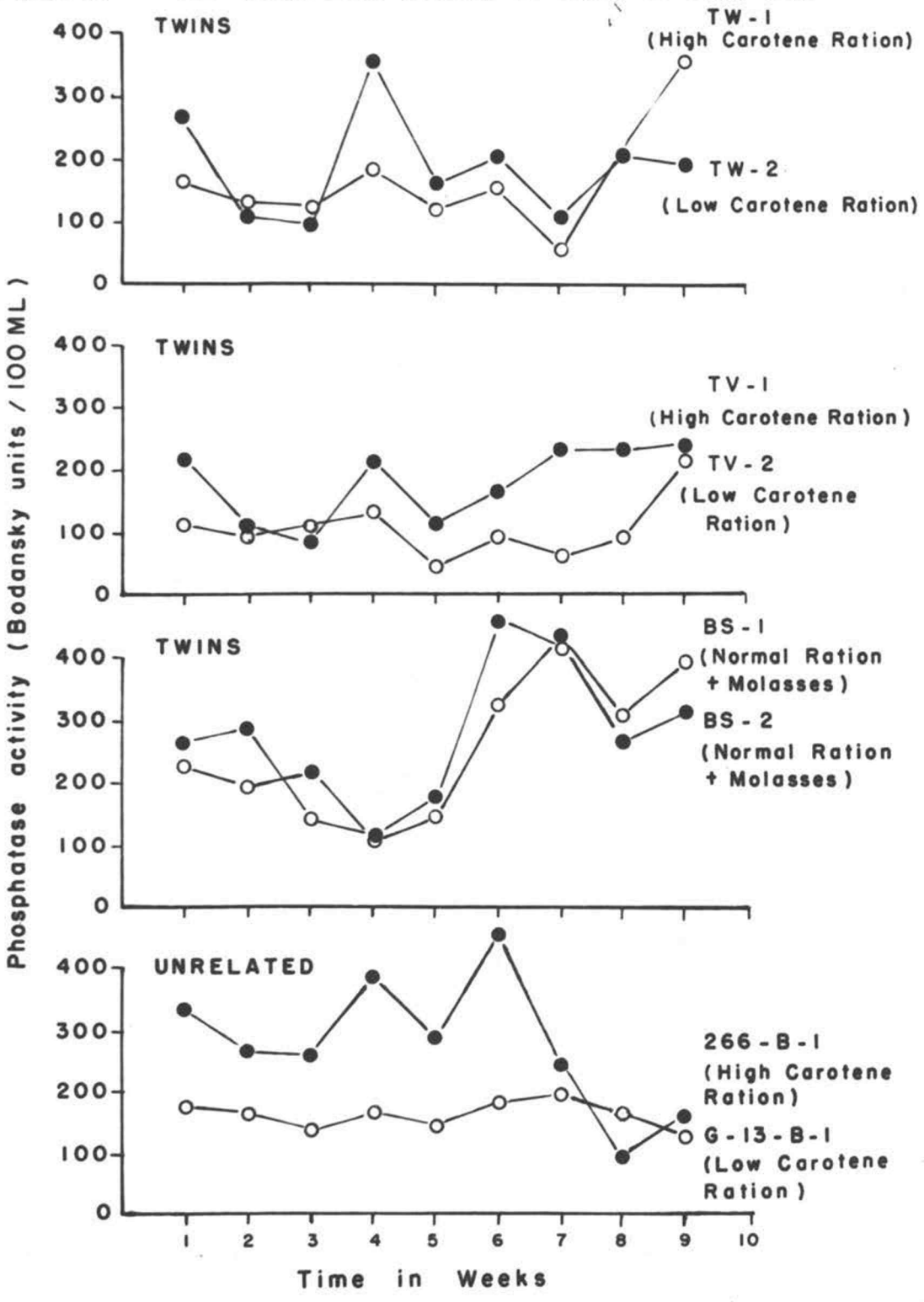

Figure 3. Alkaline phosphatase activity in rabbit semen before and after experimental ration

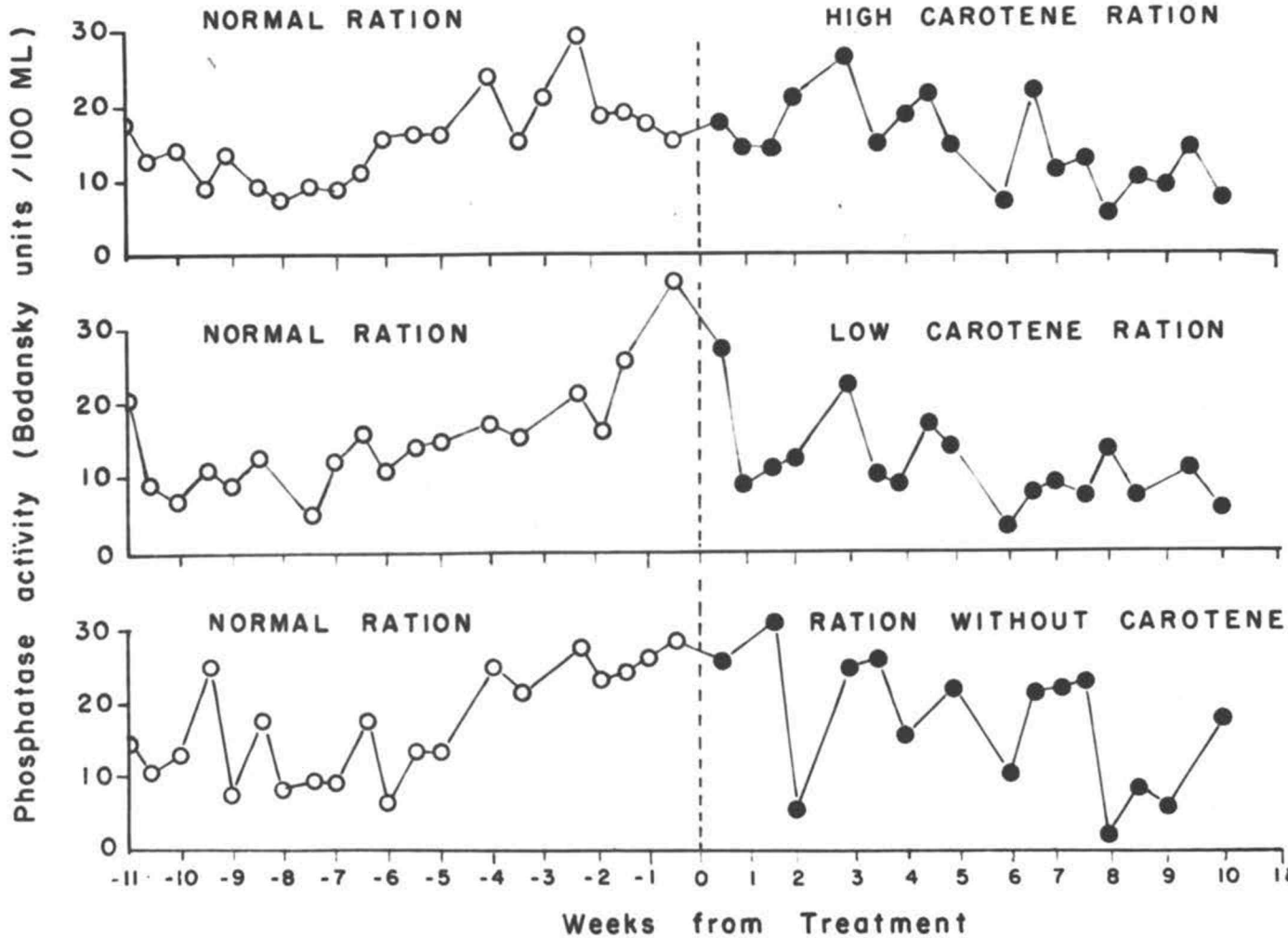

Figure 4. Acid phosphatase activity in rabbit semen before and after experimental ration