On trial: an enzyme-producing food supplement for dogs

To support clinical observation, this series of studies was undertaken to test the effects of feeding dams and their puppies a nutritional supplement that secretes enzymes

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IN 1979, a clinical report was published describing the beneficial effects of feeding a nutritional supplement (Biozyme® D—Bio-Zyme Enterprises) to horses, cows, pigs, and dogs.[1] Reported improvements included added growth and strength and improved health of pups, increased milk production by bitches, and greater ability of working dogs to withstand stress Response was reported to be most apparent among animals suffering from nutrition-related problems, *e g* anemias, parasitisms, and inadequate diets The authors' evaluations were based primarily on physical manifestation of general health status· vigor, coat, growth rate of pups, and ability to withstand stress To substantiate these clinical observations a carefully designed series of studies was conducted Results of those studies are summarized in this paper

The supplement examined is a fermentation of *Aspergillus oryzae* This product secretes enzymes including amylase, protease, pectinase, lipase, maltase, rennet, tannase, catalase, lactase, diastase, and cellulase [2] This supplement also contains vitamins and minerals (coenzymes)

The postulated mechanism of action is that the product combines with water and food in the animal's digestive tract to produce enzymes that aid in the breakdown of proteins and carbohydrates during the digestive process. This mechanism in turn may lead to better absorption with less undigested food being passed via the feces The amount of benefit a dog receives from the product should be directly related to the animal's specific deficiencies

Researchers' protocol

Organization of treatment groups

The trials were set up within the protocol for adequate testing of pet-food products indicated for feeding during gestation and lactation [3] They were conducted at a private commercial laboratory in Pennsylvania *

*White Eagle Laboratories, Inc , Doylestown, Pennsylvania

Enzyme-producing food supplement (cont d)

TABLE 1
Initial Indicators Expressed as Mean Value Per Female (n = 36)*

		Biozyme D (12)	Vit/Min (12)	Control (12)	Significance**
Hb (g/dl)		16 6 (16 3)	17 7 (20 9)	17 0 (18 3)	0 553
PCV (%)		48 3 (17 4)	50 4 (20 6)	48 8 (17 5)	0 703
Weight (kg)		11 9 (19 4)	12 4 (20 3)	10 7 (15 8)	0 547
Age (days)		1562 75(17.2)	1730 08(19 9)	1651 92(18 4)	0 815
Clinical Indicators					
Skin	Normal	10	11	10	Chi^2 = 3 06
	Slightly dry	1	0	2	P = 0 547
	Dry	1	1	0	d f = 2
Hair	Normal	10	10	8	Chi^2 = 4 29
	Slightly dry	1	1	4	P = 0 369
	Dry	1	1	0	d f = 2

*Mean rank in parentheses

**Kruskal-Wallis one-way analysis of variance

The laboratory maintains a closed colony of approximately 800 Beagle and 150 mongrel females, all dogs are kept in a controlled environment.

In order to determine if the fermented enzyme-producing culture had an effect on the animal's health separate from the benefit of vitamin-mineral supplementation alone, three treatment groups were created

- Group 1—Dogs receiving the full supplement
- Group 2—Dogs receiving the vitamin-mineral portion without the extract
- Group 3—A control group receiving no supplement.

Bio-Zyme Enterprises supplied two batches of the product, one that contained the enzyme-producing culture and one that did not. The extract constitutes 1 5979% of the complete product by weight

Classifying dogs by reproductive history

A total of 92 females expected to come into heat after November 1981 were rated according to their reproductive histories The ratings were based on a minimum of two litters, with multiparous bitches rated on their last three litters. These females were placed in three breeding categories

1. *Good*—successfully weaning an average of 6 or more healthy pups
2. *Medium*—raising 3 to 5 healthy pups
3. *Poor*—failing to raise more than 2 healthy pups

The dogs were then matched according to age and reproductive status and assigned to 12 blocks Four blocks consisted of female mongrels (2 blocks were *medium* breeders, 2 were *poor*), 8 blocks consisted of Beagles (3 *good* breeders, 3 *medium*, and 2 *poor*). The animals in each block (with two exceptions) were housed in one building to minimize environmental and handling differences The first 3 females in each block to show signs of proestrus were assigned randomly to one of the three treatment groups. The final test sample was reduced to 36 dogs.

The earliest estrus was observed on Nov 30, 1981, the latest on Apr 21, 1982 In an effort to control genetic differences in the offspring, each female in a block was assigned the same stud for breeding A back-up stud, matched as closely as possible to the first-choice male, was available for service if necessary One female in Group 2 did not conceive and was immedi-

TABLE 2
Whelping Indicators Expressed as Mean Value Per Female (n = 36)*

	Biozyme D (12)	Vit/Min (12)	Control (12)	Significance**
Hb†	79 8 (24 0)	75 3 (18 0)	73 0 (13 5)	0 050
Weight†	115 8 (22 8)	107 4 (14 4)	110 8 (18 3)	0 150
PCV†	79 6 (21 8)	78 2 (19 5)	75 3 (14 7)	0 188
Live births	6 67 (19 3)	6 50 (18 4)	6 33 (17.9)	0 948
Stillbirths	0 17 (17 2)	0 92 (19 6)	0 33 (18 8)	0 735
Congenital anomalies	0 43 (21 5)	0 00 (17 0)	0 00 (17 0)	0 042

*Mean rank in parentheses
**Kruskal-Wallis one-way analysis of variance
†As a percent of initial value at proestrus

ately replaced by a female from the original pool. The replacement female had received a commercial vitamin-mineral supplement for seven days before being returned to the pool.

Feeding regimen during gestation

All of the females in the colony were maintained on a standard commercial diet for laboratory dogs ** Vitamin-mineral supplements were discontinued on Oct. 20, approximately six weeks before the trials began. At the start of the trial each female was examined to ensure that it was healthy The animals were given a complete physical examination.*** A complete blood count (CBC) and a chemistry profile were also obtained Body weights were recorded at the start of the trial, weekly thoughout gestation, and within 24 hours after whelping. Animals were weighed to the nearest 0 25 kg on a spring scale which was calibrated with a standard 5-kg weight.

The two food supplements (Biozyme D and the vitamin-mineral portion) were assigned color-codes by personnel at Tulane University and then shipped to

TABLE 3
Whelping Indicators Expressed as Mean Value Per Female (n = 36)*

	Biozyme D (12)	Controls (24)**	Significance†
Hb††	79 8 (24.0)	74 1 (15 8)	0 013
Weight††	115 8 (22 8)	109 1 (16 4)	0 042
PCV††	79 6 (21 8)	76 7 (16 8)	0 090
Live births	6 7 (19 3)	6 4 (18 1)	0 381
Stillbirths	0 17 (17 2)	0 63 (19 2)	0 230
Congenital anomalies	0 42 (21 5)	0 00 (17 0)	0 006

*With mean ranks in parentheses
**Groups 2 and 3 combined
† Mann-Whitney U-Wilcoxian Rank Sum W Test (one-tailed)
†† As a percent of initial value at time of proestrus

**An independent analysis of this diet yielded the following results 52 3% carbohydrates, 23 7% protein, 8 11% ash, 7 65% moisture, 5 36% fat, 2 88% fiber, and 352 cal /100 grams

***All physical examinations were conducted under the supervision of Abbott S D'Ver, VMD

Enzyme-producing food supplement (cont'd)

TABLE 4
Weaning Indicators Expressed as Mean Value Per Female (n = 36)*

	Biozyme D (12)	Vit/Min (12)	Control (12)	Significance**
Hb†	86 6 (18 0)	82 3 (15 3)††	85 6 (19 2)††	0 639
Weight at proestrus†	122 4 (20 9)	109 5 (12 6)††	118 5 (18 7)††	0 121
Weight at whelping[a]	106 1 (19 3)	101 9 (16 1)††	106 3 (16 9)††	0 728
No pups weaned	5 3 (21 6)	4 4 (16 6)	4 3 (17 3)	0 446
No pups died	1 4 (18 5)	2 1 (19 0)	2 0 (18 0)	0 976

*With mean rank in parentheses
**Kruskal-Wallis one-way analysis of variance
† As a percent of value at time of proestrus
†† Calculations based on 11 females One bitch fed the vitamin-mineral combination and one control bitch lost their entire litters
[a] As a percent of value at time of whelping

TABLE 5
Weaning Indicators Expressed as Mean Value Per Female (n = 36)*

	Biozyme D (12)	Controls (24)**	Significance†
Hb††	86 6 (18 0)	83 9 (17 2)[a]	0 414
Weight at proestrus††	122 4 (20 9)	114 0 (15 7)[a]	0 072
Weight at whelping[b]	106 1 (19 3)	104 1 (16 5)[a]	0 219
No pups weaned	5 3 (21 6)	4 4 (17 0)	0 104
No pups died	1 4 (18 5)	2 0 (18 5)	NA

*With mean ranks in parentheses
**Groups 2 and 3 females combined
† Mann-Whitney U-Wilcoxian Rank Sum W Test (one-tailed)
†† As a percent of value at time of proestrus
[a] Values computed for 22 females Two controls lost their entire litters during lactation
[b] As a percent of value at time of whelping

TABLE 6
Weaning Indicators of Male Mongrel Pups Expressed as Mean Value Per Pup (n = 32)*

	Biozyme D (14)	Control (18)**	Significance†
Birth weight††	364 (19 3)	337 (14 4)	0 072
Weight (Wk 1)	585 (18 4)	529 (15 0)	0 153
Weight (Wk 2)	854 (19 1)	735 (14 5)	0 086
Weight (Wk 3)	1,073 (19 3)	930 (14 3)	0 069
Weight (Wk 4)	1,331 (19 4)	1,140 (14 3)	0 064
Weight (Wk 5)	1,647 (17 9)	1,478 (15 4)	0 229
Weight (Wk 6)	1,934 (17 4)	1,866 (15 8)	0 311
Weight (Wk 7)	2,736 (20 2)	2 365 (13 6)	0 024
Hb (g/dl)	8 8 (20 4)	8 1 (13 5)	0 019
PCV (%)	27 2 (19 6)	25 9 (14 1)	0 047

*With mean ranks in parentheses
**Vitamin/mineral and no supplement groups combined
† Mann-Whitney U-Wilcoxian Rank Sum W Test (one-tailed)
††All weights in grams

the laboratory in containers marked with only the color code Each dog had a feeding dish of the same color assigned to its treatment group The weekly ration was weighed in advance and each animal was fed daily from this ration Food consumption was calculated at the end of each week by subtracting the balance remaining from the initial amount. The food was top-dressed daily with the appropriate supplement at the rate of 3 3 g (approximately 1 tsp) per 4 54 kg body weight (manufacturer's recommendation for normal maintenance) The suggested dose for breeding and lactating dogs is 1 5 times this amount, thus the animals were underdosed per the manufacturer's recommendations Beagles weighing 8 to 10 kg received 6 6 g of supplement, whereas mongrels weighing 14 to 16 kg received 9 9 grams Stool consistency (loose or firm) and food wastage were assessed on a weekly basis

Whelping

Each female was weighed within 24 hours after whelping, and the numbers of live births, stillbirths, obvious congenital anomalies, and sex of puppies were also recorded Birth weight of the pups was recorded to the nearest gram

Lactation

Females

Each female was given a complete physical examination, and hematology and serum chemistry analyses were performed one week postpartum Food consumption was measured as described and the feces were observed for consistency through the end of the seventh week Another complete physical examination and CBC were performed on each female at the end of the seventh week of the experiment

Pups

The puppies were weighed each week and their weight recorded to the nearest gram Each was given a complete physical examination at the end of the first and seventh weeks A CBC was performed at the end of the seventh week

Puppies were introduced to solid food when they were 4 weeks old Their food was supplemented in a manner similar to the dams' diet, i.e. at a ratio of 3 3 g (1 tsp) per 4 54 kg of puppy weight (based on the combined weights of all pups in the litter) It was not

TABLE 7
Weaning Indicators of Female Mongrel Pups Expressed as Mean Value Per Pup (n = 24)*

	Biozyme D (7)	Control (17)**	Significance†
Birth weight††	342 (13 4)	319 (12 2)	0 352
Weight (Wk 1)	535 (13 6)	504 (12 1)	0 317
Weight (Wk 2)	811 (15 7)	686 (11 2)	0 077
Weight (Wk 3)	1,059 (15 0)	875 (11 5)	0 133
Weight (Wk 4)	1,323 (15 1)	1,108 (11 4)	0 120
Weight (Wk 5)	1,615 (14 6)	1,410 (11 7)	0 179
Weight (Wk 6)	1,948 (12 9)	1,892 (12 4)	0 437
Weight (Wk 7)	2,412 (13 3)	2 256 (12 2)	0 363
Hb (g/dl)	9 1 (13 9)	8 9 (11 9)	0 272
PCV (%)	29 1 (13 9)	27 7 (11 9)	0 273

*With mean ranks in parentheses
**Vitamin/mineral and no supplement groups combined
†Mann-Whitney U-Wilcoxian Rank Sum W Test (one tailed)
††All weights in grams

TABLE 8
Weaning Indicators of Male Beagle Pups Expressed as Mean Value Per Pup (n = 58)*

	Biozyme D (23)	Control (35)**	Significance†
Birth weight††	309 (31 4)	294 (28 3)	0 247
Weight (Wk 1)	523 (32 9)	468 (27 3)	0 109
Weight (Wk 2)	759 (35 4)	669 (25 6)	0 016
Weight (Wk 3)	955 (34 7)	843 (26 1)	0 029
Weight (Wk 4)	1,173 (33 8)	1,060 (26 7)	0 060
Weight (Wk 5)	1,367 (34 0)	1,219 (26 6)	0 051
Weight (Wk 6)	1,648 (33 8)	1,488 (26 7)	0 058
Weight (Wk 7)	1,919 (33 4)	1,754 (27 0)	0 076
Hb (g/dl)	8 7 (26 1)	9 2 (31 8)	NA
PCV (%)	27 7 (27 1)	28 6 (31 1)	NA

*With mean ranks in parentheses
**Vitamin/mineral and no supplement groups combined
†Mann-Whitney U-Wilcoxian Rank Sum W Test (one-tailed)
††All weights in grams

Enzyme producing food supplement (cont'd)

possible to calculate the exact amounts of food consumed by each dam and each pup during the final four weeks of the experiment

Indications of supplement performance

Initial indicators

According to the criteria variables, the health status of the three groups at the trials' onset was not significantly different (*Table 1*) All of the animals were in good health with few abnormal skin or coat conditions

Whelping indicators

The group given Biozyme D performed better than the two other groups in all variables studied except for congenital anomalies (*Table 2*) The only relationships significant at the 0 05 level were hemoglobin as a percent of its original value and congenital anomalies Because there were no apparent differences or trends between the groups receiving only the vitamin-mineral diet and the control groups, the two categories were combined in order to improve the statistical efficiency of the test. As a result, the significance of all relationships increased when the number of categories was reduced, thereby increasing the sample sizes within the groups (*Table 3*).

There were five congenital anomalies in three litters from bitches given Biozyme D. It is difficult to

TABLE 9
Weaning Indicators of Female Beagle Pups Expressed as Mean Value Per Pup (n = 54)*

	Biozyme D (19)	Control (35)**	Significance†
Birth weight††	288 (30 8)	273 (25 7)	0 129
Weight (Wk 1)	495 (29 1)	456 (26 6)	0 290
Weight (Wk 2)	671 (28 7)	638 (26 9)	0 342
Weight (Wk 3)	836 (27 2)	827 (27 7)	NA
Weight (Wk 4)	1,036 (26 0)	1,049 (28 3)	NA
Weight (Wk 5)	1,195 (25 8)	1,219 (28 4)	NA
Weight (Wk 6)	1,473 (27 9)	1,441 (27 3)	0 446
Weight (Wk 7)	1,765 (27 9)	1,753 (27 2)	0 439
Hb (g/dl)	8 5 (22 4)	9 0 (30 3)	NA
PCV (%)	27 2 (24 3)	28 1 (28 9)	NA

*With mean ranks in parentheses
**Vitamin/mineral and no supplement groups combined
†Mann-Whitney U-Wilcoxian Rank Sum W Test (one-tailed)
††All weights in grams

TABLE 10
Mean Total Weight Gain of Pups in Grams (n = 168)

	Biozyme D (63)	Vit/Min (53)	Control (52)	Significance*
Mongrels				
Male (32)	2 373 (14)	1,801 (6)	2,142 (12)	0 126
Female (24)	2,070 (7)	1,923 (13)	1,979 (4)	0 950
Beagles (112)				
Male (58)	1,610 (23)	1,321 (15)	1,565 (20)	0 171
Female (54)	1,478 (19)	1,412 (19)	1,561 (16)	0 388
Weighted average	1,791	1 556	1,729	

*Kruskal-Wallis one-way analysis of variance

develop an appropriate statistical test of so infrequent an occurrence. Yet all of the tests showed the relationship to be statistically significant Even though this number of congenital anomalies is not unusual in 36 litters, the fact remains that they all occurred in litters from Group 1 dogs Future trials and use of this product in the field should be monitored to ascertain if this rate of anomalies is a chance occurrence or if the association is valid

Weaning Indicators

Females

Females in Group 1 outperformed the other groups in all weaning indicators except weight at weaning as a percent of weight at whelping (*Table 4*) None of the relationships was statistically significant By combining the two control groups (*Table 5*), the group receiving Biozyme D outperformed the controls in all indicators, with the measure of association being stronger although none of the relationships was significant at the p = 0 05 level

Pups

The analysis of the pups was divided into four categories by breed (mongrel/Beagle) and sex Pups that received Biozyme D were larger in almost every category, with the exception of the female Beagles Few of the relationships were statistically significant

Combining the control-group categories showed that the mean weight of pups given Biozyme D were larger in 30 of the 32 weight comparisons, including time of weaning for all four breed/sex categories (*Tables 6 - 9*) No consistent trend was seen in results of hematology These data still do not indicate whether the experimental animals merely maintained their original advantage in birth weight or if they grew more during the preweaning period as well

Table 10 shows the mean total weight gain for each of the three treatment groups across the four breed/sex categories Pups in the Biozyme D group gained more in three of the four categories (p = 0 05) With the control groups combined, the weekly gains (*Table 11*) enhance the significance of the three levels where pups fed Biozyme D had an advantage in weight gain, and with the control groups combined, the deficit in the fourth level (female Beagles) is almost eliminated

Three-way analysis of variance was done on the in-

TABLE 11
Mean Total Weight Gain of Pups In Grams (n = 168)

	Biozyme D (63)	Controls* (105)	Significance**
Mongrels (56)			
Male (32)	2,373 (14)	2,029 (18)	0 034
Female (24)	2,070 (7)	1,963 (17)	0 412
Beagles (112)			
Male (58)	1,610 (23)	1,461 (35)	0 092
Female (54)	1,478 (19)	1,480 (35)	NA
Weighted average	1,791	1,646	

*Vitamin-mineral and control groups combined
**Mann-Whitney U-Wilcoxan Rank Sum W Test (one tailed)

TABLE 12
Nitrogen Balance

	Biozyme D	Control	Totals
Positive	8	11	19
Negative	4	10	14
Totals	12	21	33

Z = 0 799 Significance = 0 212 (one-tailed)

TABLE 13
Mean Food Consumption in Grams

	Biozyme D	Controls	Significance*
Mongrels	1,267 (4)	916 (7)	0 236
Beagles	1,072 (8)	723 (14)	0 084

*Two tailed t-test

formation presented in Tables 10 and 11 in order to estimate the separate effects of treatment group, sex, and breed after controlling for the effects of the other variables The effect of treatment in the analysis of the three-treatment category is statistically significant (p = 0 027) though much of the difference is obviously due to the poor performance of Group 2. Analysis of the two-treatment category shows only borderline significance (p = 0 069) for the effect of treatment

Post-trial analysis of nutrient absorption/use in females

In the final stage of the trials, the 34 females that successfully completed weaning were held in cages for three days. All feces and urine specimens and a sample of the food were sent to an independent laboratory† for proximate analysis. Subsequent analysis permitted calculation of the following indices· nitrogen absorption, nitrogen utilization, fat absorption, and carbohydrate absorption

Nitrogen absorption gives the percent of nitrogen consumed and not excreted in the feces The same principle applies to the indices of fat, carbohydrate, ash, and calorie absorption. All of these measures indicate how much of the nutrients are being absorbed by the animal as opposed to being wasted in excrement

Nitrogen utilization measures the amount of nitrogen available for metabolic purposes after loss through urine is taken into account An animal ingesting more nitrogen than it is excreting is said to have a positive nitrogen balance Otherwise, the animal is in negative balance. The measure of nitrogen utilization in these trials was crude, it did not account for loss of nitrogen through perspiration, storage in cells, and fluctuations in blood nitrogen values

Females fed Biozyme D showed better absorption/utilization in each nutrient category, although the only statistically significant results were for nitrogen utilization (p less than 0.05). On the basis of their utilization scores, animals were classified as being in a positive or negative nitrogen balance (*Table 12*) A higher proportion of the Biozyme D females were in positive nitrogen balance and the Biozyme D group also consumed more food during the three-day collection period (*Table 13*).

† Nutrition International, Inc , East Brunswick, New Jersey

TABLE 14
Clinical Indicators

	Hair		Skin	
	Biozyme D	Control	Biozyme D	Control
FEMALES				
Gestation (8th week)				
Normal	12	20	12	20
Abnormal	0	4	0	4
	p = 0.278*		p = 0.278*	
Post-whelping				
Normal	10	18	10	18
Abnormal	2	4	2	4
	p = 1.000*		p = 1.000*	
Weaning				
Normal	12	21	12	21
Abnormal	0	1	0	1
	p = 1.000*		p = 1.000*	
PUPS				
Weaning				
Normal	62	102	62	102
Abnormal	1	3	1	3
	p = 1.000*		p = 1.000*	

*Fisher's Exact Probability

Benefits of enzyme-producing supplement

The trials were done to provide rigorous testing of the product. As noted, previous clinical use had demonstrated that the greatest response was in animals suffering from nutrition-related problems. All of the females used in this trial were healthy, parasite-free breeding stock from a commercial laboratory. They were housed in a controlled environment and fed the same high-quality commercial laboratory diet.

The animals received only the normal maintenance dosage of Biozyme D, which is 50% less than recommended for pregnancy and lactation. The good health of the animals is attested by the few that were graded abnormal according to clinical indicators (*Tables 1 & 14*). In each category during all stages of the trial, animals fed Biozyme D rated better in terms of these clinical indicators than did controls. Numbers in the abnormal category for both treatment groups are so low that none of the results were statistically significant.

Animals in the Biozyme D group tended to outperform controls at every stage of the trials in terms of weight maintenance, live births, stillbirths, puppy survival, puppy growth (except for the female Beagles), clinical indicators, and the results of the proximate analysis. However, few results were statistically significant. The unexpected positive association with congenital anomalies is difficult to explain and bears further monitoring. Throughout gestation and weaning, females in Group 1 maintained their hemoglobin concentrations better than did the controls (*Tables 3 & 5*). No consistent relationship existed between treatment group and blood values for the pups at weaning.

The pattern of consistent results across the trials supports the hypothesis that supplementation with Biozyme D was associated with improvement of health in the study population. A strong case is made for further studies, using larger samples, to determine appropriate dosage and potential applications, especially in the areas of

- Growth—pups in the Biozyme D group were larger at birth and generally grew more than did the controls.
- Nutrition therapy—the results of the fecal analysis suggest potential use of this product as an adjunct for the therapy of nutrition-related disorders including
  1. diarrheal diseases in which inflammation of the mucosa hinders absorption,
  2. parasite infestations that have led to debilitation, and
  3. stressful situations that increase demand on the animal's metabolism
- Reproduction—the difference in the average number of pups weaned as seen in these trials may have implications for animal breeders.

REFERENCES

1. Knowles, R. P., Basham, H. H. Clinical Impressions of the Use of an Enzyme Additive in Large and Small Animals. *VM/SAC 74* (12): 1733-1735, 1979.
2. Raper, K. B., Fennell, D. I. *The Genus Aspergillus*. Williams and Wilkins, Baltimore, Md., 1965. pp. 338-395.
3. Association of American Feed Control Official Publication, Charleston, W. Va., 1981.