R=H) was shown by acid hydrolysis to be composed of D-glucuronic acid and D-galactose. Complete methylation of the methyl ester methyl glycoside (IV, R=CH_3) gave methyl-[methyl-6-*O*-(2,3,4 - tri - *O* - methyl - β - D - glucosyl) uronate] - 2,3,4 - tri - *O* - methyl - D - galactoside (V) which upon reduction with lithium aluminum hydride (*1, 5*) yielded methyl 6-*O*-(2,3,4-tri-*O*-methyl-β - D - glucosyl) - 2,3,4 - tri - *O* - methyl - D-galactoside (VI). The structure of the latter was deduced from the observation that upon hydrolysis it afforded about equal amounts of 2,3,4-tri-*O*-methyl-D-glucose (identified as the 1,6-di-*p*-nitrobenzoate) and 2,3,4-tri-*O*-methyl-D-galactose (identified as its aniline derivative).

These findings emphasize the highly complex structure of the alfalfa hemicellulose. Thus 4-*O*-methyl-D-glucuronic acid was previously shown to be attached by α-(1→2) linkages to D-xylose units. In the present paper, D-glucuronic acid is shown to be attached to D-xylose by α-(1→2) linkages and to D-galactose by β-(1→6) linkages. The present work also revealed that D-galacturonic acid units are an integral part of the hemicellulose molecular complex and that they are joined by α-(1→2) linkages to L-rhamnose. D-Glucuronic acid units linked by β-(1→6) bonds to D-galactose and D-galacturonic acid units joined by α-(1→2) linkages to L-rhamnose are structural features common to the plant gums and mucilages (*10*).

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FEED SUPPLEMENTS

Enzyme Supplementation of Rations for Dairy Calves

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An evaluation was made of the effects of adding supplementary digestive enzymes to calf starter rations, containing either steam-rolled or dry-cracked grains. Measurements were made of blood glucose level, rumen pH, proportion of rumen volatile, fatty acids, feed consumption, and growth rate of calves during a 16-week period. Neither the addition of supplementary enzymes nor steaming of the grains had any appreciable effect on feed consumption or growth rate to either 8 or 16 weeks of age. The average blood glucose level of all groups of calves declined from approximately 80 mg. per 100 ml. at 11 days of age to a level typical of an adult ruminant by 32 days of age. Differences among groups with respect to proportions of rumen volatile, fatty acids were of insufficient magnitude to be reflected in growth rate.

DURING THE LAST few years it has been shown (*3, 5, 7, 9, 18*) that very young animals secrete a limited amount of certain digestive enzymes known to be necessary for the utilization of rations ordinarily consumed by mature animals of the same species. Moreover, Larson *et al.* (*13*) observed that very little digestion of starch occurred in calves which were about 9 months of age when different experimental diets were fed directly into the omaso-abomasal cavity, presumably because insufficient amylase was secreted to utilize the starch in these diets efficiently.

In the evaluation of supplemental enzymes for calves, most investigators have added the enzymes to milk replacers or gruel mixtures which upon ingestion would be expected to pass directly into the abomasum (*6, 14, 20, 27*). In these studies, the addition of pepsin, malt diastase, papain, animal diastase, or pancreatin powder to rations containing various ingredients has been without benefit for dairy calves.

More recently, enzymes have been added to the rations of cattle and sheep under conditions where the ingested feed would pass into the rumen. Burroughs *et al.* (*2*) and Nelson and Catron (*17*) have reported experiments in which the addition of various supplementary enzyme preparations to rations for beef steers increased the rate of gain and improved feed conversion efficiency. Other workers (*12, 19, 21, 24, 25*) have observed no substantial benefit from such additions to rations for fattening lambs or beef cattle. In the work by Burroughs *et al.* (*2*), the digestibility of dry matter, organic matter, protein, and cellulose was not influenced by the addition of a combination of proteolytic and amylolytic enzymes (Agrozyme); however, Grainger and Stroud (*8*) observed an increase in the digestibility of dry matter by wethers upon the addition of gumase, amylase, or a combination of these enzymes along with protease, to a semipurified ration. The addition of the three enzymes separately, or in combination, resulted in a significant increase in crude cellulose digestibility, while only the combina-

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tion of the three enzymes increased crude protein digestibility and nitrogen balance.

Only a limited amount of work has been done to determine the effects of adding supplementary enzymes to the dry feed for dairy animals. Leatherwood, Mochrie, and Thomas (*15*) reported that the growth of dairy calves, which were 2 months of age or older at the start of the trials, was not increased by the addition of a fungal cellulase to the ration. Also, the apparent digestibility of the ration constituents was not affected by the addition of the enzyme. Similarly, Wing and Wilcox (*28*) reported that the growth of dairy heifers receiving a supplemental enzyme preparation containing protease, diastase, and a gum-splitting enzyme was not significantly greater than that of a similar group of animals receiving no supplemental enzymes in the ration.

No work has been reported wherein supplemental enzymes were added to the grain ration of very young dairy calves. Therefore, the present experiment was conducted to determine the effects of adding a preparation containing both proteolytic and amylolytic enzymes (Agrozyme) to calf-starter rations for dairy calves. Since Shaw *et al.* (*23*) had reported that steam heating of the concentrate ration for beef steers markedly increased the efficiency of body weight gains, the effect of the supplemental enzymes was determined using starter rations containing either steam-heated or dry-cracked grains.

Experimental Procedure

Animals. Thirty-two male Holstein calves, weighing between 75 and 105 pounds at birth, were used for the experiment. Each calf received colostrum for a 2-day period after birth and was then assigned at random to one of four treatment groups when either 3 or 4 days of age. The calves were housed in individual stalls with wood shavings for bedding.

Preparation of Rations. The ingredients used in making up the experimental calf starters were all vegetable products (Table I). A commercial bacterial preparation (Agrozyme) containing proteolytic and amylolytic enzymes was added to one starter ration having steamed corn and oats, and also to another having dry-cracked corn and oats. Thus, the addition of the enzyme preparation constituted one variable and the steaming of the grains used in the rations was another variable, making a 2 × 2 factorial arrangement of treatments. In steaming the grains for two of the starters, sufficient steam was allowed to condense on the grain to make it as high in moisture content as practical considerations of mechanical handling would permit; therefore, it is estimated that the grains were heated to approximately 190° F. Sufficient dehydrated alfalfa crumbles were added to make the final mixtures used during the first 8 weeks of the experiment contain a 1 to 4 ratio of hay to concentrates, and the mixtures used during the second 8-week period to contain a 1 to 1 ratio.

Treatments. Each calf was fed whole milk at the daily rate of 10% of its initial body weight during the first 14 days in the experiment, and at the rate of 8% of initial weight for an additional 10 days. Fifty milligrams of chlortetracycline, in the form of Aurofac D, were administered daily in the milk to aid in preventing infections. The respective calf starters were fed free-choice throughout the experiment, and they constituted the sole diet after the calves were weaned abruptly at 28 days of age. The calves were weighed on 2 consecutive days at the beginning of the experiment and at the end of the first and second 8-week periods. Interim weights were recorded weekly. The amount of feed consumed was recorded daily during the first 8 weeks of the experiment and at weekly intervals thereafter.

Samples of venous blood were taken weekly at approximately 5 hours after feeding during the first 12 weeks and were analyzed for glucose by a modification of the method of Athanail and Cabaud (*1*). Rumen samples were taken by means of a stomach tube at the second, fourth, sixth, eighth, and tenth weeks of the experiment and the pH was determined immediately. The rumen samples taken at the fourth week (when the calves were 32 days old) were analyzed for the proportion of volatile fatty acids (acetic, propionic, and butyric), using a silicic acid chromatographic procedure (*11*).

Results and Discussion

There were no significant differences among the four treatment groups with respect to feed consumption or weight gains to either 8 or 16 weeks (Table II). Nevertheless, the over-all performance of the animals in terms of growth rate exceeded the Beltsville growth standards for Holstein calves (*16*), indicating that the feeding regime followed in this experiment is satisfactory for raising dairy calves.

No beneficial effects resulted from the addition of supplementary enzymes to the calf starters in this experiment; however, Nelson and Catron (*17*) observed that the degree of response of fattening steers to commercial enzyme preparations was influenced by the level of supplementation. The lack of any significant change in the proportion of rumen volatile fatty acids (VFA) as a result of the addition of enzymes to the calf starters in the present experiment (Table III) parallels the findings of Ward, Richardson, and Tsien (*25*) with respect to the total concentration and proportion of rumen VFA in beef cattle fed amylolytic and/or proteolytic enzymes.

Steaming the grains resulted in an increase in the molar percentage of propionic acid and a corresponding decrease in the butyric and higher acids, statistically significant at the 10% level of probability (Table III). As noted

Table I. Composition of Calf Starters[a]

Ingredient	Starter No. 1	2	3	4
	Lb./Ton			
Corn, dry-cracked	..	..	480	480
Oats, dry-cracked	..	..	480	480
Corn, steam-rolled	480	480	..	..
Oats, steam-rolled	480	480	..	..
Wheat bran	160	160	160	160
Corn, distiller's solubles	110	110	110	110
Dried molasses	80	80	80	80
Soybean meal	225	230	225	230
Dicalcium phosphate	20	20	20	20
Trace mineral salt	20	20	20	20
Antibiotic-vitamin premix[b,c]	20	20	20	20
Dehydrated alfalfa crumbles	400	400	400	400
Agrozyme[d]	5	..	5	..

[a] During the second 8 weeks, the same grains were used along with sufficient hay pellets to give a 1:1 ratio of hay to grain. Sufficient Agrozyme was added to give the same concentration of enzymes as that in the starters used during the first 8 weeks.

[b] Amounts of vitamins added per pound of ration: vitamin A, 3750 I.U.; vitamin D_2, 470 I.U.; thiamine, 15 mg.; riboflavin, 28 mg.; pantothenic acid, 7.5 mg.; niacin, 17 mg.; vitamin B_{12}, 0.0075 mg.; *d*-biotin, 0.15 mg. Antibiotic per pound of ration: aureomycin, 25 mg.

[c] Only vitamins A and D were added to the starters used during the second 8 weeks.

[d] Agrozyme contains both proteolytic and amylolytic enzymes.

Table II. Average Feed Consumption and Weight Gains of Calves[a]

Treatment Group	No. of Animals	Starter Consumed, Lb. 8 weeks	16 weeks	Av. Daily Gain, Lb. 8 weeks	16 weeks
Steamed grains and Agrozyme	8	137	523	1.34	1.67
Steamed grains, no added enzymes	8	135	524	1.22	1.62
Cracked grains and Agrozyme	8	131	531	1.17	1.48
Cracked grains, no added enzymes	8	127	539	1.35	1.68

[a] Differences among treatment groups were not statistically significant ($P > 0.10$).

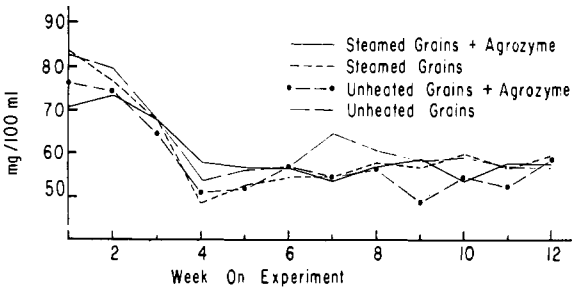

Figure 1. Blood glucose levels of calves receiving different calf starters

Table III. Volatile Fatty Acids in the Rumen Fluid of Calves at 32 Days of Age

Group	Total Acid, Meq./100 Ml.	Acids, Mole %		
		Acetic	Propionic[a]	Butyric and higher
Steamed grains and enzymes	9.60	51.3	38.3	10.4
Steamed grains	8.05	49.7	36.4	13.8
Cracked grains and enzymes	7.07	48.5	35.8	15.7
Cracked grains	7.73	52.7	31.1	16.3

[a] Difference between steamed and cracked grains statistically significant at 10% level of probability; difference between enzymes *vs.* no enzymes not significant ($P > 0.10$).

Table IV. pH of Rumen Contents of Early Weaned Calves Receiving Different Starters

Starter	Week on Experiment				
	2	4	6	8	10
(1) Steamed grains and enzymes	6.23	5.50	5.69	5.99	5.70
(2) Steamed grains	6.44	5.53	5.58	6.09	5.80
(3) Cracked grains and enzymes	6.50	5.65	5.50	5.80	5.60
(4) Cracked grains	6.36	5.74	5.65	5.85	5.89

above, however, these changes in the proportion of rumen VFA were not reflected in differences in the growth rate of the calves. Using different rations and older animals, Shaw *et al.* (*23*) observed that steaming of corn produced marked changes in the proportion of rumen VFA and a significant increase in weight gains. However, in the latter work the steers which received steam-heated corn also received ground and pelleted hay, while the animals with which they were compared received ground corn and alfalfa hay chopped into 2-inch lengths. This difference in fineness of the hay may have accounted for a large part of the difference in rate of gain of the animals involved, since Shaw (*22*) has shown that more marked changes in rumen VFA occur when heated grains are fed along with ground and pelleted hay than when long or chopped hay is fed.

Although there were no differences of consequence between treatment groups in the pH of the rumen contents at any time of sampling, the pH of the contents of all the calves decreased from the second to the fourth week of the experiment, and subsequently increased somewhat (Table IV). The decrease in pH was associated with the initiation of fermentation within the rumen, as evidenced by pH values approximating 5.9 for calves which consumed grain during the first 2 weeks as compared to values around 6.7 for calves which consumed no grain during this period. The subsequent rise in rumen pH with advancing age, also observed by Conrad, Hibbs, and Frank (*4*), is very likely associated with increased absorption of the VFA from the rumen as it develops (*9*), and/or with an increase in buffering capacity of the rumen as a result of increased salivary output (*10*).

The blood glucose levels for the four groups of calves were similar, there being a rapid decline from about 80 mg. per 100 ml. at 1 week on the experiment to a level typical of an adult ruminant after 4 weeks (Figure 1). A change in metabolites available to the animal upon abrupt weaning to dry feed is believed to be the primary factor involved in this unusually rapid decline in blood glucose, since Warner, Harrison, and Loosli (*26*) have presented data showing that the nature of the diet definitely influences the level of blood glucose attained as the calf advances in age.

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