EFFECTS OF SUPPLEMENTAL ENZYMES ON NITROGEN BALANCE, DIGESTIBILITY OF ENERGY AND NUTRIENTS AND ON GROWTH AND FEED EFFICIENCY OF CATTLE.

T. W. PERRY, E. D. PURKHISER AND W. M. BEESON

Purdue University, Lafayette, Indiana

Research with supplemental enzymes has attracted a great deal of attention, since Jensen et al. (1957) demonstrated an improvement in the dietary value of barley for chicks by supplementation with a mixture of enzymes. Later, Willingham et al. (1960) showed that this chick response was limited to barley grown in certain geographical locations of the United States. In general, the response of pigs to supplemental enzymes has been very slight (Nielson et al., 1959; Combs et al., 1960).

Burroughs et al. (1960) summarized the data collected from 10 feedlot experiments on the evaluation of a commercial enzyme preparation for fattening cattle. Gains were increased an average of 7%, and efficiency of feed conversion was improved 6%. Dyer (1961) reported an average daily weight increase of 91 gm. per calf and a decrease of 350 gin. of feed per kg. of gain in three trials. Nelson and Catron (1960) reported an apparent synergism between several enzymes and diethylstibestrol, based on the data from eight experiments.

The objectives of the trials reported in this paper were to investigate the effects of supplemental enzyme sources on growth rate, digestibility of nutrients and nitrogen balance by beef cattle.

Experimental Procedure

Three feeding trials and six digestion trials were conducted with supplemental enzyme concentrates. The following descriptions of the enzyme preparations were supplied by the respective manufacturers:

Multiple Enzyme No. 1 (MK-124). A multiple enzyme preparation of bacterial origin. The supplement was composed of 90% dialyzable (enzymes and other high molecular weight compounds) fractions. The enzymes and percent by weight (as compared with appropriate control activity) which were present in multiple enzyme No. 1, respectively, were as follows: amylase, 1%; cellulase, 15%; hemicellulase, 0.3%; limit dextrinase, 1%; and proteinase, 1.6%.

Multiple enzyme No. 2. This enzyme supplement was a standardized product obtained by culturing a selected strain of Bacillus subtilis under conditions for maximum production of amylase. It was buffered, activated and stabilized with sodium phosphate and calcium sulfate. It was standardized by diluting the enzyme concentration to an amylase level of 55 mg./mg. (the amount of enzyme which will give 55 mg. of maltose per mg. of enzyme under arbitrary assay conditions). The diluent used was ground cereal grain. In addition to amylase, enzyme No. 2 contained appreciable but variable amounts of protease.

Multiple Enzyme No. 3. This product contained a feed grade of multiple digestive enzymes. The activity was derived from plant and primary fermentation origin and was produced by culturing nonpathogenic microorganisms on media adapted to the production of multiple enzymes. The enzyme quantities per gram in this supplement, respectively, were as follows: protease, 7,500 P.V. units; amylase, 12,480 D.V. units; and "gumase", 28 units.

Multiple Enzyme No. 4. This product contained mainly amyloglucosidase which splits starch into glucose. The supplement used in the study reported herein was obtained by the fermentation of a special strain of Aspergillus niger, and it assayed about 160 units per gram. One unit was defined as the amount of enzyme that will catalyze, under the assay conditions, the production of 1 gm. of dextrose in 1 hr.

1 Department of Animal Sciences Journal Paper No. 2664, Purdue University Agricultural Experiment Station.
2 Present address: Department of Animal Sciences, University of Kentucky, Lexington, Kentucky.
3 Multiple enzyme No. 1 (Agrozyme-MK-124), supplied by Merck and Co., Rahway, N. J.
4 Multiple enzyme No. 2 (HT-550F), supplied by Miles Chemical Co., Clifton, N. J.
5 Zymo-Pabst, supplied by Pabst Brewing Co., Milwaukee, Wisconsin.
6 Diazyme, supplied by Miles Chemical Company, Clifton, N. J.
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This product also contained traces of unidentified enzymes.

Protease. This was a standardized product containing a specific quantity of proteolytic enzymes and appreciable but variable amounts of amylase. It was obtained by culturing a selected strain of Bacillus subtilis under conditions favorable for maximum production of protease. The enzyme concentrate was standardized by diluting it to a protease level of 200 NU (Northrop Units) per gram. Cereal flour provided the carrier for the enzymes. The assay involved colorimetrically determining the extent of peptide hydrolysis of a specific casein substrate and converting the values to Northrop Units by using a standard of known Northrop potency.

Cellulase. An enzyme product obtained by cultivating a special strain of Aspergillus niger was the source of cellulase employed. Assay of the enzyme material indicated that, in addition to cellulase, it contained traces of several other enzymes.

Feeding Trial I. Two lots of 14 yearling cattle each were fed a full feed of shelled corn, 908 gm. of Purdue Supplement A 9 and limited sorghum silage as a roughage for 230 days. One lot served as a control, and a second lot received 3.4 gm. of multiple enzyme No. 1 per head daily.

Feeding Trial II. In a 210-day pasture fattening trial, 47 yearling cattle were divided into three lots of 12 and one lot of 11 each. An attempt to get the cattle on a full feed of ground ear corn by hand feeding twice daily was unsuccessful; thus, at the end of 42 days the cattle were switched to a mixed ration consisting of eight parts of ground ear corn to one part of Purdue Supplement A. This mixed ration was supplied ad libitum. Three products were compared: multiple enzymes No. 1, No. 2 and No. 3. Purdue Supplement A served as the carrier for the enzyme products. Because of variations in total feed consumption, the average intake of the enzyme products varied accordingly. The average intakes of the multiple enzyme products per steer daily were as follows: No. 1, 3.9 gm. and No. 2 and No. 3, 3.7 gm. each.

Feeding Trial III. Seventy-one yearling steers, divided into 11 lots of six and one lot of five each, were used in replicated treatments to compare two levels of multiple enzyme No. 1, 3.4 gm. and 1.1 gm. per head daily, and 3.4 gm. per head daily of each of enzymes No. 2, No. 3 and No. 4, with a control treatment receiving no supplemental enzymes. The rations fed in drylot for 117 days consisted of from 4.5 to 5 kg. of ground shelled corn, 7 to 8 kg. of corn silage and 0.9 kg. of Purdue Supplement A per head daily.

Digestion Trials I through VI. Two sets of identical twin beef animals were used in each digestion trial. An equalized-paired feeding system was employed in six digestion trials. The basal ration employed was ground corn cobs, according to the appetite of the animal of each pair consuming the least amount, plus 0.9 kg. of Purdue Supplement A. In two of the trials a minimal level of corn was substituted for the supplement, since a proteolytic enzyme was being tested. Treatments are listed in table 2.

Two sets of identical twin calves were used in each digestion trial. The trials were conducted in replicates with the experimental diets identical within replicates, but the rations were switched in each replication. An equalized paired-feeding system was employed in all digestion trials. Calves were placed in the metabolism stalls at least 1 week before the initial collection. Each time the twins were used on a subsequent trial, they were switched to the opposite stall to cancel any position effect, and an adjustment period of at least 1 week preceded each collection period. The duration of the collection period was 10 days. Nitrogen balance was determined only during digestion trials I and II and V and VI. Portions of the feces, which were collected and dried for all other analyses, were used for determining fecal nitrogen excretion. Urinary nitrogen was determined by using 5-ml. aliquots of fresh samples each day. Analyses were performed until duplicate values were obtained with no greater difference in nitrogen than 0.01% for urine and 0.03% for feces.

Results and Discussion

Feeding Trial I. During the 230 days of this trial, calves receiving 3.9 gm. of multiple enzyme No. 1 daily gained 36 gm. more per day than the control group. However, this difference was not significant. Feed requirements per kilogram of gain for the two groups were similar (9.38 vs. 9.35 kg.). The enzyme-supplemented calves consumed slightly more corn than the controls (table 1).

9 Protease (Milezyme P Concentrate 200-F8868) furnished by Miles Chemical Co., Elkhart, Ind.
8 Cellulase (Cellulase 200-F8894) furnished by Miles Chemical Co., Elkhart, Ind.
9 Contained: soybean meal, 65%; molasses, 14%; dehydrated alfalfa meal, 14%; steamed bonemeal, 5.2%; cobalt salt, 1.7%; and vitamin A, 22,000 I.U. per kg.
Feeding Trial II. This trial was designed to compare the effects of three multiple enzyme supplements (No. 1, No. 2 and No. 3). The average daily gains of the four lots were similar; the greatest difference between lots was 41 gm. The control group was more efficient in terms of feed required per kilogram of gain, except that the consumption of pasture forage was not included in any of the calculations (table 1).

Feeding Trial III. Average daily gain of calves in all six lots was approximately 0.9 kg. per day, with only slight variations. The calves fed multiple enzyme No. 3 and both groups receiving No. 1 gained 0.94 kg. per day, whereas the average daily gain for the remaining cattle, including the controls, was 0.89 kg. per day. However, the difference was not significant.

Feeding Trial IV. Enzyme concentrates suppressed average daily feed consumption from 309 to 808 gm. Since all other ingredients were held at a constant level, these values represented differences in consumption of corn. Differences in average daily gains of the various experimental groups ranged from 64 to 186 gm. The depressed growth rates resulting from supplemental enzymes varied from 6.4% for multiple enzyme No. 4 to 18.7% for No. 1. Analysis of the data indicated multiple enzyme No. 1 significantly (P<.05) reduced gains, whereas the differences for other treatments were not significant (table 1).

Digestion Trials I and II. Digestibility of dry matter, crude protein and energy was higher (P<.05) for all calves receiving supplemental enzymes than for their respective controls. Differences in digestibility of these constituents between the control and enzyme-treated animals varied from 0.56 to 6.67%. The coefficients of apparent digestibility of NFE were almost identical within one treatment and control set of twins, but within the remaining experimental groups differences were consistently in favor of the enzyme treatments. The amount of nitrogen retained was higher for the steers receiving enzymes in both trials but lower in the heifer comparisons.

Analysis of the data indicated there was no significant difference in the effects of multiple enzyme No. 1 and No. 2, thus permitting a pooling of the results from the two enzyme treatments (table 2).

Digestion Trials III and IV. Statistical analysis of the combined data from the two trials showed that cellulase had no significant effect on digestible energy or on any of the coefficients of apparent digestibility. However,

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a Multiple enzyme No. 1, Agrozyme; No. 2, Takamine HT-550F; No. 3, Zymo-Pabst; No. 4, Diazyme. Enzyme mixtures were fed at levels of 3.4 gm. per head daily except for cattle in lots 2, 3 and 4 of Trial II, which received 3.9, 4.2 and 3.7 gm., respectively. Lots 7 and 8 of Trial III received 1.1 gm. of multiple enzyme No. 1, per head, daily.

b Feed intake in Trial II was not included in the calculation.

c All feeds were converted to 13% moisture equivalent.

* Significantly (P<.05) different from the control.
this analysis revealed there were greater variations within sets of twins than between twin pairs in ability to digest crude protein, ether extract, NFE and energy. Although the mean-square values for variations within sets of twins were not larger than those between pairs of twins for crude fiber and dry matter digestibilities, total variations between animals in inherent capacity to utilize these constituents of the feed were quite large. For these reasons a second statistical analysis was performed.

Since each animal was maintained on both the control and treatment rations during the separate periods and since there were no significant differences in the results from different periods, each animal was used as its own control for this analysis. The interactions for variations within the animals were not significant; therefore, the data were pooled. The pooled analysis of variance showed that cellulase significantly (P < .05) increased digestibility of the crude fiber fraction. The same enzyme had no significant effect on any of the other ration components analyzed (table 2).

**Digestion Trials V and VI (Protease).** The effects of protease enzyme on nitrogen retention and on apparent digestibility of nutrients and energy were measured with the same two sets of identical twins used in the two preceding digestion trials.

The average daily fecal nitrogen excreted by both the control and treatment heifers was not less than 26 gm. nor greater than 27 gm. in both trials. Similarly there were only slight variations within the steers during both of their trials. Therefore, differences in nitrogen retention were due almost entirely to the amount of nitrogen excreted in the urine. Statistical analysis showed the differences in nitrogen excreted between control and treatment were not significant (table 2).

Apparent digestibility of dry matter varied only slightly during both trials and was not affected consistently by protease. Since the digestion coefficients for crude protein were calculated from the nitrogen balance data, differences between coefficients followed the same pattern as for grams of fecal nitrogen.

Since, according to Chambers and Synge (1954), nitrogen balance is quite variable among animals, each animal was used as its own control in statistical analysis. The pooled statistical analysis showed that protease had no significant effect on the amount of nitrogen retained or apparent digestibility of crude protein. Similarly, the digestibility of dry matter, crude fiber, ether extract, NFE and energy balance was unaffected.

The failure to show relatively consistent growth stimulation from enzymes in these experiments is in contrast to the work reported by Burroughs et al. (1960), in which supplemental enzymes stimulated gains an average of 7% in 10 feedlot experiments.

The finding that cellulase increased the digestibility of crude fiber is consistent with the observations by Bowden and Church (1959), who showed that the in vitro digestion of purified cellulose, and of the cellulose of barley straw was improved by a fungal cellulase. In addition, Leatherwood et al. (1960) noted that cellulase greatly enhanced the cellulolytic activity of rumen fluids in vitro.

The results of the digestion trials with protease indicated it had no significant effect.
Summary

Four experiments were conducted on the effects of multiple enzyme preparations on growth rate and feed efficiency of cattle fed a full feed of corn plus 908 gm. of a 32% cattle supplement and a limited amount of roughage. Subsequently, the effects of cellulase, protease and multiple enzyme products on ration digestibility were measured in six digestion trials with identical twin calves. The rations used in all digestion trials were high in ground corn cobs.

In general, the enzyme treatments were ineffective in producing a significant improvement in growth rate or efficiency of feed conversion.

Multiple enzyme preparations significantly (P<.05) improved apparent digestibility of crude protein, dry matter and energy of a high fiber ration.

Although the cellulase preparation (4.0 gm. daily) tended to increase the digestibility of dry matter, crude protein, NFE and energy, only the digestibility of crude fiber was significantly (P<.05) increased by this enzyme.

The addition of 1 gm. of protease per calf daily to a ration composed of ground corn cobs, ground corn and protein supplement was of no measurable value in increasing nitrogen retention or apparent digestibility of nutrients, dry matter or energy.

There were greater variations within than between sets of identical twin calves in digestibility of certain ration components. This observation casts some doubt on the validity of using small numbers of twins or of nontwins in digestion studies without using a reversal design.

Literature Cited


