Determination of the Nutritional Requirements of the Calf's Gut

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Treatment of diarrheic calves mainly relies on electrolyte solutions to replace the fluid lost in the scours. Antibiotics are also used, but they only work against a few of the many causes of scours. These treatments do not promote healing of the gut. Instead, they keep the calf alive so that the body can slowly heal itself. We have shown that scouring calves have damage to the lining of the gut and reduced absorption of nutrients. We know that holding animals off feed results in atrophy of the gut. If we knew which nutrients the gut uses, we could speed healing by adding the required nutrients to an electrolyte mix. The purpose of our study was to determine the intravenous nutritional requirements of the calf's gut by infusion of several potential nutrient and energy sources.

Five healthy male Holstein calves were acquired from local dairy. All calves were obtained within a week of age. They had been fed colostrum (5% of the body weight at birth). The calves were then fed twice daily fresh cow's milk (10% of the body weight). The femoral artery, jugular vein and the portal vein were cannulated to measure nutritional gut uptake. Calves were allowed at least five days to recover from surgery. Blood flow was measured using an ultrasonic probe placed around the portal vein. Experiments consisting of infusion three nutrients and a control were carried out both at rest (fasting) and in the working state. To mimic work, the calves were fed an electrolyte solution mix without an energy source. No milk was fed for 16 h before each infusion. There was at least one day of rest between each experiment. In each experiment, four different infusions of glucose, acetate, glutamine, and saline (control) were administered intravenously at a rate of 200 mmol/L/h over 1 h. The order of these infusions were randomized. Blood samples were collected in the last 15 min of an infusion. There was a period of at least 30 min between each infusions to allow the calf to adapt to the new nutrient. At the end of the experiments, the calves were euthanized and the proper placement of the catheters were confirmed on necropsy.

Plasma glutamine, all the essential amino acids, -hydroxybutyrate, acetate and pyruvate concentrations were quantitatively determined by reverse-phase high performance liquid chromatography. Plasma ammonia and glucose concentrations were determined by spectrophotometry.

Our study shows that glucose and glutamine are important sources of energy for the gut in the fasted state prior to any infusions. No significant intestinal uptake of acetate, -hydroxybutyrate, or any essential amino acids were found in all the infusion studies. However, when glutamine was infused there was an increased intestinal glutamine uptake which was associated with ammonia production. This is important because glutamine infusion stimulates use of glutamine by the gut (as shown by additional production of ammonia a breakdown product of glutamine). Infusing glucose was not associated with increased glucose uptake in both groups. Glutamine has been categorized as a nonessential amino acid. Recently, glutamine has been suggested to be beneficial in the regeneration of the damaged gut. This work has been performed in healthy calves, but may have implications in the development of treatment to promote gut healing in diarrheic calves.

Dectomax Discovery: A Review of the Scientific Approach to Long Duration Endectocide Activity

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Abstract

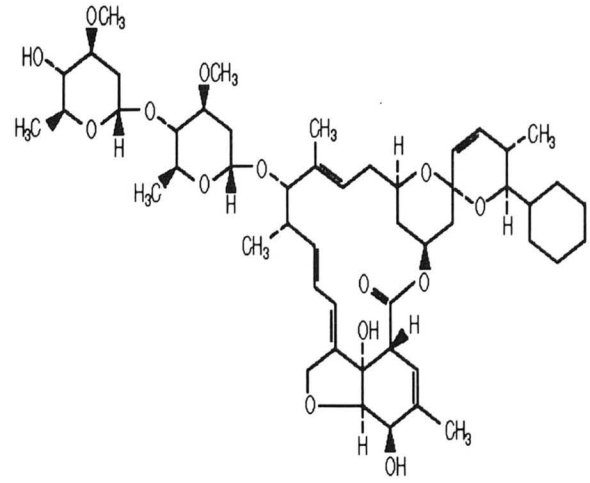
Approximately 10 years ago, PfizerAnimal Health resolved to discover and develop a novel parasiticide active against internal and external parasites that was superior to currently marketed parasiticides in spectrum of activity and time of protection from reinfection. Several scientific teams of researchers were assembled

to achieve this goal through a series of approaches including innovative fermentation research. These scientists discovered a series of key enzymes in a complex biochemical pathway that controlled the production of avermectins by *Streptomyces avermitilis*. Modification of one of these enzymes through mutational techniques allowed the scientists to alter the known end-products, and with subsequent novel substrate feeding, allowed them to create a series of novel avermectin analogs. Biological screens were then utilized to select the "best" analog against parasites in non-target and target hosts. Concomitant with the biological testing, pharmacokinetic profiles were generated to select the "best" formulation of the "best" analog for therapeutic activity and time of protection from reinfection. The end result was Dectomax, a novel endectocide for treatment and long-acting protection against economically important, internal and external parasites of cattle.

The gold-standard of antiparasitic products for the past decade has been a semi-synthetic analog of a tertiary end-product of fermentation by an actinomycete, *Streptomyces avermitilis*. This natural fermentation product was isolated and characterized in 1977, and since then no additional naturally occurring avermectins have been discovered. This product is considered the gold-standard because it has excellent therapeutic activity against nearly all economically-important parasites of cattle, sheep, horses and swine. In addition, because of its non-polar nature, it remains in the plasma for an extended period of time and protects against reinfection when animals remain in an environment where they are continuously challenged.

Pfizer Animal Health resolved to discover an even more effective antiparasitic product. Pfizer fermentation chemists began this process by looking for novel avermectins through analyses of the enzyme pathways in the terminal metabolic steps in the avermectin synthesis. They found a decarboxylase that was critical to the formation of the branched-chain fatty acid at the 26 position of the naturally-occurring avermectin B1. Using targeted mutagenesis to make a decarboxylase-free mutant, they then were able to redirect end-product synthesis to make previously unknown analogs. Following that discovery, additional substrates were utilized to direct the synthesis of a series of novel end products for further antiparasitic testing and analog selection.

Since Pfizer now had a series of analogs to test, biological systems were set up to test first therapeutic activity and then protective activity against important parasites. To aid in the process of selecting a molecule that had the longest protective activity, pharmacokinetic profiles of the analogs were also utilized. When this process was completed, a 25-cyclohexyl avermectin B1 analog, doramectin (Figure 1), was seen to be superior for both therapeutic and protective activity when all molecules tested were in an aqueous micelle formulation, and the pharmacokinetic (PK) profile confirmed a solid relationship between plasma residence time and protection time.



Source: A. C. Goudie et al. / Veterinary Parasitology 49 (1993)

Figure 1. The Structure of Doramectin

A final product is not just the molecule, but it is the molecule in its commercial formulation. Therefore, a series of studies were subsequently designed to develop an injectable formulation given subcutaneously that would enhance the basic activity of the molecule with careful attention to the length of protection provided. The initial screening was done in an aqueous micelle that allowed relatively rapid absorption and excretion of doramectin. Since the molecule required a non-polar solvent, a series of oils and oil mixtures were then utilized to obtain the optimal maximum plasma concentration (Cmax) and increase the plasma residence time (t 1/2) with a goal of a greater bioavailability as estimated by the area under the PK curve (AUC). This end-point was achieved using a sesame oil:ethyl oleate mixture (Figure 2).

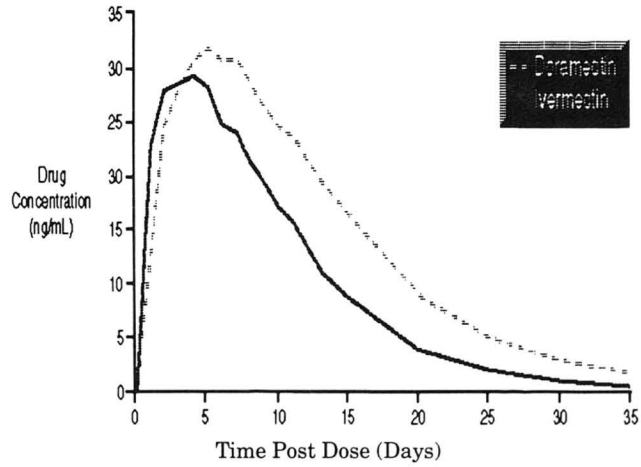
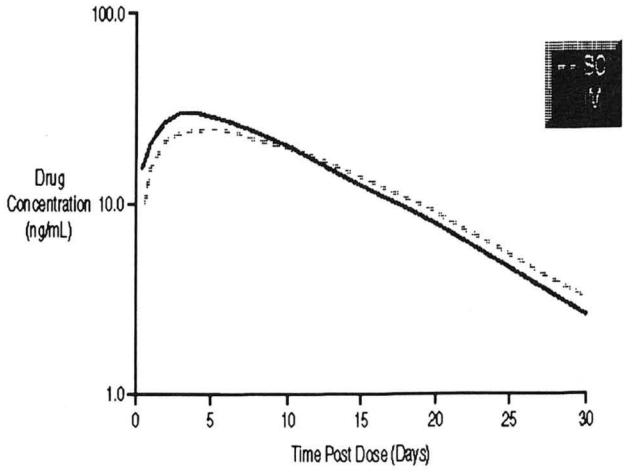


Figure 2. Mean Concentration of Doramectin and Ivermectin in Bovine Plasma After Subcutaneous Administration of 200 μg/kg

An added bonus to this formulation was seen when subcutaneous (SC) administration was compared with intramuscular (IM) administration. The shape of the curve and the AUC of these administrations were identical (Figure 3). If an injection intended for SC administration were inadvertently given IM, doramectin would be equally

bioavailable with either route and no reaction would be expected at the injection site.



Source: Nowakowski et al, 1995. Journal of Veterinary Pharmacology and Therapeutics, In Press

Figure 3. Mean Concentration of Doramectin in Bovine Plasma After Intramuscular (IM) or Subcutaneous (SC) Administration of a Single Dose of 200 µg/kg

With the successful completion of the early discovery program and the formulation development, world wide trial programs of therapeutic and protective efficacy have been conducted in at least 20 countries with over a total of 1,000 trials. An example of laboratory protection from reinfection data can be seen on Table 1 with 3 important nematode species. Protection periods of 21 to 42 days with both internal and external parasites under artificial challenge conditions allow for the development of parasite control programs with minimum treatment frequencies.

Table 1. Duration of Persistent Efficacy of Doramectin Against Nematode infections of Cattle

Post-treatment Challenge period (Days)	Percentage Reduction in Worm Burden (Doramectin vs Control)		
	Ostertagia. ostertagi	*Cooperia oncophora*	*Dictyocaulus viviparus*
14	99.9	99.2	-
21	99.9	90.7	100
28	93.7	-	99.9

Source: Weatherley et al, 1993. Veterinary Parasitology 49: 45-50

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Equivalent Persistent Efficacy of Ivermectin, Abamectin, Doramectin and Moxidectin in Cattle

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As one of the major goals in strategic parasite control is to minimize the level of pasture contamination, post treatment fecal egg count (FEC) reduction provides a good indicator of the relative utility of antiparasitic compounds under field conditions. A series of 8 studies, involving more than 500 cattle, was undertaken over a two year period in Latin America, Ireland and South Africa to compare the efficacy of commercial endectocides in maintaining reduced FECs in cattle grazing naturally infested pastures. Within each study, cattle of similar breed and age were ranked and blocked on the basis of either pre-treatment FECs or body weights and randomly allocated among treatment groups. One group was untreated (in the trial in Ireland, the control group was treated with oxfendazole) while each of the others was allocated to treatment with ivermectin, moxidectin, doramectin or abamectin. All treatments were administered according to label recommendations to provide a minimum dose of 200 mcg/kg. In each trial, all groups shared the same pasture. Fecal samples were collected at approximately weekly intervals between weeks 3 and 9 post treatment and processed to determine the number of nematode eggs. Eggs tended to appear earliest in moxidectin-treated groups, and from week 5 eggs generally appeared at a low but increasing rate in feces of all medicated groups. In the South American and South African trials there was no significant difference in FECs among the treated groups at weeks 6, 7, 8 and 9, but counts in treated groups were significantly less ($p<0.05$) than those in the untreated controls. In the trial in Ireland, only the ivermectin-treated group showed significantly reduced fecal egg counts, relative to the moxidectin-treated group. Based on larval differentiation, challenge in all studies was predominantly *Cooperia*, with substantial proportions of *Ostertagia* present and some *Haemonchus*. The results of these studies demonstrate that on the basis of fecal egg count reductions under varied conditions of natural challenge on three continents, there is no functional difference in the persistent activity of subcutaneously administered ivermectin, doramectin, moxidectin or abamectin against gastrointestinal parasites of cattle.