Clotting Factor in Bovine Preruminant Nutrition

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Introduction

During the process of digestion in the preruminant calf, the casein protein in cows' milk clots in the abomasum due to the action of the enzymes pepsin and rennin (chymosin) and hydrochloric acid. The importance of this clotting mechanism has been studied, but the physiological implications are not fully understood. Today in the United States, many calves are raised very successfully on milk replacers that do not contain casein protein because skim milk powder, which is the main source of casein, is prohibitively expensive. Instead, whey proteins are the predominant source of milk proteins in calf milk replacers. Research indicates that whey proteins, which do not clot in the abomasum, are as efficacious as casein proteins in the diet of the preruminant calf.

The subject of "clotting" has become topical due to an attempt through the National Dairy Heifer Evaluation Program (which is a part of the National Animal Health Monitoring System) to evaluate milk replacers on farms by a rennet clotting test. This test was developed in Europe to determine the presence of heat denatured skim milk in calf milk replacers and has no relevance to milk replacers formulated on the basis of whey proteins.

This paper reviews the physiology of digestion in the calf, discusses the implications of milk clotting and addresses the issues of modern milk replacer formulations.

Digestion In The Calf

The digestive system of the preruminant calf is well suited to digest milk and the digestive processes have been extensively reviewed[1].

In the young calf, milk by-passes the poorly developed rumen and reaches the abomasum (or milk stomach) via the esophageal groove. Closure of the groove is a reflex reaction in response to one or a number of stimuli, including the presence of liquid milk, or even the presence of the calf feeder alone. Casein, which constitutes approximately 80% of the protein in cows' milk, begins to clot in the abomasum within 1 to 10 minutes after ingestion of a meal. Clotting occurs primarily through the action of the proteolytic enzyme chymosin, with a small contribution from pepsin and HCl. The pH of the abomasum increases to 6.0 to 6.5 immediately after milk feeding and then gradually decreases to 1.5 to 2 by 8 to 10 hours after feeding. Coagulation activity of rennin is greatest at pH 6.5, whereas proteolytic activity is

greatest at 3.5 for rennin and 2.1 for pepsin[2].

Clotting occurs as a result of hydrolysis of specific peptide bonds in k-casein in the presence of calcium. This results in release of a glycoprotein from the k-casein. Clotting of the casein entraps most of the milk fat in the curd, but lactose and whey proteins are excluded as the clot contracts. Further motility of the abomasum helps to release whey proteins from the curd. For calves given one or two feeds daily most of the whey passes from the abomasum within 4 hours after ingestion.

The milk fat is retained in the abomasal clot and is subjected to the action of the lipid enzyme pre-gastric esterase, which is found in saliva. Pre-gastric esterase is more specific to butter fat than other fats used to replace it in calf milk replacers.

As the clot is digested by the enzymes the partially digested casein and lipid are released into the small intestine. Significant release of partially digested casein begins about 4 hours post-feeding. The result of abomasal clotting is thus a fairly continuous flow over 24 hours of partially digested proteins, peptides and lipid products, with the main passage between 4 and 10 hours after a feed. Some of the clot may be retained for as much as 16 hours after feeding and therefore becomes part of the clot from the next meal.

As material passes from the abomasum to duodenum it is acted on by intestinal and pancreatic enzymes. The greatest secretion of the pancreatic enzymes occurs 2 hours after feeding.

It might seem that abomasal clotting is an essential part of the digestion of cows' milk by the young calf, but the calf and lamb are unique in their mothers' milk forming a firm clot in the abomasum. Milk of other species such as the pig and human does not form a firm clot[3].

With the predominance of casein in cows' milk it might be argued that the ability of casein to form a clot in the abomasum is a specific adaptation for its digestion and that coagulation and partial abomasal digestion would have little effect on the other proteins which do not clot and are excluded from the curd. It has been shown that degradation of casein to peptides in the abomasum is extensive, whereas only a portion of the α-lactalbumin and none of the β-lactoglobulin are hydrolysed in the abomasum[4].

Arguments have been used to support the assumption that suppression of curd formation would result in more rapid flow of undigested protein into the duodenum, overwhelming the digestive capacity of the intestine and causing diarrhea. It is now generally accepted that merely increasing the rate of entry of material into the duodenum does not cause diarrhea.

Prevention and Importance of Clotting

Experimental procedures to test the importance of clotting have included acidification to prevent coagulation, chelation of calcium ions with citrate, infusion of milk replacer into the duodenum, pretreating milk or milk replacer with pancreatin, and use of an oxalate-sodium hydroxide buffer.

In diets containing high levels of skim milk powder (>60%) prevention of clotting by addition of citrate or hydrochloric acid did result in decreased digestibilities of dry matter, crude protein and lipids. For citrate the depression occurred during days 14 through 20, but not days 23 to 34, whereas for hydrochloric acid the depression occurred through day 48[5]. The use of acid in these trials can be criticized because of interference with intake and other digestive mechanisms. Studies to prevent clotting by addition of pancreatin secretion prior to feeding are difficult to interpret because the protein was already partially hydrolysed and denatured prior to feeding.

A more thorough series of experiments by Brisson and coworkers has been undertaken using an oxalate and sodium hydroxide buffer to prevent coagulation of milk replacers[6]. Oxalate chelates calcium ions and thus prevents the chymosin-mediated coagulation of casein; however, addition of more calcium ions restores the clotting ability and thus the protein is not denatured.

To test the effects of the oxalate buffer alone on growth and metabolism of calves two groups (3-5 days of age) were fed a non-clotting milk replacer based on skim milk which had been heat treated to specifically prevent clotting. One group of calves was given the milk replacer pre-treated with the oxalate buffer. Oxalate treatment did not affect blood concentration of any metabolites except calcium (which of course the oxalate had chelated and precipitated). It was concluded that the oxalate buffer did not alter digestion or metabolism in young calves.

There then followed a series of experiments by the same group of workers who used low-heat skim milk powder (which did clot), fed with or without the oxalate buffer[7,8]. Flows of protein nitrogen, total nitrogen and fat were more rapid after feeding the non-clotting replacer, indicating a more rapid gastric emptying. Digestibilities of dry matter, crude protein and lipid measured during week 3 of life were not different between calves fed the control and non-clotting milk replacers.

A growth trial was also conducted in which four groups of 7 calves were fed either whole milk or milk replacer, both with and without oxalate buffer to inhibit coagulation. The milk replacer contained 40% skim milk powder. Calves were fed one of the four diets for 28 days. Inhibition of coagulation did not decrease gains from either whole milk or milk replacer. Diarrhea was not a problem with any treatment.

The conclusion from this group of experiments is that while inhibition of coagulation affects patterns of nutrient flow from the abomasum, there is minimal effect on nutrient digestibilities or body weight gain in calves less than 3 weeks of age.

Much of the confusion over the effects of "non-clotting" proteins in milk replacer has arisen out of the problems of using severely heated skim milk powder as the protein source. In the early years of milk replacer development (1950's and 1960's) use of skim milk powders subjected to severe heating during the drying process resulted in poor calf performance and high incidence of diarrhea. A series of studies by Roy[9] and colleagues established that the underlying cause of excessive scours and poor growth in

calves fed severely heated skim milk powder was the denaturation of whey proteins. Excessive heat denatures the whey proteins, exposing sulfhydryl bonds, and appears to cause an interaction between β-lactoglobulin and β-casein that prevents chymosin induced coagulation. Denatured whey proteins also bind ionizable calcium, making it unavailable for the clotting of casein.

The increase of clotting time or lack of clotting with high heat treated skim milk led to decreased abomasal outflow during the first hour after feeding, increased abomasal pH and increased passage to the small intestine of casein that had not been first hydrolysed to peptides. The overall biological value of the proteins, however, was not decreased indicating that the denaturation of the proteins did not alter utilization of absorbed amino acids. Roy concluded that the use of high-heat skim milk powders was associated with as much as a 30% decrease in weight gain during the first 3 weeks of life.

We still do not have a complete understanding of why heat-treated skim milk causes problems. Roy speculated that the more rapid abomasal outflow of undigested protein at an increased pH might allow overgrowth of pathogenic bacteria that would cause diarrhea. It likely that the full reasons for non-clotting of heat-treated skim milk and its relation to diarrhea and calf performance are complex, but it is clear that the simple prevention of clotting of undenatured protein has little effect on calf performance and health. Therefore, much of the concern over "non-clotting" has no bearing on any situation other than incorporation of high levels of heat-treated skim milk in milk replacers.

Tests For Clotting of Milk Replacers

The experiments of Roy and co-workers in the 1960's emphasized the importance of having undenatured whey proteins in skim-milk powder to ensure that the skim milk powder clotted. It became common to measure the concentration of undenatured whey proteins in skim-milk powder. Two tests are used for this purpose. In the United Kingdom, the Rowland method of noncasein nitrogen determination was used, whereas in the USA the Harland-Ashworth method was used[10,11]. Heat-denatured whey proteins lose their solubility and will precipitate like casein in either of these tests. Therefore, low whey-protein nitrogen (WPN) values in reconstituted skim-milk powders denote heat damage. The American Dry Milk Institute grading system classifies milk powders as high-heat (<1.5 mg WPN per g powder), medium-heat (>1.5 <6.0 mg WPN per g powder), or low-heat (>6.0 mg WPN per g powder).

In the late 1960's it also became common to measure the in vitro rennet-clotting ability of skim-milk powders and milk replacers[12]. Because the undenatured whey-protein tests could not be applied to milk replacers containing whey or non-milk proteins the rennet coagulation test became standard for evaluating the "quality" of milk replacers. By the late 1970's "quality" had become synonymous with "ability to clot in vitro" and, presumably, in vivo. Many factors were found to be important in establishing a standardized rennet clotting test, such as temperature of water used for reconstitution, pH, and rennet concentration.

Modern Milk Replacer Formulation

Within the last decade, rapid advances in milk protein technology have created new opportunities for different milk protein sources in the milk replacer industry. At the same time, new processing techniques for a wide range of non-milk proteins have been developed and there are now a number of non-milk proteins available for use in milk replacers.

Skim milk has continued to be a very high-priced protein source. Demand for its use in animal feeds has been restricted on an economic basis except where it carries government subsidies for incorporation in milk replacers (as in Europe and in the US in the mid 1980's).

The specific technological advance that has made the largest impact on milk replacer formulation is the ability through the ultra-filtration process to remove lactose and the other soluble components from raw whey. By doing this, whey proteins can be concentrated up to high levels. The resultant whey protein concentrate is then evaporated at a low temperature to prevent protein denaturation and spray-dried again at low temperatures. This whey protein concentrate powder contains highly soluble proteins. By definition these proteins do not clot in the abomasum.

In recent studies researchers have fed preruminant calves milk replacers based on whey protein concentrate and other high protein whey by-products, which have a low degree of heat denaturation. Glas reported that European use of all-whey protein milk replacers had increased tremendously, especially in the Netherlands[13]. Calf performance compared favorably with more traditional milk replacer, and calves tolerated high concentrations of lactose, nitrates, sodium and potassium in such replacers providing that whey proteins are not denatured and that milk coagulating enzymes are excluded from the whey preparations.

There are a number of studies reported that have examined calf performance where skim milk powder was replaced with whey protein concentrate[15].

Results of three major studies comparing skim milk versus whey protein concentrate and involving over 600 calves have been reported by Milk Specialties. Calves received only milk replacer during the trial periods so the results are not confounded by intake of dry feed. There were no significant differences in growth rate or feed conversion for calves fed skim milk or whey protein concentrate. Growth rates in the three trials were 0.80 v 0.75, 0.6 v 0.6 and 0.57 v 0.56 kg/d for skim milk versus whey protein concentrate respectively[17].

In addition to the growing use of whey proteins, many of the modern milk replacer formulations contain up to 50% replacement of milk proteins with proteins from the soybean or other plant proteins, including wheat and potato. It is clear that as long as amino acids are balanced and antinutritional factors are reduced, calf performance is very satisfactory!

Summary

"Non-clotting" of a milk replacer is only of concern if the

cause is severe heat treatment of the skim-milk powder which has resulted in denaturation of the whey proteins. Lack of clotting of a milk replacer which is formulated without casein protein is of no concern from a physiological or calf health standpoint.

References

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