ABSTRACT

Two experiments involving 3- to 5-d-old dairy calves were carried out. In Experiment 1, lime-treated corn flour (Nixtamal) supplied 50 to 100% of carbohydrates in a milk substitute based on sodium caseinate, lard, and cerelose. In Experiment 2, partially hydrolyzed fish protein concentrate replaced 50% of proteins in milk substitutes based on skim milk powder, lard, and 35% Nixtamal. Increasing the proportion of carbohydrates supplied by Nixtamal was associated with a linear decrease of postprandial serum glucose and insulin. Postprandial fluctuations in blood glucose were less in calves fed Nixtamal than in controls. Nixtamal probably was trapped within the casein clot in the abomasum, leading to delayed rate of passage of Nixtamal carbohydrates into the intestine. Replacing skim milk protein with hydrolyzed fish protein in diets containing Nixtamal had no effect on blood glucose or insulin but elevated free essential amino acids, which promoted glucagon secretion. More uniform concentrations of blood essential amino acids and glucose were related to lower blood urea at 54 d in calves fed diets based on hydrolyzed fish protein and Nixtamal, than that of control calves. It is suggested that more uniform postprandial blood glucose concentrations might reduce amino acid degradation for energy purposes and stimulate protein synthesis. Young dairy calves may adapt to milk substitutes based on Nixtamal and hydrolyzed fish protein despite changes in the concentration patterns of several blood components.

INTRODUCTION

Successfully replacing skim milk powder with other protein and energy sources in milk substitutes for preruminant calves is of practical importance. To that end, a lime-treated corn flour, Nixtamal, may replace up to 50% of skim milk carbohydrates without detrimental effects on growth rate and feed efficiency (7). As alternative protein sources, various fish protein products appear promising (7, 14, 21). Because both Nixtamal and partially hydrolyzed fish proteins are cheaper sources of energy and protein than skim milk powder, it was of interest to investigate further these ingredients in the diet of young dairy calves.

Skim milk powder obtained by low heat processing retains its ability to form a coagulum in the abomasum of young calves (5, 23). Coagulation tests in vitro show that Nixtamal remains associated with the casein-fat clot when it is incorporated in a milk substitute based on low heat skim milk powder, but a viscous flocculate is formed when partially hydrolyzed fish protein concentrate (HFP) is the main source of protein in such substitutes (7). The lack of coagulation of a milk substitute in the abomasum is associated with modifications in the flow rate of certain components into the small intestine (5, 13, 22) and with changes in the concentrations of some blood components (23).
TABLE 1. Composition of milk substitutes used in Experiment 1

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>27C (%)</th>
<th>54C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nixtamal*</td>
<td>. .</td>
<td>27</td>
<td>54</td>
</tr>
<tr>
<td>Sodium caseinate</td>
<td>28</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>Lard</td>
<td>21</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Cerelose</td>
<td>49</td>
<td>25</td>
<td>. .</td>
</tr>
</tbody>
</table>

* Nixtamal at 27% (27C) or 54% (54C).

Each diet contained .7% soya lecithin and 1.3% vitamin-mineral mixture. The vitamin-mineral mixture supplied (mg/kg DM): CuSO4, 25.0; CoSO4·7H2O, .52; MnO2, 63.5; ZnO, 50.3; KI, .33; SeO2, .14; FeSO4·7H2O, 498; MgO, 1160; biotin, .75; folic acid, .1; thiamine, .6; nicotinic acid, .22; ascorbic acid, 5.0; pyridoxine, 1.3; riboflavin, 2.2; calcium panthotenate, 70.0; niacin, 220; vitamin K, 35; vitamin B12 88 μg/kg; vitamin A, 40,000 IU/kg; vitamin D3 8000 IU/kg, and vitamin E, 52 IU/kg.

The objective of this study was to replace clot-forming skim milk powder with Nixtamal and HFP in milk substitutes for dairy young calves and measure the effects on postprandial concentrations of blood glucose, insulin, glucagon, triglycerides, free amino acids, and urea.

MATERIALS AND METHODS

Fifteen 3- to 5-d-old Holstein male calves in Experiment 1 and 20 in Experiment 2 were randomly allocated to three and four treatments, respectively (Tables 1 and 2). They were housed in individual wooden cages with slatted floors and placed in a room where temperature was 20°C and relative humidity was 60%. All animals were fed the experimental diets exclusively for 8 wk. Amounts of milk substitute and water were adjusted to age and individual body weight as previously described (7). Reconstituted milk substitutes were offered in open pails at body temperature twice daily at 0830 and 1630 h.

In Experiment 1, lime-treated corn flour, designated Nixtamal (Compania de Subsistencia Populares, Mexico, supplied by Universidad Nacional Autonoma de Mexico, Mexico, D. F. 04510, Mexico) was used at the rate of 27 or 54% DM basis, to replace cerelose partly or completely in milk substitutes based on casein and lard (Table 1). The preparation of Nixtamal and its chemical composition were previously reported (7). Lard was added to milk substitutes as a homogenized spray-dried high fat premix (7).

In Experiment 2, Nixtamal and HFP (CPSP-90 Coop'érative de Traitement des Produits de la Pêche, Boulogne s/Mer, France, supplied by Sopropeche Canada, Inc. Hamilton, Ont., Can.) replaced skim milk powder to the extent of 66.6% of milk substitutes originally based on skim milk powder, cerelose, and lard (Table 2). The chemical composition of the HFP was 85.2% CP, 6.8% ether extract, and 6.2% ash (DM basis). Milk substitutes were prepared as in Experiment 1; low heat skim milk powder instead of sodium caseinate was used in the high fat premix.

Blood samples were drawn by venipuncture from the jugular vein on d 8 and 54. On these days, the second feeding was delayed until blood sampling was completed. In Experiment 1, blood samples were taken prior to morning feeding and at .5, 1, 2, 3, 5, and 7 h postfeeding. In Experiment 2, blood was collected before morning feeding and at .5, 1, 2, 4, 6, 8, and 10 h postfeeding. Chilled evacuated tubes were used for blood collection. In Experiment 2, 400 K.I.U. (Kallikrein inactivating units) of aprotinin (Trasylol, Miles Pharmaceutical, Rexdale, Ontario, Can.) were added immediately after sampling. Blood was allowed to clot for 2 h at 4°C and then chill centrifuged at 3000 x g for 20 min. Serum was stored in 10-ml serum bottles (Fisher Scientific, Monetre, Que., Can.) at -20°C until needed for analyses.
TABLE 2. Composition of milk substitutes use in Experiment 2.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>35C/0F</th>
<th>35C/10.5F</th>
<th>35C/15F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nixtamal (C)</td>
<td>. . .</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Hydrolyzed fish protein (F)</td>
<td>. . .</td>
<td>. . .</td>
<td>10.5</td>
<td>15.0</td>
</tr>
<tr>
<td>High fat premix</td>
<td>37.0</td>
<td>33.5</td>
<td>36.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Skim milk powder</td>
<td>42.0</td>
<td>29.5</td>
<td>12.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Cerelose</td>
<td>19.0</td>
<td>. . .</td>
<td>4.5</td>
<td>11.0</td>
</tr>
</tbody>
</table>

Chemical composition, % DM

Protein (N x 6.25) | 23.1 | 22.2 | 24.3 | 24.1 |
Lipid            | 17.9 | 17.7 | 19.2 | 18.8 |
Ash              | 5.3  | 4.4  | 4.7  | 4.5  |

Amino acid (AA), g/kg DM

Total essential AA | 124.6 | 116.3 | 115.9 | 114.8 |
Lysine           | 16.4  | 14.4  | 15.6  | 16.0  |
Branched-chain AA | 50.6  | 47.5  | 43.9  | 41.9  |

1 Abbreviations: 35C = 35% Nixtamal; 0F, 10.5F, and 15F = 0, 10.5, and 15% partially hydrolyzed fish protein concentrate as CPSP-90, respectively.
2 Each diet contained .7% soya lecithin and 1.3% vitamin-mineral mixture. The vitamin-mineral mixture supplied (mg/kg DM): CuSO4, 25.0; CoSO4·7H2O, 52; MnO2, 63.5; ZnO, 50.3; KI, 33; SeO2, .14; FeSO4·7H2O, 498; MgO, 1160; biotin, .75; thiamine, .6; nicotinic acid, 22; ascorbic acid, 5.0; pyridoxine, 1.3; riboflavin, 2.2; calcium panthotenate, 70.0; niacin, 220; vitamin K, 35; vitamin B12 88 μg/kg; vitamin A, 40,000 IU/kg; vitamin D3 8000 IU/kg, and vitamin E, 52 IU/kg.

The nitrogen content of milk substitutes was measured by Kjeldahl method using a Kjel-Foss Automatic 16210 (A/S N. Foss Electric, Denmark). Coagulation or flocculation time of milk substitutes was measured using chymosin (EC 3.4.23.4; Renin, Sygma R-7751) according to Emmons et al. (9). Plasma glucose in Experiment 1 was measured using a commercial kit (Glucose GOD-PAP No. 166391, Boehringer Mannheim, Canada Ltd.), and in Experiment 2 according to the method of Richterich (25). Serum insulin was analyzed by radioimmunoassay using rat insulin as standard, and polyethylene glycol to separate free and antibody bound insulin (6). Serum triglycerides were determined using a commercial kit (Peridochrome Triglycerides No. 575429, Boehringer Mannheim Canada Ltd.). Glucagon was assayed with the 39 K antibody of Unger (26); porcine glucagon was used as standard (Novo Research Institute, Copenhagen, Denmark) and polyethylene glycol for the separation procedure (6).

In Experiment 2, blood samples from all animals in each treatment were pooled, and free serum amino acids and urea were measured using the pooled samples. One milliliter of serum was mixed with .05 g sulfosalicylic acid and allowed to stand on ice for 1 h. The mixture was then centrifuged at 10,000 x g for 15 min at 2 to 4°C. The supernatant was passed through a .22-μm filter (Millipore Filter Corp., Bedford, MA) and kept frozen at −20°C until analyzed. The HFP samples were hydrolysed under N in 5.4 M hydrochloric acid at 120°C for 24 h. Amino acid content of HFP and free amino acid content of blood serum were measured using an LKB amino acid analyzer (LKB Biochrom Ltd., Cambridge, Engl.) equipped with an ultrodata 4440 integrator. The essential amino acid (EAA) content of skim milk and Nixtamal was calculated from reported values (1).

All data were analyzed as a split-plot design. Age, treatment, and time of sampling effects were tested and a priori comparisons were carried out (16). Responses of postprandial serum parameters to treatments were analyzed by comparing mean areas under the postprandial curves above fasting values. The postprandial period was divided in two distinct intervals, from 0 to 3 h and 3 to 7 h in Experiment 1 and from 0 to 4 h and 4 to 10 h in Experiment 2.
RESULTS AND DISCUSSION

General Trends

Data on weight gain, feed intake, and health for both Experiments 1 and 2 were previously reported (7). In general, serum glucose and insulin increased (P<.001) up to 2 h postfeeding and then decreased gradually to prefeeding values (Figures 1 and 2). Adding Nixtamal to the diet tended to reduce the amplitude of postfeeding increases in both serum glucose and insulin. The concentration of serum glucose followed patterns similar to those previously reported (2). Serum glucagon remained at the same concentration throughout the postprandial period (Figure 2), in agreement with reports indicating that mixed diets fail to alter plasma glucagon concentrations in other species (10).

Serum triglycerides varied with treatments (Figure 2). In control calves, they were high before morning feeding, decreased to reach minimum concentrations from 2 to 6 h postfeeding, and then increased to peak at about 8 to 10 h postfeeding. This general trend was similar to that previously reported for calves fed a skim milk and lard diet (2). In animals fed milk substitutes containing Nixtamal and HFP, serum triglycerides increased immediately after feeding to peak at 2 to 4 h postfeeding, then gradually decreasing to prefeeding value (Figure 2).

Serum urea in control animals was high before feeding, decreased to lowest values 4 h postfeeding, then increased until the end of the period (Figure 3). Because urea is the primary end product of amino acid catabolism, the postfeeding decrease in serum urea could be related to an increase in protein synthesis during that period. The decrease in serum free EAA observed in these animals 4 h postfeeding (Figure 3) and the high concentrations of blood glucose...
Figure 2. Effect of age and postprandial time on serum glucose, insulin, glucagon and triglycerides in calves fed control (●), 35% Nixtamal (35C) and 0% hydrolyzed fish protein concentrate (OF) (○), 35C/10.5F (■), or 35C/15F (■) diets in Experiment 2.
at this moment (Figure 2) support this hypothesis. In calves fed milk substitutes containing Nixtamal and HFP, serum urea did not decrease to reach minimum levels at 4 h postfeeding as in control animals (Figure 3, 35% Nixtamal, 10.5% HFP and 35% Nixtamal, 15% HFP).

**Age Effect**

Postprandial serum glucose, insulin, and triglycerides were higher ($P<.01$) at 54 than at 8 d (Tables 3 and 4; Figures 1 and 2), but glucagon was markedly lower ($P<.001$). Free EAA and urea tended to decrease between 8 and 54 d (Figure 3). The increase in glucose associated with age was likely due to higher feed intake at 54 than at 8 d. Effectively, feed intake increased from 10 to 18 g milk substitute powder per kilogram body weight between 8 and 54 d in Experiment 1, and from 13 to 19.5 g in Experiment 2.

Serum insulin increased from 8 to 54 d to a much greater extent than the corresponding increase in serum glucose (Figures 1 and 2). In several species, including calves (12, 15), glucose is the major physiological stimulus for insulin release. The relationship between blood glucose concentration and insulin release is sigmoidal (15), and the threshold concentration for the stimulating action of glucose lies between 70 and 90 mg/dl (15). This characteristic of insulin response might explain differences observed in insulin at 8 and 54 d. Thus, at blood glucose concentrations lower than the threshold concentration, the basal secretion rate of insulin remained constant (Figure 1, diet 54% Nixtamal), but when glucose concentrations exceeded this concentration, insulin secretion was greatly stimulated (Figure 1 and 2).

The increase in serum triglycerides associated with age was smaller than expected in view of higher lipid intake at 54 than at 8 d. Because insulin inhibits lipolysis and promotes accumulation of fatty acids across the membrane of adipose cells (9), the high concentration of circulating insulin at 54 d was likely to enhance lipid synthesis, consequently preventing an increase in serum triglycerides. This anabolic effect of insulin could be related to accelerated growth rate of calves at this age (7).

Glucagon secretion is generally inhibited by high serum glucose and insulin (11). The age related decrease in serum glucagon observed in Experiment 2 is likely the consequence of higher serum glucose and insulin at 54 than at 8 d (Figure 2).

Despite a twofold increase in feed intake and body weight gains of approximately 700 g/d at 54 d compared with practically no gain at 8 d (7), free EAA in blood was lower at 54 than 8 d (Figure 3). This is likely related to the rate of protein synthesis; an important proportion of absorbed amino acids would be used for anabolic processes, and only a small proportion would be degraded for energetic purposes. The reduction in blood urea at 54 d from that of 8 d (Figure 3) tends to support this suggestion. Furthermore, insulin in serum reached concentrations 10 times higher at 54 than at 8 d, whereas blood glucagon decreased. Consequently, the insulin:glucagon ratio, a predictor of the metabolic status, was markedly higher at 54 than at 8 d. Increases of this ratio have been generally associated with greater utilization of nutrients for anabolic processes (26).

**Skim Milk Replacement with Nixtamal**

Increasing the proportion of carbohydrates from Nixtamal at the expense of skim milk powder, in Experiment 1, was associated with a linear decrease ($P<.05$) of postprandial serum glucose and insulin, measured as the area under the response curve (Table 3). In Experiment 2, the decrease was linear for glucose but not for insulin (Table 4). This lowering effect of Nixtamal on serum glucose and insulin, when present, was greater during the first postprandial period, 0 to 3 h postfeeding, than during the second period, 3 to 7 h (Figures 1 and 2). This phenomenon was likely due to the slower digestion of Nixtamal complex carbohydrates than digestion of lactose and cerelose in the control diet. Effectively, Nixtamal contains 78.5% N-free extract of which only 2.9% is free sugars (7).

In monogastric animals, the intake of complex carbohydrates as starch results in lower serum glucose and insulin than the intake of monosaccharides or disaccharides and affects the amount and distribution of glucose within the intestinal tract (4, 17, 20). Thus, it can be assumed that Nixtamal released glucose in the digestive tract more slowly and in more distal
Figure 3. Effect of age and postprandial time on serum essential amino acids and urea in calves fed control (●), 35% Nixtamal (35C) and 0% hydrolyzed fish protein concentrate (OF) (○), 35C/10.5F (■), or 35C/15F (□) diets in Experiment 2.
TABLE 3. Serum glucose and insulin in Experiment 1.1

<table>
<thead>
<tr>
<th>Diet and effects</th>
<th>Glucose2</th>
<th>Insulin2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 d</td>
<td>54 d</td>
</tr>
<tr>
<td>Diet</td>
<td>A4</td>
<td>B</td>
</tr>
<tr>
<td>Control</td>
<td>40.0</td>
<td>32.5</td>
</tr>
<tr>
<td>27C3</td>
<td>25.3</td>
<td>28.5</td>
</tr>
<tr>
<td>54C3</td>
<td>2.8</td>
<td>9.6</td>
</tr>
<tr>
<td>CV</td>
<td>15.5</td>
<td>11.9</td>
</tr>
</tbody>
</table>

Main effects

| Age               | **       | ***      | **       | ***      |
| Diet (D)          | **       | ***      | **       | ***      |
| Period (P)        | ***      | **       | ***      | **       |
| D × P             | *        | **       | *        | **       |
| Contrast          | Linear5  | **       | ***      | **       |

1Serum glucose and insulin responses to treatment were expressed as mean areas under postprandial curves above fasting levels.

2Glucose (mg/dl), insulin (μU/ml), and time (h) were used to calculate response areas.

3Nixtamal at 27% (27C) or 54% (54C).

4Period A = 0 to 3 h, period B = 3 to 7 h, respectively.

5For Nixtamal.

*P<.05.

**P<.01.

***P<.001.

regions of the small intestine than either cerealose or lactose in the control diet. Moreover, in monogastric animals, some enteric hormones such as gastrointestinal polypeptide (GIP), which are physiologically important in potentiating insulin release, are unevenly distributed in the intestinal tract, and an infusion of glucose stimulates the secretion of serum GIP to a greater extent when it is made into the proximal portion of the intestine rather than in a more distal region (8, 17). Thus, postprandial concentrations of insulin are likely to be affected by the nature of dietary carbohydrates and the rate of luminal digestion. Our data suggest that young calves fed milk substitutes exclusively would react like monogastric animals.

Diets containing casein as the sole source of protein coagulated in vitro, despite the inclusion of 27 or 54% Nixtamal in diets (Experiment 1). Milk substitutes containing 35% Nixtamal and low heat processed skim milk powder also coagulated (Experiment 2), but Nixtamal increased the coagulation time from 1 min to between 3 and 4 min, and reduced curd firmness as appreciated visually upon shaking the reaction tube. Nixtamal was insoluble but remained in suspension associated with the casein clot (7). Postprandial fluctuations of blood glucose in calves fed diets containing Nixtamal (27, 54, or 35%) were smaller than those in control calves. At 54 d, in both experiments, a slight increase in blood glucose was observed at about 5 to 6 h after feeding the Nixtamal diets (Figures 1 and 2). We suggest that Nixtamal was trapped in the abomasal clot, consequently leading to a delay in the rate of passage of carbohydrates into the intestine. Reported data (3) indicating that corn starch is retained in the abomasum in association with the casein clot support this suggestion.

Detecting only a small rise in blood glucose following the intake of the Nixtamal diets is not necessarily indicative of negligible glucose absorption or utilization. Slow digestion rate of Nixtamal might result in absorption processes
TABLE 4. Serum glucose, insulin, glucagon, and triglycerides in Experiment 2.1

<table>
<thead>
<tr>
<th>Diet and effects</th>
<th>Glucose 2</th>
<th>Insulin 2</th>
<th>Glucagon 2</th>
<th>Triglycerides 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 d</td>
<td>54 d</td>
<td>8 d</td>
<td>54 d</td>
</tr>
<tr>
<td>Control</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>35C/0F</td>
<td>40.5</td>
<td>28.1</td>
<td>65.5</td>
<td>22.0</td>
</tr>
<tr>
<td>35C/10.5F</td>
<td>16.4</td>
<td>11.1</td>
<td>34.5</td>
<td>9.9</td>
</tr>
<tr>
<td>35C/15F</td>
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<td>10.5</td>
<td>39.0</td>
<td>6.1</td>
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<tr>
<td>CV</td>
<td>19.8</td>
<td>11.5</td>
<td>44.3</td>
<td>17.4</td>
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Main effects

<table>
<thead>
<tr>
<th>Age</th>
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<th>Period (P)</th>
<th>D x P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NS</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

Contrasts

| Control vs. 35C/0F | NS       | NS       | NS     |
| Control vs. 35C/15F| **       | NS       | NS     |

1Serum component responses to diet were expressed as mean areas under postprandial curves above fasting levels.
2Glucose (mg/dl), insulin (µU/ml), glucagon (pg/ml), triglycerides (mg/dl), and time (h) were used to calculate response areas.
3Nixtamal at 35% (35C) and 0, 10.5 or 15% hydrolyzed fish protein concentrate (0F, 10.5F, or 15F).
4Period A = 0 to 4 h, period B = 4 to 10 h, respectively.

*P<.05.
**P<.01.
***P<.001.

Significant linear increase in glucagon associated with partially hydrolyzed fish protein at 8 d in both periods.
occurring over an extended time and in the utilization of glucose by the small intestine mucosa and/or the liver, as absorption takes place, thus preventing the appearance of a glucose peak in blood. This would be in agreement with the observation that, in a calf fed a starch diet for 10 wk, only small changes are found in the systemic glucose but increases are marked in the portal concentrations both soon after feeding and 6 h postfeeding (27).

In view of these data, we suggest that there was a reduced rate of glucose absorption immediately after feeding Nixtamal and flattened out fluctuations in the rate of delivery of nutrients to the metabolic systems throughout the postprandial period.

Hydrolyzed Fish Protein Effect

Calves fed diets containing Nixtamal in Experiment 2 had lower blood glucose than controls. Replacing up to two-thirds of skim milk proteins with HFP in diets containing Nixtamal had no effect on blood glucose (Figure 2). However, blood insulin in calves fed 35% Nixtamal and the highest (15%) HFP diet (35% Nixtamal, 15% HFP) remained at a concentration as high (P>0.05) as that of controls (Figure 2). In calves fed the 35% Nixtamal, 15% HFP diet, free EAA in serum increased rapidly after feeding, in contrast to no increase in controls (Figure 3). Observations were similar for calves fed a milk substitute containing HFP at a substitution rate of 74% on protein basis, when free amino N in blood markedly increased during the first 4 h postfeeding (12). Because amino acids enhance insulin release in some species when glucose and other carbohydrates are available simultaneously in the diet (15), we suggest that in calves fed HFP as the main source of protein, insulin secretion was stimulated by elevated free amino acids in blood soon after feeding.

Increasing the substitution rate of skim milk proteins with HFP was associated with an increase in serum glucagon (Figure 2). Response areas were greater at both ages but were significant (P<0.05) only at 8 d (Table 4). Since blood free EAA were higher in calves fed diets based on HFP than in controls, during the first 6 h postfeeding, it might be inferred that glucagon secretion was potentiated by free amino acids in blood as in several other species (11).

In control calves, blood triglycerides reached highest concentrations immediately before feeding, decreased sharply until 2 h postfeeding, remained low from 2 to 6 h, and then steadily increased until the end of the postprandial period (Figure 2). This would agree with reported data (2). Low blood triglycerides from 2 to 6 h postfeeding, followed by an increase from 6 h until the end of the postprandial period, suggest that lipids were originally trapped in the abomasal clot to be released, digested, and absorbed more extensively from 6 h on.

Curd firmness was reduced by Nixtamal, but no clot was formed when HFP replaced 50 or 67% of skim milk protein (35% Nixtamal with 10.5 or 15% HFP). It might be expected that under these conditions the profile of triglycerides concentrations in blood would be modified, from those of the control. Effectively, in this case triglycerides were low before feeding but increased immediately thereafter (Figure 2), thus indicating fast absorption of lipids during the first 2 h postfeeding. This was confirmed by a greater (P<0.01) triglyceride response area during the first postprandial period at both ages in calves fed diet 35% Nixtamal, 15% HFP, as compared with controls (Table 4). Furthermore, at the end of the postprandial period, blood triglycerides were elevated in controls but low in calves fed HFP diets (Figure 2), indicating that triglyceride absorption was still taking place in controls but not in calves fed HFP diets. These observations, in agreement with others (12), suggest that the rate of triglyceride absorption was related to the ability of the milk substitute to coagulate in the abomasum.

In calves fed milk substitutes containing HFP (35% Nixtamal and 10.5 or 15% HFP), free EAA in blood increased at 1 h postfeeding, remained constant up to 6 h, and decreased to prefeeding values at the end of the postprandial period. In control calves, free EAA decreased after feeding, increased at 1 h, then decreased until 6 h postfeeding, and finally increased at the end of the period (Figure 3). Differences in serum free amino acid concentration profile between calves fed HFP diets and controls may be related to differences in clotting properties of the milk substitutes used. Other data (5, 23) have shown that free EAA concentrations in
blood increase during the first hour after the ingestion of a milk substitute lacking the property to clot in the abomasum, whereas EAA concentrations do not change after the ingestion of milk substitutes that do clot. It is suggested that this parameter might be a reliable indicator of in vivo curd forming ability of a milk substitute.

In other mammals, when readily available carbohydrates are ingested with protein, insulin secretion is potentiated (18). High blood glucose and insulin promote the utilization of amino acids for synthetic purposes. In the present work, the control diet contained twice as much readily available carbohydrates in the form of glucose and lactose as Nixtamal plus HFP diets (Table 2). Under these conditions, glucose and insulin were at their highest postprandial concentrations 2 h postfeeding, but free EAA decreased markedly at that time and reached their lowest concentration between 4 and 6 h postfeeding. However, in calves fed Nixtamal plus HFP diets, free EAA blood concentrations tended to remain more uniform throughout the postprandial period; insulin and glucose were generally lower than in controls (Figure 2). Therefore, it would appear that protein synthesis was taking place at an accelerated rate between 4 and 6 h postfeeding in control calves.

Blood urea decreased in all cases after feeding while insulin and glucose attained their highest concentrations (Figures 2 and 3). Insulin secretion promotes the passage of amino acids to tissues, thus stimulating protein synthesis and reducing amino acid degradation for energetic purposes. Therefore, a decrease in urea during the first period postfeeding could be expected. At the end of the postprandial period, however, blood urea increased in control animals but not in calves fed Nixtamal plus HFP diets. Because more blood EAA were available in control animals at that time than in calves fed Nixtamal plus HFP, it might be suggested that more amino acids were catabolized for energetic purposes, thus causing an increase in blood urea. It is possible therefore that maintaining uniform free EAA in blood and uniform glucose concentrations during the entire postprandial period might reduce amino acid degradation for energy purposes, thus stimulating protein synthesis. Data from N balance trials (24) tend to support this hypothesis.

Considering blood responses in both trials, and growth results previously reported (7), it appears that young dairy calves may adapt satisfactorily to milk substitutes based on lime-treated corn flour (Nixtamal) and HFP concentrate, despite changes in the concentration of several blood components.

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concentrate in calf milk replacers. J. Dairy Sci. 65:784.