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Early Weaning of Calves Using Feedstuffs. A Rationalization Based on Inhibition of Lipolysis

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The ability of broll (a combination of the wheat-milling byproducts bran and pollard, i.e., a mixture of wheat bran, husk, and flour) and blackstrap molasses (an ingredient of calf feed) to inhibit calf pregastric lipase (CPGL)-catalyzed hydrolysis of tributyrylglycerol (TBG) has been studied in vitro. Lipolysis was measured at pH 6.5 and 37 °C (CPGL at 0.02 mg/mL) with stirring at 300 rpm. The broll soaked in Bis-Tris buffer (50 mM, pH 6.5) at 4 °C for either 24 h or 15 min, and then added to an emulsion containing TBG, before initiation of the reaction by addition of CPGL, exhibited 22% inhibitory effect. A solution of blackstrap molasses (50%, v/v) exhibited inhibitory effects of 50% in the absence and 45% in the presence of Bis-Tris buffer. The initial rate of lipolysis in the presence of the dialyzed molasses retentate (10%, v/v) increased a little, compared with the same amount of crude molasses, from a mean value of 69% to a mean value of 74%. The results have been discussed in terms of the chemical nature and composition of broll and molasses and their roles as components of feedstuffs used in development of the rumen in early weaning of calves.

KEYWORDS: Feedstuffs; inhibition of lipolysis; calf pregastric lipase; weaning of calves; molasses

INTRODUCTION

Pregastric enzymes are secreted from the pharyngeal and epiglottal regions of the tongue by all mammals during suckling and swallowing (*1*). These enzymes play a fundamental role in fat digestion in mammals (*2*). Young calves secrete copious amounts of saliva, especially when suckling, and it seemed reasonable to assume that the secretions of the oral and esophageal passage serve an important role in the assimilation and utilization of milk by the animal and particularly in the cleavage of short- and medium-chain fatty acids from milk fat (*1*).

Lean (*3*) has stated that "In contrast to adult cattle, where the rumen dominates the gastro-intestinal tract and represents 85% of the volume of the stomach, a calf's abomasum represents only 70% of the volume of the combined stomachs". Milk is channelled through the esophageal groove via the developing omasum to the abomasum. As milk is delivered to the abomasum, a casein clot, containing entrapped fat, is formed under the influence of rennin, hydrochloric acid, and (to a lesser extent) pepsin, within 3−4 min of meal intake. The amount of pregastric lipase/esterase produced by the calf affected the degree of hydrolysis of the fats in milk. The clot is gradually disintegrated by the action of the gastric enzymes sloughing off layers of partially digested proteins and fat globules, leading to passage to, and further digestion by, the small intestine. Most of fats are digested before leaving the abomasum. Apart from lactose, galactose, and glutose, the calf cannot absorb many carbohydrates (*3*). Lean (*3*) has also pointed out that "the pre-ruminant calf cannot digest starch, dextrin, maltose, or sucrose. The transitional period from non-ruminant to ruminant is governed largely by the diet which the calf receives. While the calf has ample access to milk it will essentially remain dependent on milk". To advance the formation of the rumen, calves are given access to palatable dry diets and begin to ingest these foods from approximately 7 days of age.

The aim of every dairy farmer, in a country such as New Zealand with a market-driven economy, is to wean the calves as soon as possible, and at the same time to achieve a rapid weight gain for the calf. This feeding regime suggests that the role played by pregastric lipase in the nutrition of a calf, during the transition from feeding with milk to feedstuffs' formulations, may be important. This study aims to assess the effect of two major components of an animal feedstuff, broll and blackstrap molasses, on the activity of calf pregastric lipase (CPGL). CPGL has been partially purified, and its molecular weight is reported as 50 kDa (*4*). The substrate for lipolysis was the short-chain fatty acid triglyceride tributyrylglycerol (TBG).

EXPERIMENTAL PROCEDURES

Materials. Broll (a gift from Northern Roller Mills, Auckland, New Zealand) is collected after the milling of flour from wheat, when the mill is cleaned, and it contains a mixture of husk, bran, and flour. The digestible contents of broll are dry matter (88.1%), digestible energy (11.5% MJ/kg), and digestible protein (107 g/kg). It is made up of 18 separate amino acids, usually at concentrations of 2−8 g/kg except for glutamic acid (25 g/kg). As well, it contains ash (43.4 g/kg), neutral detergent fiber (359 g/kg), acid detergent fiber (104 g/kg), and fat (38.8 g/kg) (*5*).

Blackstrap molasses (donated by Agri Feeds, Tauranga, New Zealand) was a diluted blackstrap molasses (diluted from 83−90 to 79.5 °Brix). Dry matter (74.5 g/100 g) was measured according to the

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AOAC method (925.45) and contained up to 15% ash. The dietary cation (K^+, Na^+)/anion (Cl^-, SO_4^{2-}) difference (DCAD) was +211. The lower is the DCAD, the better for developing feeding before calving (preventing milk fever) (*6*). Sucrose, glucose, and fructose contents were 48, 7, and 9%, respectively, and crude protein was 6.2%.

Crude calf enzyme extract was supplied by the New Zealand Rennet Co. (Eltham, New Zealand) and partially purified, as described previously for lamb pregastric lipase (*7*), to yield an activity equal to 7.85 μmol/min/mg (8.3 mM TBG, 37 °C, pH 6.5). The purification factor was 4.1. Tributyrin (98%) from Sigma (St. Louis, MO) was used as the substrate for the hydrolytic reactions. 1,2-Diacyl-*sn*-glycero-3-phosphocholine (L-α-lecithin; type II-S, from soybean (Sigma) and sodium caseinate emulsifier (New Zealand Dairy Research Institute) were added to prevent phase separation of the hydrophobic lipid substrate. The buffer used in this study wasbis[2-hydroxyethyl]iminotris-[hydroxymethyl]methane (Bis-Tris) (Sigma). Water was of Milli-Q grade and had a conductivity of <18.2 $M\Omega^{-1}$ cm^{-1}. Fast flow Q-Sepharose was from Pharmacia, and the dialysis tubing (12−14 kDa) used to dialyze the solution of molasses was from Medicell International (London, U.K.).

Instrumentation. The pH-stat titrator was a 736 GP Titrino from Metrohm (Switzerland) equipped with a 6.0234.100 pH electrode and a 728 Metrohm stirrer. Stirring speed was measured with a transistorized stroboscope from Electronic Applications (U.K.). Sonication was achieved with a KT 50 Micro Ultrasonic Cell Disrupter from Kontes (Vineland, NJ).

Preparation of Broll Suspensions in Bis-Tris Buffer. Broll (1 g) was first suspended in Bis-Tris buffer (25 mL, 50 mM, pH 6.5), sonicated for 1 min, and stored at 4 °C, for either 15 min or for 24 h before use.

Tributyrin Assay in the Presence of Broll. A TBG assay is used in the food industry for standardizing the commercially available pregastric enzyme solutions. TBG is relatively insoluble in water, and its exposure to the enzyme is as an emulsion (*7*). Because CPGL is active at the interface between aqueous and lipid phases, both an emulsifier and sonication are needed for the assay. The standard emulsion was prepared by dispersing sodium caseinate (2.4 g) and L-α-lecithin (200 mg) in 100 mL of Milli-Q water with a magnetic stirrer, before the volume was adjusted to 200 mL by the addition of water. This preparation was well stirred until all of the solids were dissolved and had a "use-by" date of no more than 3 days. The total volume of the initial titration solution was 50 mL and was made up as follows: substrate emulsion, obtained by adding TBG (100 mg) into the casein−lecithin emulsifier (25 mL); various broll-containing samples (25 mL) or Bis-Tris buffer (25 mL, control sample); enzyme solution (50 μL, 20 mg/mL).

The activity of CPGL was assessed by measuring the release of butyric acid from TBG as determined by the amount of NaOH needed to maintain a constant pH of 6.5. Only one butyric acid moiety per molecule of TBG is released during the time period under investigation. The remaining two butyric acid moieties are released much more slowly (*7*).

The TBG assay was monitored by the pH-stat TiNet 2.4 program on the 736 GP Titrino using a procedure established previously (*8*). The temperature was maintained at 37 °C and the pH at 6.5. The pH of all samples was readjusted to 6.5 before lipase addition. Then the enzyme solution was added, the titrator was activated, and the volume of NaOH required to maintain pH 6.5 was recorded automatically. For broll-containing samples, the activity of CPGL was assessed as described above, except that the emulsion containing TBG was mixed with the broll suspension just before addition to the titration cup. The activity of the control sample was determined similarly, but the broll suspension was replaced with Bis-Tris buffer.

Under all experimental conditions, the concentration of TBG was <10 mM, thus ensuring that measured rates were true initial rates. All experiments were carried out at a fixed stirring speed (300 rpm). Each experiment was performed at least in triplicate; each datum point thus represents the mean of a minimum of three (control values only) and maximum of nine observations.

Tributyrin Assay in the Presence of Molasses. Blackstrap molasses (25 mL, stored at 4 °C) was mixed with standard emulsion (25 mL) for 15 min. This mixture was sonicated for 1 min, and the temperature and pH were then adjusted to 37 °C and 6.5, respectively. CPGL (50 μL, 20 mg/mL) was added to activate the TBG assay. The activity for a control sample, prepared by replacing molasses with Milli-Q water (25 mL), was also determined.

In a separate set of experiments, the standard emulsion was replaced with Bis-Tris buffer (50 mM, pH 6.5) instead of Milli-Q water to dissolve L-α-lecithin and sodium caseinate. The tributyrin assay was carried out, as described above, but using this Bis-Tris emulsion instead of the standard emulsion. A control sample, prepared by mixing Bis-Tris buffer (25 mL, 50 mM, pH 6.5) with standard emulsion (25 mL), was also used for determination of the activity of CPGL.

Dialysis of Molasses Solution. The dialysis tubing was prepared by boiling twice, for 15 min, in 1 mM Na_2EDTA/10 mM $NaHCO_3$. The tubing was then rinsed in 50 mM NH_4HCO_3 (to remove any residual free acid), followed by rinsing with Milli-Q water. Blackstrap molasses (30 mL) was used directly in the dialysis. The crude blackstrap molasses (5 mL) plus Milli-Q water (38 mL), or the dialyzed molasses (43 mL), was mixed with concentrated Bis-Tris emulsion (see below) (7 mL) for use in the TBG assay. Concentrated Bis-Tris emulsion, prepared by dispersing sodium caseinate (8.64 g), L-α-lecithin (720 mg), and Bis-Tris (7.53 g) in 100 mL of Milli-Q water with a magnetic stirrer, before the volume was adjusted to 200 mL by the addition of water, was 3.6-fold more concentrated than the Bis-Tris emulsion used in the assay in the presence of crude molasses. This higher concentration ensured that after addition of this emulsion to the medium used for assay of activity in the presence of dialyzed molasses, the concentration of emulsifiers was maintained at a constant value during all lipolytic assays. The solution of blackstrap molasses increased manyfold in volume during the dialysis process. The volumes used in the TBG assays allowed for this dilution and ensured that the same concentration of molasses was used in the determination of enzyme activity. A control sample, prepared by mixing Milli-Q water (43 mL) with concentrated Bis-Tris emulsion (7 mL), was also used in the TBG assay.

RESULTS AND DISCUSSION

In an earlier study (*9*), we identified the ability of brans to inhibit CPGL-catalyzed hydrolysis of TBG. Lipolysis was measured under the conditions used in this investigation, that is, pH 6.5 and 37 °C (CPGL at 0.02 mg/mL) with stirring at 300 rpm. Brans tested were coarse and fine red and white wheat brans and oat bran. The brans, soaked in Bis-Tris buffer (50 mM, pH 6.5) at 4 °C, before exposure to CPGL, exhibited inhibitory effects of 17−30% after being soaked for 24 h and 13−20% after being soaked for 15 min. On exposure to Bis-Tris buffer, water-soluble components were solubilized rapidly from coarse red wheat bran, and these were detrimental to the activity of CPGL. The activity profiles of the soaked bran and of the aqueous extract (supernatant from soaked bran suspension) merged together at ∼20 h. The effect of the incubation time between coarse red wheat bran and CPGL on the initial rate of lipolysis was apparent immediately after exposure of CPGL to bran, with the most pronounced effect occurring within 2−3 min of incubation. There was no difference in lipolysis activity of CPGL in the presence of either the aqueous extract of coarse red wheat bran or the dialyzed extract. The results were discussed in terms of the inhibitory components within the brans, for example, (1→3, 1→4)-β-glucan, and their roles in inhibiting pregastric and other lipase-catalyzed hydrolysis of dietary lipids.

In this study, we have extended the range of potential inhibitors of lipolysis to broll and molasses, both used extensively in feedstuffs within New Zealand. Feed wheat cultivars, which have become more available recently, have the advantage of containing both more energy and protein per kilogram than barley. Because of this higher energy content, wheat is useful for making up energy dense creep, weaner, grower, and lactating sow diets (*5*).

Broll, a combination of the wheat-milling byproducts bran and pollard, is a mixture of wheat bran, husk, and flour and is widely used by the New Zealand feedstuff industry. It is stated that the husk (hull) comprises the interlocked palea and lemma, which are modified leaves (bracts), and that their several cell layers comprise the outmost epidermis, which has a thick cuticle, above the hypodermis with thick, lignified, and sometimes silicified walls, a layer of thinner-walled parenchyma, and an inner epidermis (*10*). There is evidence that the husk is responsible for an appreciable amount of the inhibitory effect seen in lipid hydrolysis. A study on the husks of field beans has shown that the husks are mostly carbohydrates in composition, with cellulose being the predominant polysaccharide. A diet with tannin-free husks slightly lowered the digestion of lipid (*11*), and this lowering was believed to be due to inhibition of digestive enzymes, possibly through their adsorption onto the husks. Diets with tannin-rich husks caused a large reduction in the digestion of lipid, mainly due to inactivation of the digestive enzymes by the formation of tannin−enzyme complexes (*11*).

Molasses is a nutritive sweetener with a desirable texture and mouthfeel (*12*). Blackstrap molasses is commonly added to calf feed, not only for palatability and binding but also to help break down fibers so that they may assist in the development of the rumen through critical enzyme action (cellulase and lignase) (*6*). It acts as an energy source for rumen microbes, and it improves rumen fermentation. In young milk-fed calves for whom the rumen is not yet established, the feeding of meal and higher fibers, such as hay, is introduced slowly, allowing the calf to convert over to a ruminant. This process includes stimulation of the gut muscles by "effective fiber" with a "scratch factor", which in turn causes them to regurgitate and chew their cud. The saliva from the cud contains sodium bicarbonate, which aids buffering of the stomach, preventing acidosis. Molasses, as a stockfeed, provides rumen microbes with an energy source or instant food, being ~50% sugars, causing them to multiply and digest fibrous feeds more effectively.

Determination of the Inhibitory Effect of Broll on the Activity of CPGL. Figure 1 shows the inhibitory effect of broll on the activity of CPGL against TBG at 37 °C and pH 6.5. The broll sample had been soaked in Bis-Tris buffer at 4 °C for periods of 15 min or 24 h before it was exposed to the enzyme. It is clearly seen that the time of exposure does not affect the degree of inhibition. Both samples exhibited ~22% inhibitory effects, which may reasonably be attributed to a combination of the effects from the bran and the husk (due to the relatively high composition of fiber in broll).

Determination of the Inhibitory Effect of Blackstrap Molasses on the Activity of CPGL. Figure 2 shows the effect of blackstrap molasses mixed with emulsion prepared by using either Milli-Q water or Bis-Tris buffer on the activity of CPGL against TBG at 37 °C, compared with the corresponding control sample. In both cases, a marked inhibitory effect, 50% in the absence and 45% in the presence of Bis-Tris buffer, was observed.

Apart from sucrose (48 g/100 g) and dry matter (up to 15% ash), the most abundant species in molasses is crude protein (6.2 g/100 g). It has been documented that the activity of bile salt stimulated lipase (an enzyme with lipolytic properties similar to those of CPGL) is inhibited by the presence of proteins (*13*). Thus, it is likely that the protein present in molasses is responsible for the observed inhibitory effect. This supposition is supported by the very small effect on the activity resulting after dialysis of a solution of molasses.

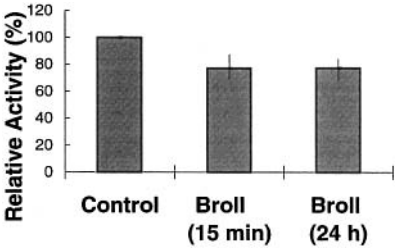

Figure 1. Inhibitory effect of broll (20 g/L), soaked in Bis-Tris buffer at 4 °C for 15 min or 24 h, on the activity of CPGL (50 μL, 20 mg/mL). Initial titration volume was 50 mL, pH 6.5, 37 °C, stirring speed = 300 rpm. Error bars represent the range of values.

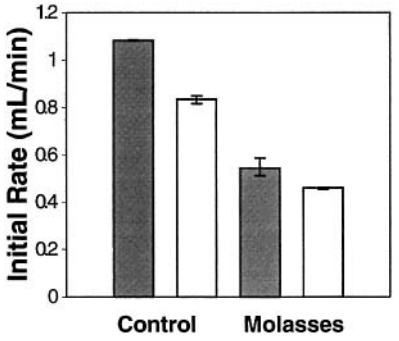

Figure 2. Inhibitory effect of blackstrap molasses (25 mL) on the activity of CPGL (50 μL, 20 mg/mL) in the absence (shaded bars) and presence (white bars) of Bis-Tris buffer. Initial titration volume was 50 mL, TBG 100 mg, 37 °C, pH 6.5, stirring speed = 300 rpm. Error bars represent the range of values.

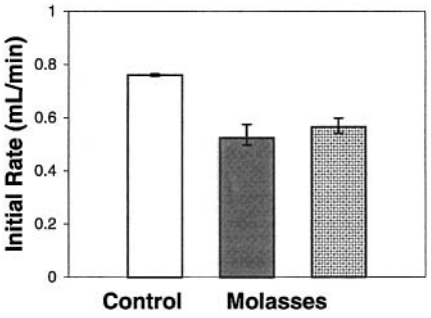

Figure 3. Initial rates of CPGL (50 μL, 20 mg/mL) catalyzed hydrolysis of TBG (100 mg) in the presence of crude black strap molasses before (solid bar) and after (stippled bar) dialysis with Milli-Q water. TBG assay: 37 °C, pH 6.5, stirring speed = 300 rpm. Error bars represent the range of values.

Effect of Dialysis of Blackstrap Molasses on the Activity of CPGL. This set of experiments was designed to identify whether the components responsible for the inhibitory effects of blackstrap molasses were macro- or microcomponents. **Figure 3** shows the effect of dialysis on the inhibitory ability of blackstrap molasses. During dialysis, the volume of the molasses dialyte increased very rapidly, and it was twice necessary to remove a sample from the dialysis tubing before continuing. Overall, there was an 8-fold increase in volume of original dialyte. Thus, of the original 30 mL of molasses, only 3.75 mL remained in the 31.6 mL dialyte after 24 h of dialysis. The inhibitory effect decreased a little from 31.0% (range = 24.3−34.6%) to 25.6% (range = 21.3−28.8%). This decrease probably resulted from the removal of some sugars (sucrose, glucose, and fructose), salts, and other reducing substances by dialysis. Reducing sugars may be responsible for some inhibition, although the addition of sucrose to a suspension of coarse

Table 1. Confidence Intervals at the 95% Confidence Level of Experimental Data in Figures 1–3

initial rate data used in analysis (mL/min)			no. of observations (n)[d]	confidence intervals (μ)
broll[a]		control	3	0.81 ± 0.04
		soaked for 15 min	6	0.62 ± 0.06
		soaked for 24 h	9	0.63 ± 0.04
molasses[b]	Bis-Tris	control	4	1.08 ± 0.00
	absent	molasses	4	0.54 ± 0.05
	Bis-Tris	control	4	0.84 ± 0.02
	present	molasses	4	0.46 ± 0.01
molasses[c]		control	3	0.76 ± 0.05
		before dialysis	4	0.52 ± 0.06
		after dialysis	5	0.57 ± 0.03

[a] Data shown in Figure 1. [b] Data shown in Figure 2. [c] Data shown in Figure 3. [d] Observations retained at 90% confidence level after application of Q test (*15*).

red wheat bran had no effect on its inhibitory action on the CPGL-catalyzed hydrolysis of TBG (*9*). The removal of salts will cause a change in ionic strength and ionic charge, which may also have affected the initial rate of lipolysis. It has been shown that salts inhibit the lipolysis of TBG catalyzed by lamb pregastric lipase (*14*). The remaining inhibition would have been caused by proteins and carbohydrates whose molar mass was >12−14 kDa.

Statistical Treatment of Data. Before calculations of mean values, Q tests were performed to determine whether a questionable experimental result should be rejected. These tests were conducted at the 90% confidence level, which means the outliers were discarded with 90% statistical confidence (*15*).

Table 1 shows the confidence intervals of each experimental result at the 95% confidence level. These confidence intervals, which predict how well the mean values are likely to agree with the true values, were calculated from the equation

$$\mu = \bar{x} \pm ts/\sqrt{n}$$

where μ is the confidence interval, $\bar{x}$ is the average experimental datum value (in this case the initial rate datum for enzyme activity in mL/min), t is a statistical constant that depends both on the confidence level and on the number of measurements involved (*15*), n is the number of observations, and s is the standard deviation.

Rationalization of Using Feedstuffs for Early Weaning. High-quality, nutritious, and easily digested calf feeds are specially formulated to give calves the head start they need to reach their full potential. They must contain a balance of protein, fiber, fat, and salt and generally be suitable for calves reared for both weaner and dairy replacements. They usually contain an anticoccidial to prevent coccidiosis and are complete feeds that require no added roughage, vitamins, or minerals. Thus, they represent a nutritionally balanced compound feed for calves because they include the roughage required for early rumen development, molasses (for palatability), and high protein (18−20%). They meet the growth requirements of calves without inhibiting postweaning growth.

As expected, we found that broll, with its high fiber content, restricted the lipolysis of a simple lipid, TBG, a process likely to be replicated for the lipids present in the milk ingested by a suckling calf, thereby strengthening an alternative pathway for nutrition during weaning. The surprising result obtained in this investigation is that molasses also adopts this repressive role. Until now, it has been thought the role of molasses was to enhance palatability and to increase caloric intake. Now we find that broll and molasses both play a more significant role in the preweaning nutrition of calves than has hitherto been understood.

ABBREVIATIONS USED

Bis-Tris, bis[2-hydroxyethyl]iminotris[hydroxymethyl]methane; CPGL, calf pregastric lipase; Da, dalton; DCAD, dietary cation anion difference; L-α-lecithin, 1,2-diacyl-*sn*-glycero-3-phosphocholine; rpm, revolutions per minute; TBG, tributyrylglycerol.

LITERATURE CITED

(1) O'Connor, C. J.; Barton, R. H.; Butler, P. A. G.; MacKenzie, A. D.; Manuel, R. D.; Lai, D. T. Ruminant pregastric lipases: experimental evidence of their potential as industrial catalysts in food technology. *Colloids Surf. B: Biointerfaces* **1996**, *7*, 189−205.

(2) Villeneuve, P.; Pina, M.; Graille, J. Determination of pregastric lipase specificity in young ruminants. *Chem. Phys. Lipids* **1996**, *83*, 161−168.

(3) Lean, I. Calf and heifer nutrition. In *Nutrition of Dairy Cattle*; The University of Sydney Post-Graduate Foundation in Veterinary Science: Sydney, Australia, 1987; pp 168−169.

(4) O'Connor, C. J.; Lai, D. T. Pregastric enzymes: how do they work and what do they do? *N. Z. BioSci.* **1996**, 17−23.

(5) Morel, P. C. H.; Pluske, J.; Pearson, G.; Moughan, P. J. *A Standard Nutrient Matrix for New Zealand Feedstuffs*; Monogastric Research Centre, Institute of Food, Nutrition and Human Health, Massey University: Palmerston North, New Zealand, 1999; pp 18−20.

(6) Arnold, M. Personal communication, 2000.

(7) Barton, R. H.; O'Connor C. J.; Turner, K. W. Characteristics of tributyroylglycerol hydrolysis mediated by a partially purified lamb pregastric lipase. *J. Dairy Sci.* **1995**, *79*, 27−32.

(8) O'Connor, C. J.; Barton, R. H. Modifications of emulsions for determination of the rates of lipase-catalysed hydrolysis and intramolecular acyl transfer reactions of lipids. *J. Mol. Liq.* **1997**, *72*, 263−277.

(9) O'Connor, C. J.; Sun, D.-X.; Smith, B. G.; Melton, L. D. The inhibitory effects of dietary fibres on the lipolysis of tributyrin catalysed by calf-pregastric lipase. *Br. J. Nutr.* **2001**, submitted for publication.

(10) Fincher, G. B.; Stone, B. A. Cell walls and their components in cereal grain technology. In *Advances in Cereal Science and Technology*; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1986; Vol. 8, pp 207−275.

(11) Longstaff, M.; McNab, J. M. The inhibitory effects of hull polysaccharides and tannins of field beans (*Vicia faba* L.) on the digestion of amino acids, starch and lipid and on digestive enzyme activities in young chicks. *Br. J. Nutr.* **1991**, *65*, 199−216.

(12) Whistler, R. L.; BeMiller, J. N. Sweeteners. In *Carbohydrate Chemistry for Food Scientists*; American Association of Cereal Chemists: St. Paul, MN, 1997; p 225.

(13) O'Connor, C. J.; Butler, P. A. G.; Sutton, B. M. Bile salt stimulated lipase: interactions with proteins. *J. Bioact. Compat. Polym.* **1988**, *3*, 390−402.

(14) O'Connor, C. J.; Lai, D. T.; Barton, R. H. Lamb pregastric lipase activity: catalyzed hydrolysis of dibutyryl-1,2(3)-propanediols and butyryl-1(2)-propanol and inhibition of the catalyzed hydrolysis of tributyrylglycerol in the presence of EDTA, LiCl, NaCl and ethanol. *J. Bioact. Compat. Polym.* **1998**, *13*, 124−135.

(15) Hargis, L. G. Representing and manipulating data. In *Analytical Chemistry*; Prentice Hall: Englewood Cliffs, NJ, 1988; pp 50−57.

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