The Free and Total *myo*-Inositol Contents of Early Lactation and Seasonal Bovine Milk

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ABSTRACT

A high performance anion-exchange chromatographic method employing pulsed amperometric detection was applied to the determination of endogenous free and total *myo*-inositol in bovine milk. The contents and trend variability of *myo*-inositol in milk from extensively pasture-fed cows during early lactation and across a production season were evaluated. This information will improve understanding of the expression of innate *myo*-inositol in bovine milk, and enhance manufacturers formulation capability related to the production of cow's milk-based paediatric products.

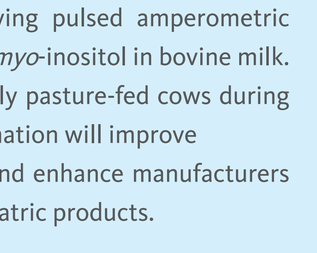
INTRODUCTION

The cyclohexitol sugar alcohol *myo*-inositol forms the structural basis of biochemically significant compounds including multiple inositol phosphates, phosphatidylinositol and phosphatidylinositol phosphates.

Although present in foods as free *myo*-inositol, it also exists in multiple phosphorylated forms and analytical methodology should include contributions from both phosphatidylinositol and the lower inositol phosphates, although there is no consensus whether phytate merits inclusion.

Milk is a significant source of *myo*-inositol, both free and bound, and since human milk contains higher levels of *myo*-inositol than bovine milk, it is commonly added to infant formulae to ensure against potential early neonatal deficiency.

High performance anion-exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD) is a highly sensitive and specific platform for the determination of *myo*-inositol in foods, infant formulae and human milk in free and/or bound forms.



myo-inositol

ANALYTICAL TECHNIQUE

SAMPLE PREPARATION

Free *myo*-inositol:

- Dilute 10 mL liquid milk sample to approximately 25 mL with water
- Vortex and sonicate for 30 min
- Adjust pH to 4.5 ± 0.2 and make to 50 mL with water
- Clarify through a glass fibre filter and filter through a 0.45 µm PTFE syringe filter
- Dilute 50-fold with water in autosampler vial

Total *myo*-inositol:

- To 10 mL liquid milk sample, add 10 mL of HCl (36%) and autoclave at 120°C for 6 h
- Cool and adjust pH to 7.0 ± 0.2 and make to 50 mL with water
- Clarify through a glass fibre filter and filter through a 0.45 µm PTFE syringe filter
- Dilute 50-fold with water in autosampler vial

Analysis:

Chromatography:

- Dionex ICS 5000 system with ICS-5000 ED detector
- CarboPac MA1 250 x 4 mm analytical column and CarboPac MA1 guard column
- Phase A: 40 mM NaOH under N2
- Phase B: 1.0 M NaOH under N2
- Gradient: 100% A (0 – 10 min), 100% B (10 – 20 min), 100% A (20 – 40 min)
- Flow: 0.4 mL/min
- Column temp: 30°C
- Injection: 25 µL

Detection:

- ED detector with Ag/AgCl reference cell and PTFE-Au disposable working electrode
- Standard quad potential waveform (integrate at +0.10 V, 0.20 – 0.40 sec)

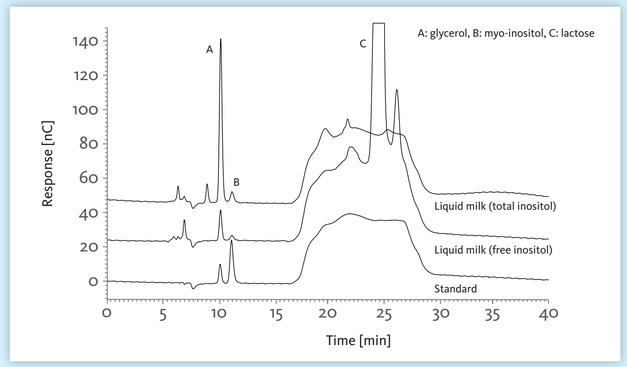
Quantitation:

- 6-level *myo*-inositol external standard linear calibration

RESULTS AND DISCUSSION

Analytical method:

Free and total *myo*-inositol contents were quantitated subsequent to separate preparation schemes, with both analyses completed under identical chromatographic conditions, as illustrated.



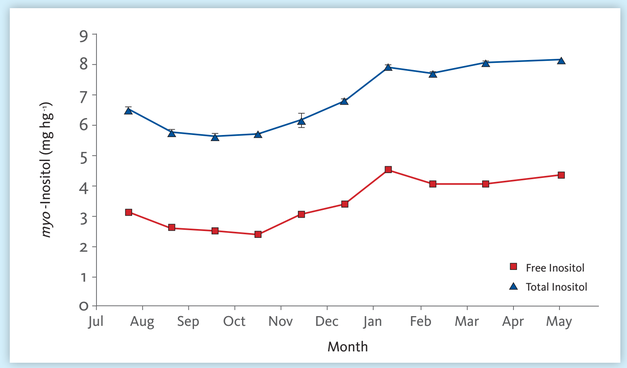
Qualitatively, the principal chromatographic difference between determinations of free *myo*-inositol and total *myo*-inositol is the presence of the dominant late-eluting lactose peak when free *myo*-inositol is the measurand.

Application to bovine milk:

The composition of ruminant milk is generally influenced by the physiological stage of lactation and the present study investigated the distribution of *myo*-inositol during the transition from colostrum to mature milk over 28 days subsequent to parturition and the data are presented below.

There was a minor increase in free *myo*-inositol during the early colostral phase, followed by a relatively stable content following the transition to mature milk, while the total *myo*-inositol content remained essentially constant across the entire 28-day post-partum period.

Dairy husbandry in New Zealand exploits extensive pasture grazing, which facilitates the investigation of natural seasonal changes in herd milk. The free and total *myo*-inositol contents in bulk raw bovine herd milk were quantified over an entire production season, with the data presented below.



There was a minor increasing seasonal trend in both free *myo*-inositol and total *myo*-inositol and consequently, a relatively constant free to total ratio, which possibly suggests a greater influence from systemic active transport relative to mammary biosynthesis across the season. This systematic trend may also be an outcome of the synchronised herd calving husbandry model that is intended to maximise herd lactation volume coincident with peak early summer grass growth in New Zealand.

CONCLUSIONS

- Free and total *myo*-inositol in bovine milk was 2.3—4.5 and 5.3—8.7 mg/100g, respectively
- The proportion of free to total *myo*-inositol in bovine milk was 0.4—0.5
- The influence of lactation and season on endogenous milk *myo*-inositol contents was studied
- The data will support enhanced control of infant formula manufacture regarding *myo*-inositol

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