Influence of Concentrate Feeding Frequency and Intrinsic Factors on Diurnal Variations of Blood Metabolites in Dairy Cows

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Introduction

The health of dairy cows can be monitored by their ability to maintain normal blood concentrations of numerous metabolites. Therefore, clinical biochemistry analyzes, using metabolic profile tests, were introduced in the early seventies.[36] Since then, many researchers have been using biochemical analyzes as a diagnostic aid in subclinical conditions such as reproduction disorders [24,40] and in the assessment of nutritional status or feeding deficiencies.[24,40,45]

Blood glucose (**GLC**) is not a direct indicator of nutritional input, but concentrations are measured for detection of breakdown of its homeostasis.[37] Blood glucose increases when rumen propionate concentration increases.[1,11,34] Glucose concentrations give an indication of the activity of the rumen biomass[51] and of the efficiency of gluconeogenesis in the liver. Ketone bodies[15] and especially β-hydroxybutyrate (**BHB**) have also been used as indicators of the energy deficiency.[22,23,27,31,37] Urea nitrogen (**BUN**) is a useful indicator of the nutritional energy-protein balance.[26,41,42] Plasma urea nitrogen is typically elevated in the energy-deficient cows compared to those fed relatively high amounts of concentrates.[20]

Non-esterified fatty acids (**NEFA**) appear in blood as a consequence of the mobilization of body fat. During early lactation, glucose oxidation is reduced as a sparing mechanism for lactose synthesis. Therefore, fat oxidation is enhanced to provide the cow with energy[4] and NEFA concentrations in blood are elevated. Cholesterol (**CHOL**) was found to be associated with improved fertility in cows.[43,44] Furthermore, cholesterol concentrations are elevated with high dietary fat[13,46,47] and with

propylene glycol administration.[17] It is also reported as an indicator of good fibre supply.[25] The concentration of total serum protein (**PROT**) gives an indication concerning the long term energy and protein supply.

Diurnal fluctuations are known to be an important source of variations of many blood metabolites. Diurnal variations of glucose have been considered negligible by some authors,[8,30,37] whereas Blum *et al.* showed a significant 24-hour pattern, depending on energy supply.[7] Also β-hydroxybutyrate showed diurnal variations which appeared to be of major importance,[7,9,30,49] with a typical increase after feeding. Conflicting information exists about the diurnal variations of BUN concentrations.[20] Some authors found higher,[21,39] some others lower[32,35] concentrations of urea nitrogen in the morning milk compared to the afternoon. During the day, NEFA concentrations decreased after feeding.[10,14,37] The diurnal pattern of cholesterol was considered negligible,[5,7,48,49] whereas total protein showed either significant[48] or not significant[7] diurnal variations.

The results of some of these studies are contradictory. An explanation for the contradictions could be the relatively small number of cows used in many of these studies, leading to an overexpression of individual cow effects. Another hypothesis for this discrepancy is that the feeding dynamics (solubility of the components, feeding strategy) have an important effect on the daily pattern.

The purpose of this study was to determine the influence of the concentrate feeding frequency and some intrinsic factors on the diurnal variations of the blood metabolites glucose, β-hydroxybutyrate, urea nitrogen, non-esterified fatty acids, cholesterol and total protein

in two herds with different concentrate feeding frequencies. These informations should yield knowledge for a better standardization of the sampling procedure when biochemical analyzes are to be used as diagnostic aids.

Materials and Methods

Two herds with similar production levels and demographic characteristics, but with different feeding strategies were chosen. The first herd had a traditional feeding system (**TRAD**) with two meals of roughage and concentrate per day, whereas in the second herd, two meals of roughage and up to six concentrate feedings per day with an automatic computerized distribution of concentrate (**ACD**) were given. Time of first feeding was 06h30 in herd TRAD and 06h00 in herd ACD, both beginning with grass silage.

In each herd, 20 cows divided in two groups of 10 (group 1: 40 to 110 days post partum (**DIM**); group 2: 150 to 230 DIM) were randomly selected. Blood samples for serum and plasma were taken through venipunction of the caudal vein four times daily (08h30, 11h00, 13h30 and 16h00).

Glucose (GLC), non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) were analyzed in fluoride plasma, whereas urea nitrogen (BUN), cholesterol (CHOL) and total protein (PROT) were analyzed in serum.

To characterize the diurnal pattern of each metabolite, a multivariate repeated measures analysis of variance model was fitted to test the interactions of time (**TIME**) with the factors feeding strategy (**TYPE**), lactation group (**GROUP**) and the intrinsic factors parity (**LN**), days post partum (**DIM**) and daily milk yield (**KGMI**). Furthermore, the differences between each sequential sample and between the 8h30 and the 16h00 samples were tested with a Wilcoxon rank sum test for paired samples.

Results and Discussion

Glucose, β-hydroxybutyrate and Urea Nitrogen
For those three parameters, a rapid (within hours) reaction to the feeding of concentrate could be observed (Figures 1-3). The diurnal variations showed a significant difference between the two herds, which is most probably related to the frequency of concentrate feeding. Frequent distribution of small amounts resulted in a constant level of the parameters, whereas the patterns for the herd with only two larger concentrate meals showed marked differences between morning and afternoon.

These three parameters remain useful diagnostic tools in the evaluation of the protein and energy metabolism, but the influence of diurnal variations and type of feeding has to be taken into account.
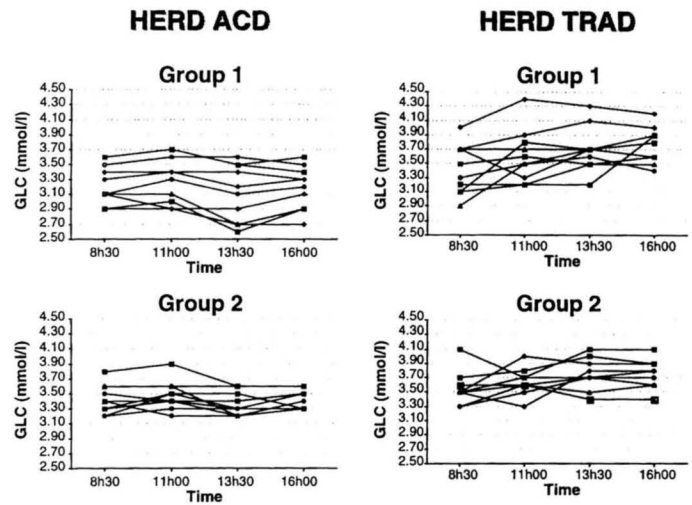
Figure 1. Glucose concentrations (GLC) of the individual cows in the herd with automatic concentrate distribution (ACD) and the herd with traditional feeding (TRAD) for the two production groups.
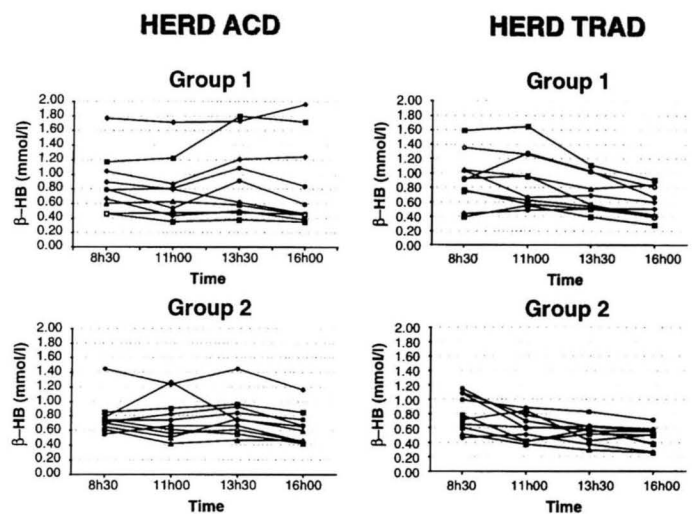
Figure 2. Concentrations of β-Hydroxybutyrate (β-HB) of the individual cows in the herd with automatic concentrate distribution (ACD) and the herd with traditional feeding (TRAD) for the two production groups.

Non-esterified Fatty Acids, Cholesterol and Total Protein
In contrast, the diurnal patterns of NEFA (Figure 4) and CHOL were similar for both herds, but more marked differences (although statistically not significant) in the shape of the curves were observed between the two production groups.

Time of sampling did not seem to be important for cholesterol because of the absence of significant diurnalvariations. However, the number of days in lactation have an important influence on the value of this parameter.[13,19,46] This has to be taken into account to determine sampling strategy or for analyzing and comparing CHOL concentrations in dairy cows.
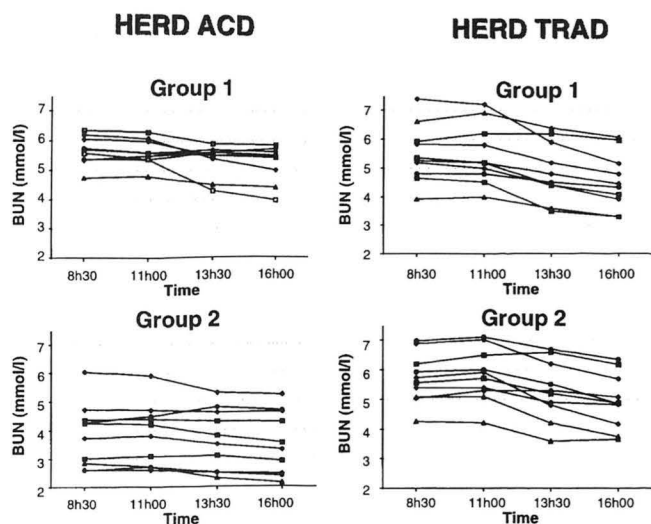
Figure 3. Urea nitrogen concentrations (BUN) of the individual cows in the herd with automatic concentrate distribution (ACD) and the herd with traditional feeding (TRAD) for the two production groups.
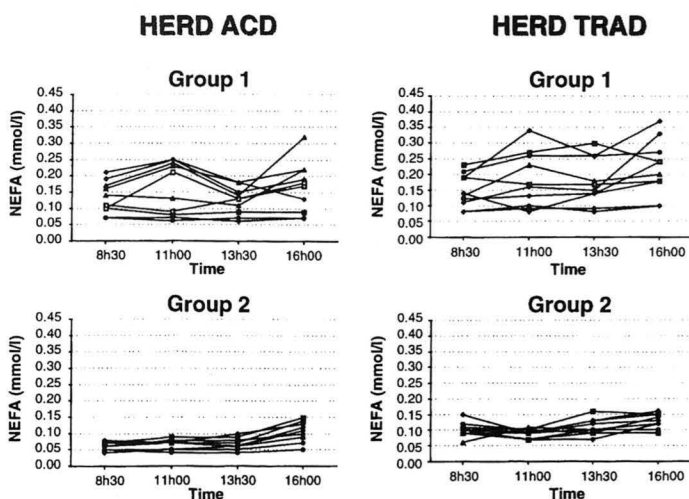

Figure 4. Concentrations of non-esterified fatty acids (NEFA) of the individual cows in the herd with automatic concentrate distribution (ACD) and the herd with traditional feeding (TRAD) for the two production groups.

Although the diurnal variations and differences between the two herds for PROT concentrations were statistically significant, the absolute value of these differences was considered biologically negligible.

Influence of production group

Only slight differences were observed between the two production groups within herds. For all the metabolites except for BHB, the stage of lactation (DIM) and the level of production (KGMI) did not have a major impact on diurnal variations. However, some effects were

shown through the shapes of the NEFA curves which were different between both productions groups.

Conclusions

Under practical conditions, the strategy of sampling should focus on cows in the group at risk according to the clinical diagnosis or suspicion. In several studies, a relationship has been found between feeding and the metabolic situation on one hand and fertility on the other hand. Due to the marked diurnal variations, sampling strategy must be planned according to the problem that will be investigated. In a situation of insemination only once a day for example, biochemical analyzes should be performed at the same time as inseminations occur.

In order to obtain consistent and comparable results of biochemical analyzes, time after feeding has to be standardized for sampling. Under normal conditions, 4 to 5 hours after the beginning of feeding, the metabolites are most sensitive to changes potentially caused by metabolic disorders. At this time, concentrations of GLC and NEFA should then be at their nadir and BHB at its peak. If at this time NEFA are elevated, it can be concluded that the cow has not been able to compensate the energy deficiency and had to mobilize increased amounts of body fat. At the same time, the cow is at highest risk to show a breakdown of GLC homeostasis, which could be falsely normal at another time of the day. Blum[7] showed that BHB increased for a longer period after feeding in energy deficient cows than in cows fed high energy diets. Therefore, this time of sampling should allow the detection of truly ketotic cows in minimizing the impact of a dietary induced increase of BHB in healthy cows.

These findings emphasize the need of a standardization of the time of sampling during the day and are a possible explanation why non standardized samples will provide uninterpretable results. The necessity of a standardization of the collection of samples in order to allow an appropriate interpretation of the analyzes has already been reported.[32,37]

As the dynamic changes during the day can be different between herds, a double sampling in the morning and in the afternoon could be a further use of biochemical analyzes in order to better understand the metabolic situation of a herd, especially if the morning and evening feeding strategies are different. For economic reasons, pooling of the individual samples has been proposed.[45,51]

The present study shows different diurnal patterns in herds with different feeding practices, indicating that the feeding strategy, or the com-

position of the food, or both of them, have a major impact on blood metabolites.

References

1. Abdul-Razzaq, H. A., R. Bickerstaffe, and G. P. Savage. 1988. The influence of rumen volatile fatty acids on blood metabolites and body composition of growing lambs. Aust. J. Agric. Res. 39: 505-515. 2. Andersson, L., and K. Lundström. 1984. Milk and blood ketone bodies, blood isopropanol and plasma glucose in dairy cows; methodological studies and diurnal variations. J. Vet. Med. Ser. A 31: 340-349. 3. Anonymous. 1996. SYSTAT® 6.0 for Windows®: Statistics. Chicago IL, SPSS® Inc. 4. Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63: 1514-1529. 5. Beynen, A. C., and L. G. M. van Gils. 1983. Diurnal patterns of the concentrations of cholesterol, triglycerides, glucose, nonprotein nitrogen and urea in the serum of veal calves fed a milk replacer supplemented with cholesterol. Z. Ernahrungswiss. 22: 50-58. 6. Bines, J. A., I. C. Hart, and S. V. Morant. 1983. Endocrine control of energy metabolism in the cow: diurnal variations in the concentrations of hormones and metabolites in the blood plasma of beef and dairy cows. Horm. Metab. Res. 15: 330-334. 7. Blum, J. W., F. Jans, W. Moses, D. Fröhli, M. Zemp, M. Wanner, I. C. Hart, R. Thun, and U. Keller. 1985. Twentyfour-hour pattern of blood hormone and metabolite concentrations in high-yielding dairy cows: effects of feeding low or high amounts of starch or crystalline fat. J. Vet. Med. Ser. A 32: 401-418. 8. de Boer, G., A. Trenkle, and J. W. Young. 1985. Glucagon, insulin, growth hormone, and some blood metabolites during energy restriction ketonemia of lactating cows. J. Dairy Sci. 68: 326-337. 9. Borrebaek, B., K. Halse, B. Tveit, H. K. Dahle, and L. Ceh. 1990. Plasma glucose, ketone bodies, insulin, glucagon and enteroglucagon in cows: diurnal variations related to ketone levels before feeding and to the ketogenic effects of feeds. Acta Vet. Scand. 31: 5-15. 10. Bowden, D. M. 1971. Non-esterified fatty acids and ketone bodies in blood as indicators of nutritional status in ruminants: a review. Can. J. Anim. Sci. 51: 1-13. 11. Burgwald-Balstad, L. A., J. S. Caton, V. I. Burke, and K. C. Olson. 1995. Influence of forage level and naloxone injection on feed intake, digestion, and plasma hormone and metabolite concentrations in dairy heifers. J. Anim. Sci. 73: 2677-2686. 12. Carlsson, J., and J. Bergström. 1994. The diurnal variation of urea in cow's milk and how milk fat content, storage and preservation affects analysis by a flow injection technique. Acta Vet. Scand. 35: 67-77. 13. Carroll, D. J., M. J. Jerred, R. R. Grummer, D. K. Combs, R. A. Pierson, and E. R. Hauser. 1990. Effects of fat supplementation and immature alfalfa to concentrate ratio on plasma progesterone, energy balance and reproductive traits of dairy cattle. J. Dairy Sci. 73: 2855-2863. 14. Coggins, C. R. E., and A. C. Field. 1976. Diurnal variation in the chemical composition of plasma from lactating beef cows on three dietary energy intakes. J. Agric. Sci. (Camb.) 86: 595-602. 15. Dohoo, J. R., and S. W. Martin. 1984. Subclinical ketosis; prevalence and associations with production and disease. Can. J. Comp. Med. 48: 1-5. 16. Folman, Y., H. Neumark, M. Kaim, and W. Kaufmann. 1981. Performance, rumen and blood metabolites in high-yielding cows fed varying protein percents and protected soybean. J. Dairy Sci. 64: 759-768. 17. Formigoni, A., M. C. Cornil, A. Prandi, A. Mordenti, A. Rossi, D. Portetelle, and R. Renaville. 1996. Effect of propylene glycol supplementation around parturition on milk yield, reproduction performance and some hormonal and metabolic characteristics in dairy cows. J. Dairy Res. 63: 11-24. 18. Frohli, D. M., and J. W. Blum. 1988. Nonesterified fatty acids and glucose in lactating dairy cows: diurnal variations and changes in responsiveness during fasting to epinephrine and effects of beta-adrenergic blockade. J. Dairy Sci. 71: 1170-1177. 19. Giesecke, D., M. S. Abdo, and J. Radtke. 1987. Plasma cholesterol and glucose tolerance in cows with high milk yield. Adv. Anim. Physiol. Anim. Nutr. 18: 31-42. 20. Gustafsson, A. H., and D. L. Palmquist. 1993. Diurnal variation of rumen ammonia, serum urea and milk urea in dairy cows at high and low yields. J. Dairy Sci. 76: 475-484. 21. Haag, T. 1988. The value of milk urea determination in the milk of cattle with special reference to systematic examinations. Inaugural-Dissertation. Justus-Liebig-University, Giessen, Germany. 22. Herdt, T. H., J. B. Stevens, W. G. Olson, and V. Larson. 1981. Blood concentrations of beta-hydroxybutyrate in clinically normal Holstein-Friesian herds and in those with a high prevalence of clinical ketosis. Am. J. Vet. Res. 42: 503-506. 23. Herdt, T. H., J. B. Stevens, J. Linn, and V. Larson. 1981. Influence of ration composition and energy balance on blood beta-hydroxybutyrate (ketone) and plasma glucose concentrations of dairy cows in early lactation. Am. J. Vet. Res. 42: 1177-1180. 24. Ingraham, R. H., and L. C. Kappel. 1988. Metabolic profile testing. Vet. Clin. North Am. Food Anim. Pract. 4: 391-411. 25. Kalchreuter, S. 1988. Cholesterol - an ideal metabolite to assess the adequacy of ruminant feeding. SVKB-Mitteilungen 26: 47. 26. Kirchgessner, M., M. Kreuzer, and D. A. Roth-Maier. 1986. Milk urea and protein content to diagnose energy and protein malnutrition of dairy cows. Archiv. Anim. Nutr. 36: 192-197. 27. Kunz, P. L., and J. W. Blum. 1985. Relationships between energy balances and blood levels of hormones and metabolites in dairy cows during late pregnancy and early lactation. Z. Tierphysiol. Tierernaehrg Futtermittelkde 54: 239-248. 28. LaCount, D. W., L. D. Ruppert, and J. K. Drackley. 1996. Ruminal degradation and dose response of dairy cows to dietary L-carnitine. J. Dairy Sci. 79: 260-269. 29. Maianti, M. G., G. Bertoni, R. Lombardelli, and V. Cappa. 1990. Variations in the blood constituents of cows subjected to various stress factors. Zootec. Nutriz. Anim. 16: 19-27. 30. Manston, R., G. J. Rowlands, W. Little, and K. A. Collis. 1981. Variability of the blood composition of dairy cows in relation to time of day. J. Agric. Sci. (Camb.) 96: 593-598. 31. Miettinen, P. V. A. 1990. Metabolic balance and reproductive performance in finnish dairy cows. J. Vet. Med. Ser. A 37: 417-424. 32. Miettinen, P. V. A., and R. O. Juvonen. 1990. Diurnal variations of serum and milk urea levels in dairy cows. Acta Agric. Scand. 40: 289-296. 33. Mudron, P., H. P. Sallmann, J. Rehage, M. Holtershinken, G. Kovac, P. Bartko, and H. Scholz. 1994. Effects of the surgical repositioning of left-sided abomasal displacement on energy metabolism parameters in dairy cows. Dtsch. Tieraerztl. Wochenschr. 101: 376-378. 34. Obara, Y., and D. W. Dellow. 1993. Effects of intraruminal infusions of urea, sucrose or urea plus sucrose on plasma urea and glucose kinetics in sheep fed chopped lucerne hay. J. Agric. Sci. 121: 125-130. 35. Oltner, R., and H. Wiktorsson. 1983. Urea concentrations in milk and blood as influenced by feeding varying amounts of protein and energy to dairy cows. Livest. Prod. Sci. 10: 457-467. 36. Payne, J. M., S. M. Dew, R. Manston, and M. Faulks. 1970. The use of a metabolic profile test in dairy herds. Vet. Rec. 87: 150-158. 37. Payne, J. M., and S. Payne. 1987. The metabolic profile test. 1st ed. Oxford University Press, Oxford New York Tokyo. 38. Pierzchala, K., J. Niezgoda, and S. Bobek. 1985. The effect of isolation on plasma cortisol, glucose and free fatty acids in sheep. J. Vet. Med. Ser. A 32: 140-145. 39. Roever, G., W. Kaufmann, J. Claus, and E. Kalm. 1982. Constituents of milk as indicators for physiological stress and its effects on fertility of cows. Zuchthygiene 17: 114-. 40. Ropstad, E. 1988. Constituents of blood and milk in relation to fertility, nutrition and metabolic status in dairy cows. Thesis. The Norwegian College of Veterinary Medicine, Oslo, Norway. 41. Ropstad, E., L. Vik Mo, and A. O. Refsdal. 1989. Levels of milk urea, plasma constituents and rumen liquid ammonia in relation to the feeding of dairy cows during early lactation. Acta Vet. Scand. 30: 199-208. 42. Rossow, N., B. Staufenbiel, and U. Jacobi. 1990. Use of milk components to monitor metabolism of dairy cows. MH. Vet. Med. 45: 686-690. 43. Ruegg, P. L., W. J. Goodger, C. A. Holmberg, L. D. Weaver, and E. M. Huffman. 1992. Relation among body condition score, milk production, and serum urea nitrogen and cholesterol concentrations in high- producing Holstein dairy cows in early lactation. Am. J. Vet. Res. 53: 5-9. 44. Ruegg, P. L., W. J. Goodger, C. A. Holmberg, L. D. Weaver, and E. M. Huffman. 1992. Relation among body condition score, serum urea nitrogen and cholesterol concentrations, and reproductive performance in high-producing Holstein dairy cows in early lactation. Am. J. Vet. Res. 53; no 1; pp 10-14; ISSN: 0002-9645; 32 ref.: 10-14. 45. van Saun, R. J. 1997. Prepartum nutrition: the key to diagnosis and management of periparturient disease. 30th Annual Convention of the AABP, Montreal, Quebec, Canada. 46. Son, J., R. J. Grant, and L. L. Larson.

1996. Effects of tallow and escape protein on lactational and reproductive performance of dairy cows. J. Dairy Sci. 79: 822-830. 47. Spicer, L. J., R. K. Vernon, W. B. Tucker, R. P. Wettemann, J. F. Hogue, and G. D. Adams. 1993. Effects of inert fat on energy balance, plasma concentrations of hormones, and reproduction in dairy cows. J. Dairy Sci. 76: 2664-2673. 48. Stampfli, G., J. Anetzhofer, and J. Stirnimann. 1980. Influence of time of day on haematological and clinico-chemical values in the lactating cow. Schweiz. Arch. Tierhlkde 122: 327-340.

49. Suphke, E., W. Junge, R. Preisinger, and E. Kalm. 1989. Substrates, enzymes and hormones in high yielding cows - relations between metabolic values and feed intake criteria. J. Anim. Breed. Genet. 106: 363-372. 50. Thomas, P. C., and M. E. Kelly. 1976. The effect of frequency of feeding on milk secretion in the Ayrshire cow. J. Dairy Res. 43: 1-7. 51 Tremblay, A. 1993. Metabolic profile test. Lecture notes, University of Montreal, Quebec, Canada.

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June 1998   608 pages   HB
ISBN 0 85199 263 3
£75.00 (US$140.00)

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