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Abstract

Despite the traditional approach of thinking of energy and protein as two separate entities when balancing dairy rations, the response of the cow to increased supply of nutrients often crosses these artificial compartments. For example, supplementation of protein as casein infusions increases not only protein yield but also milk and lactose yields. As part of the quest to enhance the biological and economic efficiency of the cow, we need to understand how and where such interactions occur. The response in lactose yield to protein supplementation is almost identical (g/g) to the response in protein yield. An obvious candidate for the link between lactose output and protein supply is glucose, as increasing protein supply through casein infusion increases the whole body (WB) rate of appearance (Ra) of glucose (sum of real portal absorption, gluconeogenesis and glycogenolysis). In ruminants, utilization of arterial glucose by the portal-drained viscera averages 22% of WB glucose utilization and real portal absorption contributes, on average for 14% of WB Ra of glucose, with variations from 0 to 37%, in relation with starch digested in the intestine. Most of the remainder of WB Ra originates from gluconeogenesis, with a liver contribution of at least 85% and the kidneys contribution at most 15% to non-detectable levels in animals fed a concentrate diet. Infusion of casein did not alter real portal absorption of glucose and neither did it decrease portal-drained viscera utilization of arterial glucose. Indeed, in one study where measured simultaneously, the increased WB Ra of glucose induced by post-ruminal casein infusion originated from an increment in liver net flux. In dairy cows, the efficiency of WB transfer of the carbons of casein into glucose is on average higher than the maximal theoretical synthesis of glucose from protein supplementation, once the increment in milk protein yield is taken into account. Stimulation of utilization of other glucose precursors or recycling of glucose could explain this high efficiency. Despite a strong relationship between casein supply, WB Ra of glucose and lactose yield, increased WB Ra of glucose does not seem to be the driving force stimulating lactose yield. Increased lactose yield is only observed when the increment in WB Ra of glucose is due to increased protein supply; the same relationship does not exist when glucose Ra is increased through energy supply. It may be that some of the essential AA are playing a key role in some metabolic pathways or are simply stimulating protein synthesis. The latter hypothesis might result in increased milk protein synthesis, 'pulling' lactose synthesis due to its osmotic role, or in stimulating 'enzyme machinery' involved in gluconeogenesis, milk protein and/or lactose synthesis. Overall, this 'simple' example demonstrates the intricate integration between protein and energy metabolism.

Introduction

Most of the models used to balance dairy rations consider protein and energy as two separate entities. Despite this dichotomy that we have imposed on the predictive models, 'cross-reactions' between energy and protein supply and the milk component responses occur. One of the 'simplest' examples is the response of milk and lactose yields to protein supplementation, in addition to the protein response. Using from the database of Doepel *et al.* (2004) only the studies where casein, casein hydrolysates or amino acids (AA) with a casein profile had been infused, the response (g/g) of lactose yield to the increased protein supply is almost identical to the response of protein yield (Figure 1).

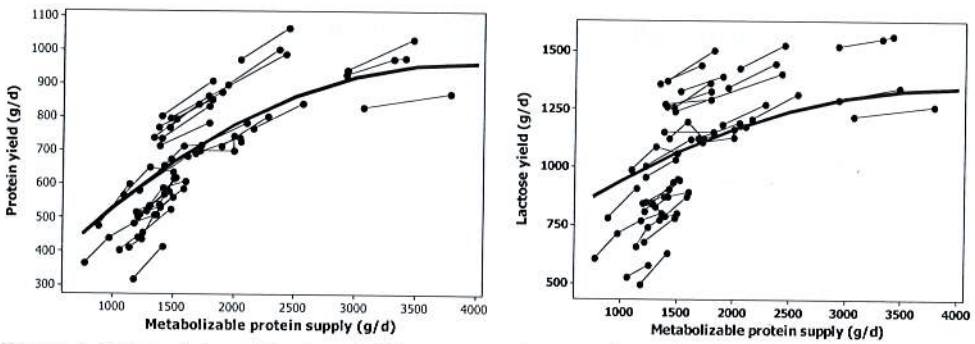

Figure 1. Milk protein and lactose yield response to increased supply of protein from post-ruminal infusions of casein, casein hydrolysates or amino acids with whole protein profile.
MPY = 196**(±32) + 0.38**(±0.03)×MP - 0.000043**(±0.000008)×MP^2+Exp., R^2adj: 98.8%,
MLY = 630**(±53) + 0.36**(±0.05)×MP - 0.000045**(±0.000013)×MP^2+Exp., R^2adj: 98.6%,
*with 32 experiments, n=80; where all units are in g/d and MPY=milk protein yield, MLY= milk lactose yield and MP=metabolizable protein supply (NRC, 2001);** indicates* P<0.01.

How does protein supplementation, or more correctly the absorbed AA, affect lactose yield? Already in 1906, the role of glucose for lactose secretion was proposed (Kaufmann and Magne, 1906) and later, it was observed that, in ruminants, more than 85% of the lactose originates from glucose (Bickertaffe *et al.*, 1974). Therefore, increased glucose availability from protein supply would be a first obvious route between protein supply and lactose output. But, where and how, in the body, and with which efficiency does an increased protein supply affect the glucose availability to the dairy cow? And with which efficiency does the dairy cow use this increased glucose availability to export milk lactose? This presentation does not pretend to be an exhaustive literature review of the whole complexity of energy and protein interaction, but rather illustrates from a simple situation that protein and energy metabolism are so closely linked that we should really integrate both their supply and requirements in our predictive models used to balance rations.

What is whole body rate of appearance of glucose?

Increasing protein supply is known to increase whole body (WB) rate of appearance (Ra) of glucose in ruminants (see review Lemosquet *et al.*, 2007). The measurement of WB Ra requires the utilization of isotopes, earlier studies being conducted with radioactive isotopes and more recent studies conducted with stable isotopes. Only studies infusing proteins post-ruminally will be considered in this review, allowing precise determination of the extra supply of AA without relying on any predictive models. Table 1 summarizes studies conducted on the effect of post-ruminal supply of casein on WB Ra of glucose in dairy cows. All these studies involved continuous infusions of labelled glucose and WB Ra of glucose was calculated using the rate of infusion and the specific activity or the isotopic enrichment of glucose in peripheral plasma. For reasons described below, the study of Hanigan *et al.* (2004) reporting glucose hepatic net flux was added to the database. The position of the isotope on the labelled molecule of glucose alters the absolute value of Ra due to variable measurements of recycling (Wolfe, 1992), but as we will be looking at increments, reported values will be directly used without correction.

First, all studies reported an increase, at least numerically, of the WB Ra of glucose when casein was provided to dairy cows. But, what does an increased WB Ra of glucose mean? The WB Ra of glucose is the sum of all the inflows of glucose in the plasma, i.e. in a fed ruminant, gluconeogenesis plus glucose intestinal absorption reaching blood circulation plus glucose from the turnover of glycogen returning to the plasma pool. Intestinal absorption of glucose plus gluconeogenesis from absorbed nutrients represent a net supply of glucose to the animal. On the other hand, glucose originating from

Table 1. Effect of post-ruminal infusion of casein (CN) and/or energy on whole body glucose rate of appearance (Ra), liver net flux and yields of milk, protein and lactose.

Treatment	Glucose Ra (g/d)	Liver net flux (g/d)	Milk yield (kg/d)	Lactose yield (g/d)	Protein yield (g/d)	Reference
Control	2,760		28.8	1,404[1]	963	Clark *et al.*,
+ CN (450 g/d Na-CN)	2,890		31.0	1,510[1]	1,072	1977
+ Glucose (450 g/d)	2,850		28.9	1,407[1]	957	
+ CN & Glucose	3,150		31.6	1,539[1]	1,104	
Control	1,931		22.1	992	607	König *et al.*,
+ CN (240 g/d Na-CN)	2,169		23.8	1,085	697	1984
+ CN (460 g/d Na-CN)	2,200		23.5	1,065	683	
Control	2,540		25.5	1,276	752	Lemosquet
+ CN (743 g/d Ca-CN)	2,830		29.8	1,448	904	*et al.*,
+ Propionate (rumen 1,043 g/d)	2,925		25.7	1,288	784	2009a
+ CN & propionate	3,270		30.5	1,464	996	
Control	2,756	1,970	30.9	1,420	951	Galindo,
+ CN (647 g/d Na-CN)	3,110	2,402	32.9	1,484	1,058	2010
Control		2,022	14.8	671	611	Hanigan *et*
+ CN (200 g/d)		2,411	16.9	768	679	*al.*, 2004
+ CN (400 g/d)		2,125	16.1	724	654	
+ CN (600 g/d)		2,773	15.9	727	656	

[1] Estimated from milk yield with a lactose concentration of 47.0 g lactose/kg milk (average of other references).

glycogenolysis, either reaching directly the plasma pool or being shuttled to the liver as lactate and then being resynthesized in glucose does not represent a net supply to the animal if the glycogen pool is not depleting but just being turned over. The relative contribution of each function to WB Ra obviously depends on the status of the animal.

Real portal absorption

In ruminants, net portal appearance of glucose is relatively limited as shown by an average 4±13 g/d, not significantly different from 0, obtained from 306 measurements (Reynolds, 2006), but with individual values bouncing below and above 432 g/d. Net appearance across the portal-drained viscera (PDV) is the result of two opposite actions: absorption of glucose from starch small intestinal digestion (+) and utilization of glucose from arterial supply by the PDV (-). First, intestinal digestion of starch and subsequent absorption of glucose yields a positive balance of glucose across the PDV. A recent meta-analysis estimated that for 1 g of starch digested in the intestine, 0.43 g of glucose is absorbed (Loncke *et al.*, 2009), in the range of values reported when starch was infused (Reynolds, 2006). Therefore, the magnitude of the real portal absorption of glucose depends, partly, on the amount of starch intake by-passing the rumen. On the other hand, the PDV uses glucose both from arterial and lumen supply. These utilizations can be estimated from uptake of lumen or systemic labelled glucose by the PDV. Very limited data exist in ruminants on utilization from lumen supply and El-Kadi *et al.* (2003) reported that most of the glucose used by the PDV would be of arterial supply. Glucose utilization from plasma arterial supply by the PDV is more documented and averaged 22% of WB utilization or 0.7 g/d/kg BW, excluding treatments involving post-ruminal glucose or starch infusions (Table 2) but showed large variations from 0.3 to 2.2 g/d/kg BW. In recent studies in lactating dairy cows (Larsen and Kristensen, 2009; Galindo, 2010), glucose PDV utilization from plasma supply averaged 423 and 397 g/d, i.e. 0.7 and 0.8 g/d/kg BW, respectively. From the meta-analysis quoted

Table 2. Glucose whole body rate of appearance (WB Ra) and tissue kinetics in ruminants (g/d).

Species (BW, kg)	Treatment	WB	Portal-drained viscera			Liver			Reference
		Ra	Net flux	Utilization	Real	Net flux	Utilization	Real	
Sheep (57)	Non-pregnant; hay	118	-17	14	-5	94	9	104	Bergman *et al.*, 1970
	Non-pregnant; grain + hay	134	-12	18	0	82	31	114	
	Non-pregnant; hay	84	-34	22	-13	79	-4	76	
	Pregnant; hay	199	-31	45	11	170	0	169	
	Pregnant; fasted	134	-17	28	11	115	-11	102	
Steers (235)	Starch (rumen; 800 g/d)	782	-130	121	-9	540	91	631	Harmon *et al.*, 2001
	Starch (abomasum (Ab); 800 g/d)	1,140	276	281	557	592	-60	531	
Dairy cows (626)	Control	2,756	337	402	739	1,970	95	2,065	Galindo, 2010
	+ Casein (CN; Ab, 647 g/d Na-CN)	3,110	281	393	674	2,402	104	2,506	
Sheep (59)	Pregnant	234	26	38	57	142	5	155	van der Walt *et al.*, 1983
	Lactating	345	20	68	111	242	-3	241	
Sheep (46)	Control	132	5	44	49				Balcells *et al.*, 1995
	+ Glucose (jugular, 65 g/d)	194	-5	47	41				
	+ Glucose (jugular, 130 g/d)	283	-13	86	73				
Sheep (33)	Control	98	-16	36	21				El-Kadi *et al.*, 2006
	+ CN (duodenum; 35 g/d)	104	-16	35	19				
	+ CN (duodenum; 70 g/d)	112	-18	43	25				
	+ CN (duodenum; 105 g/d)	116	-31	46	15				
Sheep (29)	Hay	123	-12	8	-4				Huntington *et al.*, 1980
	85% concentrate	230	35	22	57				
Dairy cows (536)	*Pre partum* (595 kg)	2,201	-134	292	158				Larsen and Kristensen, 2009
	Lactating (mean of 4, 15, 29 DIM)	3,826	-204	408	203				
	Lactating + Glucose (Ab; 1.5 kg/d)	3,624	795	439	1,234				
Sheep (44)	Grass pellets	148	-26	65	39				Piccioli Cappelli *et al.*, 1997
	+ Glucose (jugular; 130 g/d)	207	-70	54	-16				
	+ Glucose (duodenum; 130 g/d)	246	-3	96	93				
Steers (127)	Control	511	-106	139	32				Seal and Parker, 1994
	+ Propionate (rumen; 0.5 mol/d)	509	-74	58	-16				
	+ Propionate (rumen; 1.0 mol/d)	580	-122	65	-58				

above, glucose utilization by the PDV was estimated to be in a similar range, and averaged 0.5 g/d/kg BW in ruminants (346 g/d for a 650 kg cow) but also presented a fairly wide variation from 0.4 to 1.3 g/d/kg BW (Loncke *et al.*, 2009). It is not clear at this point if variations observed between studies are technical or real. In sheep, arterial utilization of glucose by the PDV increased with WB Ra of glucose (altered with intrajugular infusions of glucose), but remained a fixed proportion, 28%, of WB Ra (Balcells *et al.*, 1995). In steers, switching the site of starch digestion from the rumen to the small intestine increased both WB Ra of glucose and the proportion of WB Ra used by the PDV from 15 to 28%.(Harmon *et al.* 2001). However, in dairy cows, increasing starch intake increased WB Ra of glucose but did not affect glucose utilization by the PDV (Kristensen *et al.*, 2006). Also, estimated PDV utilization did not differ between diets providing duodenal starch and starch-free diets (Loncke *et al.*, 2009). An adequate quantification of the PDV utilization of glucose, including factors that might affect it, is much needed to determine the real amount of glucose absorbed from the diet. Nevertheless, the limited data available clearly indicate that the real absorption of glucose and its true contribution to the WB Ra is more important than usually acknowledged using only net portal appearance. Actually, from studies reported in Table 2 with no post-ruminal infusion of starch or glucose, the real contribution of absorption to WB Ra averaged 14% of glucose WB Ra in ruminants, varying from 0 to 37% in relation with intestinal starch digestion.

Gluconeogenesis

If absorption contributes, on average, to 14% of WB Ra of glucose, another important inflow has to be made. It has long been recognized that in ruminants glucose has to be provided through gluconeogenesis, due to limited absorption of glucose compared with monogastrics (Lindsay, 2006). It was only in 1970, however, that a study was conducted measuring simultaneously the WB Ra of glucose and fluxes of glucose across the PDV and the liver (Bergman *et al.*, 1970; Table 2). This confirmed the important role of the liver which contributed, on a net basis, to 82% of WB Ra in these studies, in animals mainly fed hay. As for the PDV, hepatic utilization of glucose derived from plasma should be added to this net flux. In sheep, glucose utilization by the liver from plasma (portal and arterial) supply was very small, increasing by 4% the real glucose hepatic production to 86% of WB Ra. Such a small utilization of glucose by the liver had been confirmed later in steers (Harmon *et al.*, 2001) and in cows (Kristensen *et al.*, 2006; Galindo, 2010), where it averaged between 3 and 5% of WB Ra. These very low measurements of hepatic utilization of glucose from plasma supply are in agreement with the fact that the liver has been reported to mainly use fatty acids as an energy source (Krebs, 1972). This estimation of glucose utilization by the liver might be, however, slightly underestimated because of the recycling of secondary labelled metabolite (e.g. lactate) into glucose in the liver or if cells of the liver are using newly synthesized, unlabelled glucose.

In the study of Bergman *et al.* (1970), the splanchnic contribution to WB Ra varied from 81 to 91%, still leaving slightly more than 10% of the Ra to be provided by another source. From measurements of renal net fluxes, Bergman *et al.* (1974) estimated that in sheep renal gluconeogenesis contributed on average 12% (from 8 to 16%) of WB Ra of glucose. These sheep were fed good quality leguminous hay. However, in growing beef heifers, the renal net flux of glucose averaged only between 4% (high intake) to 6% (low intake) of liver net glucose flux when fed a 75% alfalfa diet, whereas it was even were negative when the heifers were fed a 75% concentrate diet (Reynolds *et al.*, 1991). Similarly, in steers, estimation of peripheral glucose production decreased from 242 to -17 g/d, when the site of starch digestion was switched from the rumen to the small intestine (Harmon *et al.*, 2001). In lactating dairy cows, contribution from peripheral tissues was estimated to be almost null (Galindo, 2010). All together these data suggest that the contribution of the kidneys to WB Ra of glucose is related to the type of feeding and would be fairly limited in animals well fed a concentrate diet providing starch to the small intestine.

Glycogenolysis

Information on the magnitude of the contribution of the turnover of the pool of glycogen to WB Ra of glucose is scarce. In ruminants, Annisson *et al.* (1963) reported that 'There was very little incorporation of ^{14}C into glycogen during the infusion of [^{14}C]lactate or [^{14}C]glucose'. Therefore, the variation of the turnover of the pool of glycogen in response to protein supply will be considered minimal and not contributing to variations of WB Ra to increased protein supply.

Where can amino acids alter glucose kinetics?

As mentioned previously, the WB Ra of glucose is the sum gluconeogenesis plus glucose intestinal absorption plus the turnover of glucose from glycogenolysis returning to the plasma pool. Therefore, simplistically, there are three sites where an increased protein supply may affect WB Ra of glucose: across the small intestine through increased real absorption and/or across the liver or across the kidney through increased gluconeogenesis (as previously mentioned, potential alterations of the pool size and the turnover of glycogen will be considered minimal and not included in this discussion).

Absorption

Increased real portal absorption through increased protein supply could occur through two different mechanisms: 1) increased intestinal digestion of the starch and/or 2) epithelial cells of the small intestine using additional AA from the extra supply (from the lumen or from arterial supply) as an energy source thereby decreasing their utilization of glucose absorbed from the lumen. The first route is supported by the fact that increased presence of casein into the duodenum has been shown to increase post-ruminal digestion of starch in sheep, steers and dairy cows, maybe related to increased secretion of α-amylase (see review from Larsen, 2009). To support the second hypothesis, it is known that the PDV are using glucose from both arterial and lumen source (El-Kadi *et al.*, 2003) and that some AA are used as an energy source by the PDV. In young pigs the contribution of glutamate plus glutamine to CO_2 production across the PDV (54%) exceeded that derived from the sum of enteral and systemic glucose utilisation (44%; Stoll *et al.*, 1999). Such data, however, do not exist for ruminants, but there is substantial evidence that the digestive tract of the ruminants has the ability to catabolize AA to generate energy (see review by Lobley and Lapierre, 2003). In dairy cows, a substantial utilization of glutamate and glutamine by the PDV was also observed, with net portal appearance of glutamine being negative and the absorption of glutamate representing less than 10% of the small intestinal disappearance of glutamate plus glutamine (Berthiaume *et al.*, 2001). Such demand certainly is linked, in part, to use of the AA carbon as an energy source. The PDV also oxidizes the EAA. For example, leucine oxidation across the PDV has been observed in sheep (Lobley *et al.*, 2003) and increased with protein supply in dairy cows (Lapierre *et al.*, 2002). The situation is not as clear for the other EAA (see review from Lobley and Lapierre, 2003). From comparison between predicted digestive flows of EAA and measured net portal appearance in dairy cows, it has been proposed that the PDV would mainly oxidize, in addition to leucine, isoleucine and valine (Pacheco *et al.*, 2006). However, despite these two possible mechanisms to increase real portal absorption, in two studies measuring it specifically (net portal appearance plus PDV arterial utilization), casein infusion did not alter real portal absorption (El-Kadi *et al.*, 2006; Galindo, 2010).

A decreased utilization of glucose by the PDV from arterial supply alters the net flux but not the real absorption and WB RA of glucose. A decreased utilization of arterial supply would, however, spare glucose for other tissues, including the mammary gland. Recent studies have tried to determine if an extra supply of protein or candidate AA, such as glutamine, might effectively spare glucose utilization by the PDV. Indirectly, a sparing effect of glucose arterial utilization by the PDV could be observed through an increased net portal appearance of glucose. In dairy cows abomasal infusions of casein did not increase net portal appearance of glucose (Hanigan *et al.*, 2004) neither did glutamine

abomasal infusion (Doepel *et al.*, 2007). Direct measurements of glucose utilization using labelled glucose lead to the same conclusions. In sheep, the PDV appeared to metabolize increasing amounts of the branched-chain AA, glutamate and glutamine with infusion of casein, but glucose utilization by the PDV was not affected (El-Kadi *et al.*, 2006). Similarly, in a study in dairy cows, abomasal infusions of casein did not alter glucose utilization by the PDV (Galindo, 2010: Table 2). This does not preclude a contribution of AA extracted by the PDV to energy expenditure but, if it does happen, this does not increase the glucose availability to post-PDV tissues.

Altogether these data suggest that the increment in WB Ra of glucose observed with increased protein supply did not originate from increased real portal absorption. In addition, there is no evidence that, albeit some AA are oxidized by the PDV, increased supply of protein would spare glucose for peripheral tissues.

Gluconeogenesis

If the increment of WB Ra of glucose with increased casein supply does not originate from the PDV, options are for increments in gluconeogenesis from the liver or from the kidneys. Although the major glucose precursor is propionate (see reviews Huntingon *et al.*, 2006; Reynolds, 2006), most AA, except leucine and lysine, can make a net contribution to gluconeogenesis, and most of them through entry in the Krebs cycle. The contribution of AA to gluconeogenesis varies with the feeding status. In well fed sheep, AA contribution to gluconeogenesis was estimated to range between 13 to 15% (Ford and Reilly, 1969) or from 11 to 29% (Wolf and Bergman, 1972). In cattle, AA were also estimated to contribute from 11 to 30% of glucose hepatic release (Huntington *et al.*, 2006). These would be maximal estimates as it has been shown in dairy cows that part of the AA extracted by the liver is used for the synthesis of export protein: up to 20% of phenylalanine removal was used for this purpose (Raggio *et al.*, 2007). The AA can also contribute to renal gluconeogenesis in sheep. Kaufman and Bergman (1974) estimated that between 20 and 30% of renal gluconeogenesis originated from AA. Indeed in fed sheep, alanine, aspartate and glutamine were removed by the kidneys and their maximal contribution to renal gluconeogenesis averaged 12%. Also, as evidence for contribution of AA to renal gluconeogenesis, Wolf and Bergman (1972) reported that: 'conversion of ^{14}C-labelled aspartate or glutamate to glucose was 1.5-4 times greater in the whole body than calculated from hepatic uptake of the ^{14}C-labelled amino acid'. However, overall, this indicates a relatively small contribution (less than 2%) of renal neoglucogenesis from AA to WB Ra of glucose in a fed animal.

Only one study reported concurrent measurement of the response of glucose WB Ra and liver net production to increased protein supply in lactating dairy cows: the response of WB Ra of glucose to casein infusion was totally covered by the increased in hepatic net flux (Galindo, 2010; Table 1). As the increment in WB Ra of glucose was associated with the increment in liver net flux and as the data on the response of WB Ra of glucose to increased protein supply are limited in dairy cows, intention was to include studies reporting the net liver flux of glucose in response to casein infusion. However, only one additional study was added to the database (Hanigan *et al.*, 2004; Table 1). One study in non-lactating dairy cows could not be used because the profile of AA was very different from casein (Wray-Cahen *et al.*, 1997).

The advantage of studies reporting net flux across the liver is that in addition to glucose, information on the relative contribution of AA can be estimated from the liver uptake of AA. In the study of Hanigan *et al.* (2004), increasing supply of casein from 0 to 600 g/d increased liver release of glucose (Table 1), but liver removal of AA did not significantly increase. For the highest supplementation treatment, however, numerical increased removal of AA could account for about half of the increased release of glucose. In late gestation, dry dairy cows, infusion of a commercial mixture of AA increased liver net flux of glucose and if the liver uptake of alanine, glycine and glutamine was assumed to be directed totally to gluconeogenesis, these AA would have provided nearly all the 3-C units needed

to account for the extra glucose produced (Wray-Cahen *et al.*, 1997). Unfortunately, data are not yet available to estimate if the increased removal of AA by the liver matched the increased glucose hepatic release in the study of Galindo (2010).

This close relationship observed between the increment in WB Ra of glucose and increased net liver release of glucose (Galindo, 2010) suggests very limited contribution of increased renal gluconeogenesis to support the increment of glucose WB Ra that has been observed with increased protein supply.

Efficiency of transfer

Glucose formed from AA can then be used for lactose synthesis and, already in 1964, it has been reported in a dairy cow that after administration of a mixture of [^{14}C]AA, 'the milk lactose also became radioactive to an extent indicating that about 12 per cent of the lactose was formed by gluconeogenesis from protein' (Hunter and Milsson, 1964). A recent *in vitro* study, however, suggests that part of the contribution of AA to lactose synthesis might also occur within the mammary gland (Bequette *et al.*, 2006). Figure 2 illustrates the relationship between the metabolizable protein supply (MP, NRC, 2001) and the glucose 'availability' (WB Ra or liver net flux). The limited dataset did not yield a quadratic relationship between yields of protein or lactose and MP supply, as observed from the database of Doepel *et al.* (2004). However, a first striking observation is the similarity of the response between the yields of protein ($P<0.01$, 0.22±0.04 g/g; 7 experiments, n=17; R^2adj=95.7%) and lactose ($P<0.01$, 0.20±0.04 g/g; R^2adj=98.4%) to casein infusion. Therefore, in terms of milk output, casein infusion has an effect almost as strong on lactose yield as on protein yield.

Another interesting observation relies on the very high transfer of the infused casein into glucose WB Ra. According to metabolic pathways, the maximal theoretical conversion of casein into glucose would be approximately 0.60 g of glucose per g of casein. The relationship between MP supply and WB glucose Ra was linear ($P<0.01$) with a slope of 0.60±0.14 (7 experiments, n=17, R^2adj=86.7%, Figure 2), with, therefore, an observed efficiency of transfer similar to the maximal theoretical efficiency. In addition, if the increased milk protein yield is removed from the increased protein supply, the slope of the relationship increased to 0.76±0.18 ($P<0.01$). The reason for this high efficiency is not clear. It is possible that the supply of protein has positively stimulated the cow' metabolism and that in such conditions, nutrients other than the additional supply of AA (including dietary AA) have been diverted towards glucose synthesis, if that is recognized as a metabolic priority. Indeed, in cows maintained on a constant feed intake, initiation of lactation increased WB Ra of glucose (Bennink *et al.*, 1992). In view of the very small direct contribution of the glycogen pool turnover to WB Ra of glucose, it is unlikely that it would be stimulation of glycogenolysis that

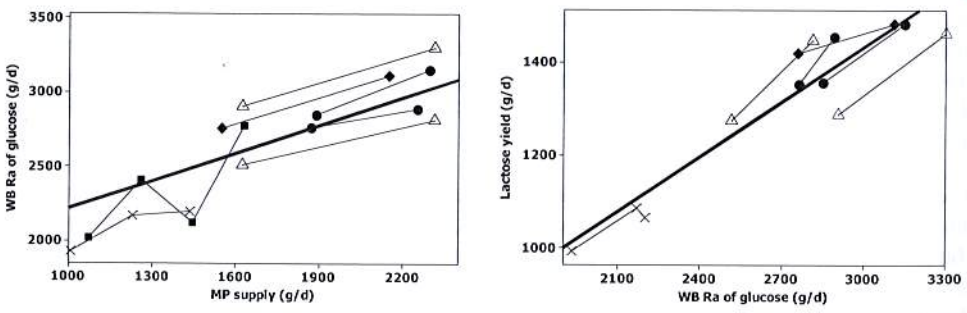

Figure 2. Relationship between whole body rate of appearance (WB Ra) of glucose, metabolizable protein (MP) supply and lactose yield in studies involving casein infusion. Data from: ● *Clark* et al., *1977,* ♦ *Galindo, 2010,* ■ *Hanigan* et al., *2004,* × *König* et al., *1984 and* Δ *Lemosquet* et al., *2009a.*

would be responsible for the high 'recovery' of glucose from protein supply. Another option could also be that part of the increased WB Ra and net hepatic flux of glucose would be the result of an increased recycling from glucose to peripheral tissue and lactate coming back to the liver being used for gluconeogenesis.

If we then look at the transfer of this increment of WB Ra of glucose into the increment in lactose yield, it averaged 0.40 (±0.06) g/g ($P<0.01$, 6 experiments, n=13, R^2adj=96.2%; Figure 2), excluding the milk lactose response from Hanigan *et al.* (2004). This value is slightly smaller than the average 0.49 value of lactose yield/WB Ra calculated for Control treatments of Table 1. This might indicate a lower marginal efficiency at higher glucose availability or also the fact that part of this increment in WB Ra of glucose is not a net increment but originates from recycling of metabolites from intermediate metabolism.

The key question then becomes: 'Is this increment in WB Ra of glucose the driving force for the increased lactose yield?' Overall, a good relationship has been reported between WB Ra of glucose and lactose output (Danfær *et al.*, 1995) and this also holds for the present dataset. However, in two studies where casein infusion increased both WB Ra of glucose and lactose yields, glucose or propionate infusions to the same cows also increased WB Ra of glucose but failed to increase yields of milk and lactose (Clark *et al.*, 1977; Lemosquet *et al.*, 2009a). This suggests that, at least in these studies, with cows still having the potential to improve their lactose yield, availability of glucose was not the critical driver of lactose and milk yields. The flexibility of the mammary gland in using different nutrients to support increased yields of lactose and protein is discussed in another contribution in the present symposium (Lemosquet *et al.*, 2010). Along the same line of reasoning, Lemosquet *et al.* (2009b) reported recently that infusion of glucose, propionate or non-EAA increased glucose WB Ra, but did not increase milk and lactose yields. This non-response of milk yield to non-EAA is in agreement with previous observations where the supply of non-EAA had little influence on milk yield (Metcalf *et al.*, 1996; Schwab *et al.*, 1996; Doepel *et al.*, 2010). Overall, this suggests that although increased glucose availability with casein supply might support the increased lactose yield, glucose availability per se is not the driving force.

Tentatively, some explanations can be proposed to explain this apparent discrepancy, but at this point, they are speculation as no direct observations are available to support or contradict them. One possibility is that stimulation of protein synthesis through increased protein supply has an overall positive effect on all proteins, including enzymes related to gluconeogenesis and lactose synthesis as well as milk protein per se. Increased milk protein synthesis would 'pull' the demand for lactose, due to its major osmotic role (Mepham, 1993), and therefore stimulate gluconeogenesis. That would imply that glucose precursors other than infused AA are used more efficiently towards glucose synthesis (through a reduced oxidation, for example) when extra protein is supplied. Another explanation relies on the potential effect of AA in stimulating protein synthesis through cell signalling pathways (Davis *et al.*, 2003). However, recently, Rius *et al.* (2009) reported that not only casein but also starch was involved in the regulation of the mTOR pathway in the mammary gland of dairy cows.

Conclusion

In summary, increasing supply of protein (casein infusion) directly increases WB Ra of glucose, mainly though an increased hepatic gluconeogenesis. However, the extra supply of AA from infused casein can not entirely explain the increase of WB Ra of glucose. This would suggest sparing of AA or other glucose precursors when metabolism is stimulated by extra protein, but demonstration of that hypothesis is still to come. The increment in WB Ra of glucose induced by casein infusion does not seem, however, to be the driving force behind the observed increased lactose and milk yields. Some essential AA are probably required to stimulate protein synthesis, either as constituent of the protein per se or playing a key role in some metabolic pathways. Overall, this 'simple' example

demonstrates the intricate integration between protein and energy metabolism in a lactating dairy cow. This clearly indicates the requirement of considering them together, rather than as two distinct entities, either when conducting trials or when balancing dairy rations.

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