Matching Quantity and Quality of Protein Sources with Rumen Bacterial Requirements

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Take-Home Messages

1. Microbial protein is the cheapest and best source of metabolizable protein.
2. Prediction of microbial protein is variable at least in part because of inefficiency resulting from continual switching from uptake of preformed amino acids versus inducing expression of bacterial enzyme pathways that synthesize amino acids.
3. More consistent prediction of efficiency of bacterial protein synthesis would reduce the variation in metabolizable protein/amino acid models.
4. Improving modeling consistency will help us formulate lower protein diets that release less nitrogen into the environment with less risk for lost milk production.
5. HMB and HMBi should have both a ruminal and a metabolic effect on milk protein production, depending on their ruminal availability (HMBi escape was estimated to be at least 58% in continuous culture fermenters) and interactions with other dietary factors.

Introduction: Rumen-Degraded Protein for Microbial Protein Synthesis…And More

Microbial protein is the major component of metabolizable protein (**MP**) in dairy cows. Its profile for limiting amino acids (**AA**) strikingly resembles that of milk protein, justifying its high MP value for high producing dairy cattle (Stern et al., 1994). I have elaborated on the variability associated with its measurement, prediction, and dietary factors limiting its supply to the dairy cow (Firkins and Reynolds, 2005; Firkins et al., 2006; Firkins et al., 2007). Predicting microbial protein synthesis remains a critical issue for efficient protein feeding to dairy cattle for one major reason: most computer models for MP still have prediction of microbial protein at their fulcrum. Why is this fulcrum so important? If microbial protein synthesis is overpredicted, then the model underpredicts the need for rumen-undegraded protein (**RUP**). However, if the model underpredicts microbial protein production, then it will underpredict rumen-degraded protein (**RDP**) requirement. Either scenario (and their correlation i.e., underprediction of RUP overpredicts RDP in feeds) inflates safety factors for dietary crude protein concentration. Decreasing RDP is associated with a decreasing dry matter intake (**DMI**) in a small but consistent way (Firkins et al., 2006). Although we are not really sure why this decrease in DMI occurs, probably the most likely explanation is a decrease in fiber digestibility in the rumen, which could increase bulk fill limitation of DMI at least some of the time during a feeding cycle for at least some cows. Of course, depressed DMI decreases the supply of all nutrients, not just AA. Therefore, the *actual* conversion of RDP into microbial protein compared with the *potential* to do so is why we need to move from *quantity* to also considering *quality* of RDP sources for efficient microbial protein production and nitrogen capture in the rumen.

Rumen-Degraded Protein and Ruminal Fiber Digestibility

The Cornell model and its derivations have at its core the concept that the fiber-degrading bacteria only require ammonia, which could come from peptides, cheaper sources of non-protein nitrogen such as urea, or even the "free" supply of blood urea nitrogen (**BUN**) that cycles to the rumen. In that model's structure, only the nonstructural carbohydrate-using bacteria benefit from preformed AA. The foundational studies and even a more current modeling effort supporting this contention (Russell et al., 2009). Such an approach is understandable because the three well characterized cellulolytic bacterial isolates (*Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *R. albus*) all require ammonia as the principal nitrogen sources plus branched chain volatile fatty acids (**VFA**), the latter of which should not be limiting their growth in dairy cows fed appropriately. Research with isotopically labeled AA supports the stimulation of fiber digestibility by addition of preformed AA (Newbold, 1999). That author explained that growth by cellulolytic bacterial cultures actually only is directly stimulated by preformed AA when fed cellobiose (the disaccharide repeating unit of cellulose) as an energy source (which is not typical of the rumen); thus, the benefit to fiber degradation was indirectly attributed to stimulating the synergistic "non-cellulolytic partners". The single exception is that the ruminococci have a clear and critical requirement for preformed phenylalanine for conversion to growth factors needed for adhesion to cellulose but not hemicellulose (Reveneau et al., 2003), prioritizing this preformed AA (Walker et al., 2005). While having some activity against hemicellulose, those three cellulolytic species are not particularly adept at using the sugars released from hemicellulolysis compared with other bacteria that also can break down hemicellulose and for which their growth can be stimulated by preformed AA (Griswold et al., 2003).

Indirect studies support the need for preformed AA for optimal fiber digestibility in the rumen of dairy cows. Both forage and most non-forage fiber sources are typically degraded at about 5%/hour (Firkins, 1997), gradually releasing sugars and oligosaccharides from both cellulose (Wells et al., 1995) and hemicellulose (Cotta and Forster, 2006) that are used by the entire bacterial community. Metagenomics approaches showed that bacterial enzymes attacking plant particles were characterized as initially arising from generalist bacteria followed subsequently by enzymes for the more recalcitrant polymers (Brulc et al., 2009). Because initial adhesion is extensively committed within 5 to 15 min, but subsequent colonization depends on growth (i.e., bacterial cell division) on that new substrate (Edwards et al., 2007), the secondary bacteria should be providing growth factors for the primary cellulolytics without outcompeting them for surface area adjacent to the plant cell walls, and the secondary bacteria should be rewarded by an increased supply of short-chain saccharides. This expectation for coordinated colonization can be supported by studies in which enzymes were exogenously applied to feed. Even addition of exogenous amylase (Kingerman et al., 2009) or protease (Colombatto and Beauchemin, 2009) with insignificant activities against fiber still stimulated NDF digestibility by dairy cattle. The authors referenced a proposed mechanism of increased surface area and stimulation of non-cellulolytics to cross-feed with cellulolytic bacteria. Thus, the provision of adequate preformed AA from RDP should help maintain the supporting microbial cast needed to help optimize the most efficient fiber digestibility rate coinciding with such a high feed intake and passage rate for the high producing dairy cow.

Central Role for Ammonia in Efficiency of Nitrogen Capture by the Dairy Cow

If we going to continue to reliably reduce the emissions of ammonia and nitrous oxide from dairy farms without depressing milk production and requiring more replacement animals (negating the benefit), we need to better understand the control points regarding how rumen bacteria use ammonia and why these control points can vary under different dietary conditions. Ruminants have a tremendous ability to convert BUN into microbial AA synthesis and supply to the animal (Lapierre and Lobley, 2001; Firkins and Reynolds, 2005). The loss of absorbed rumen ammonia from RDP is therefore hedged very well by the large return of that ammonia via BUN. There are some models that reduce metabolizable energy conversion to NEL because of the energy costs by the liver to produce urea, but the larger energy cost is actually from the liver's need to grow its metabolic machinery (more cells and more enzymes) to catabolize excess AA compared with the smaller energy cost of disposing of the ammonia as urea (Firkins and Reynolds, 2005). Therefore, if we can improve the profile of AA supplying the mammary gland to better match its needs, we could reduce the excess of some AA that need to be catabolized and that divert metabolizable energy from milk production. Similarly, if we can better meet the needs of AA for rumen microbial protein production with less catabolism of AA that are not used, we could simultaneously reduce the ammonia that is produced and lost from the rumen while potentially trapping more BUN within the rumen. The lack of understanding the processes is a major reason why ruminal ammonia concentration can vary considerably in cattle fed what might be expected to be similar diets (Firkins et al., 2007).

More Consistent Microbial Protein from RDP

Numerous studies have documented processes of proteolysis, deamination, and ammonia uptake (Walker et al., 2005). For bacteria, biosynthesis of AA requires numerous enzymes that are very energetically costly for them to synthesize. On the other hand, the ability to continue synthesizing AA in low protein diets also would be a critical competitive advantage for bacteria in the highly competitive rumen. The latter scenario would be increasingly likely as we improve carbohydrate digestibility through grain processing, more digestible forages, and better feedbunk management (i.e., more consistent consumption of the diet by more cows). That is, the more we can provide a consistent availability of substrate for the rumen microbes, the more critical it is for an increased supply of steadily available AA from RDP because the intracellular concentration should not then rate-limit protein synthesis as is more likely when relying on AA biosynthesis. My contention is that a more synchronous supply route would decrease the need for inflated RDP safety factors and allow more ration space for other ingredients.

The catch-22 with this logical approach of simply increasing AA from RDP to improve bacterial growth efficiency is that these AA could also stimulate the growth of the obligate AA-fermenting bacteria (Walker et al., 2005). This group, sometimes termed "hyperammonia producers" is in very low numbers and has to rapidly deaminate many AA molecules to ammonia to gain enough energy to grow because they do not competitively use carbohydrate as an energy source compared with other sugar fermenters (which also compete for AA for protein synthesis). The obligate AA fermenters could periodically spike in their numbers and deplete the concentration of AA that would stimulate the growth and fibrolytic capacity of the consortium of bacteria that

breaks down fiber. The current literature would support the use of an ionophore or perhaps other dietary methods to inhibit these AA fermenting bacteria, whereas another effective strategy might be to stimulate the growth of sugar-using bacteria that can effectively compete for AA against the hyper-ammonia producers (Firkins, 2010). That is, sometimes we need to "fight fire with fire". To explain, I need to discuss AA metabolism by ruminal bacteria.

Amino Acid Metabolism by Ruminal Bacteria is Convoluted

Alanine, glutamate, and glutamine are the primary AA formed from assimilation of ammonia in the rumen (Walker et al., 2005). The specific assimilation pathway depends on the actual ammonia concentration through enzyme kinetics and gene expression (Morrison and Mackie, 1997). After ammonia is attached to carbon skeletons (derived from uptake of sugars) to make alanine, glutamate, or glutamine, transamination reactions switch that amino group from those three AA to the other AA (Walker et al., 2005). These networks of AA biosynthesis pathways net can be viewed schematically in Figure 1, which is largely taken from Morrison and Mackie (1997) except that I added minor changes or additions by Paulus and Gray (1967), Gotschalk (1979), Baldwin and Allison (1983), Or-Rashid et al. (2001), and Walker et al. (2005).

Although challenging to evaluate, Figure 1 does depict very clearly how convoluted is the biosynthesis of important nitrogenous sources and how these biosynthetic reactions compete with flux of carbon for VFA and the corresponding ATP production needed to fuel those AA biosynthetic reactions (ATP usage is left off the figure for simplification). Lysine (aspartate family) and proline (glutamate family) are among the most rate-limiting AA (Demeyer and Fievez, 2004; Walker et al., 2005). Bacteria also contain up to 20% RNA and DNA, and aspartate (which also could produce lysine or other AA, including methionine) is a major precursor for pyrimidine biosynthesis for DNA and RNA. In addition, the polyamines spermidine and spermine are important in stabilizing DNA during cell division both directly and from the methionine derivative, S-adenosyl methionine (**SAM**). In contrast with the AA for which biosynthesis is rate-limiting, Atasoglu et al. (2004) concluded that phenylalanine and the branched chain AA might be the most critical preformed AA needed for optimal bacterial growth. In that study, most of the isoleucine, phenylalanine, lysine, and leucine were directly incorporated into bacterial protein without degradation.

Amino acids, as important as they are for protein synthesis, are diverted in seemingly wasteful processes to maintain bacterial function. Atasoglu et al. (2004) reported that a substantial amount of alanine must leak out or be exported from bacteria. Earlier, Blake et al. (1983) postulated a potential triple role for alanine as a short-term intracellular storage mechanism for NH_3, an excretion product to deplete excess intracellular NH_3 concentration, or a means to dispose of pyruvate (which is aminated to alanine) when readily available carbohydrate is in excess relative to availability of nitrogen. Stevenson (1978) noted the progressive excretion of AA by rumen bacteria in logarithmic growth, termination of AA efflux, and then finally uptake and apparent assimilation into protein as their exponential growth ceased. These prior authors did not distinguish L from D racemers, but D-AA accumulated in the media most in mid-log phase of a number of bacteria and declined thereafter (Bhattacharyya and Banerjee, 1974). Only L AA are incorporated into proteins, but there are some D-AA that are used in the cell walls of many

bacteria, especially D-alanine (Matsui et al., 2009). The enzymes to racemize L-AA to their respective D-AA have varying affinities for AA and would be expected to be localized near the outer cell wall. I interpret these results as evidence that rumen bacteria have evolved to buffer their metabolism for a variety of varying ammonia and preformed AA concentrations (and their interrelationships). Because transporters would be expected to have a much higher affinity for the L than D racemer (Zhang et al., 2003), I can speculate that extracellular accumulation of D-AA might help improve the intracellular L-AA profile relative to needs for protein synthesis, but there is no supportive evidence of which I am aware.

Several studies have tried to address how an imbalance of AA limits bacterial growth in vitro. In Figure 1, I show just a few of the many feedback inhibitions known for bacteria, but these would support the data from Kajikawa et al. (2005) that rumen bacterial growth was inhibited by excess threonine, also explaining why this inhibition could not be reversed by adding more lysine or methionine but was mitigated by addition of several other AA that were not in the aspartate family (to allow a better balance with the aspartate family). After surveying the literature, Walker et al. (2005) concluded that a proper mix of AA is needed to support maximal growth of bacteria, and deletion of any single AA has only a modest inhibition compared with the decreased growth resulting from removal of a complete mix of AA. Therefore, I would expect a greater benefit from trying to maintain a constant stream of AA from RDP for microbial protein synthesis as being more important than trying to manipulate the AA profile of that RDP.

Strategies to More Efficiently Convert AA from RDP into Microbial Protein

As in other aspects of feeding dairy cattle, we need to start with the basics. We need to continue to improve our ability to make sure that the ration that is printed from the computer more closely resembles what cows are eating. For example, continued efforts to improve the ratio of effective fiber to rumen-degraded starch concentrations, reduce forage sorting, improve forage quality and digestibility, improve the balance of MP and metabolizable AA, etc. We have long known that anything that improves DMI should increase microbial protein synthesis (Oldick et al., 1999), which should therefore increase the priority for RDP to enhance efficiency of microbial growth. The more consistently that more cows eat more meals per day, the less cyclicity in ruminal pH and the supply of rumen-degraded carbohydrate relative to RDP. Synchronizing carbohydrate and RDP supplies is a challenge to yield consistent benefit in the field (Hall and Huntington, 2007). Although often discussed, I don't favor trying to match fast sources of RDP with fast sources of rumen-degraded carbohydrate because the cow's feeding pattern probably is the more important mediator of synchronicity of carbohydrate and RDP. I do think that a small amount of sugars is likely to help to provide a basal population of lactate-consuming bacteria that also use AA and can outcompete the hyperammonia-producing bacteria (Firkins, 2010).

How do we properly formulate diets for structural and non-structural carbohydrates for efficient conversion of RDP into microbial protein? The Dairy NRC has made the first step in providing a sliding scale in integrating these two feed fractions i.e., the lower the forage NDF, the lower should be the non-fiber carbohydrate. Improvements in effective NDF values and chemical measurements of starch and sugar help refine this system. But we should be accounting better for differences in ruminal starch availability using book values as in our third trial with liquid feeds

(Firkins et al., 2008) or by more sophisticated approaches. The CNCPS or CPM models are estimating rates of degradation of different carbohydrate fractions, which is very helpful, but there is only so far that in vitro degradations can simulate in vivo situation.
Huhtanen and Hristov (2009) evaluated the 2001 NRC model and concluded that the percentage of RDP has a minor impact on milk protein. However, these RDP values were mostly extracted from the NRC model's feed library because few studies actually measured RDP of all feeds. Because of the strong collinear effect of RDP on both DMI and milk protein based on a meta analysis after adjusting for the random effect of different trials (Firkins et al., 2006), I have looked in the literature for studies that actually measured RDP the way that NRC recommends (and have not yet found other studies that met the same criteria for inclusion). I would note that adjustment of data for the "trial effect", I noted a quadratic relationship between RDP (% of DM, X) and milk protein (kg/d, Y variable): $Y = 0.14 + 0.194X - 0.0089X^2$. I also verified that much of this response is indeed a result of the relationship between RDP and DMI. What this quadratic relationship shows is that as RDP is progressively decreased, you would expect a progressively decreased production of milk protein. For example, dropping from 10.0 to 9.0% RDP should lose 25 grams/day of milk protein/cow/day; and from 9.0 to 8.0, an additional 43 grams/day. An average response of 25 grams/day would account for 76 kg (168 lb) of milk protein per month for a 100-cow herd, but the actual response would be somewhat more if you factor in decreased DMI. But the real problem arises when you consider that this is just an average response. The actual response would range from no loss whatsoever (i.e., cows will do fine on 9.0% RDP) to a 2- or 3-fold worse response than average. Going back to the conclusion of a small response from RDP based on book values (Huhtanen and Hristov, 2009) partially misses the point. A mean follows a bell-shaped curve in which actual RDP being greater than average would have no response, but actual RDP being lower than average ought to have a worse response than average. How would a nutrition advisor know the that actual RDP fed to a herd is lower than his/her formulation until after the result already showed up (and brought to his/her attention by a less-than-enthused client)? Therefore, if you can't actually measure RDP of feeds or if the cost of measurement reduces the benefit from actually knowing the true RDP, the natural inclination will continue to be to inflate a protein safety factor. For these reasons, I have been looking for ways to improve the efficiency of conversion of AA from RDP into microbial protein to circumvent this natural inclination.

HMB or HMBi to Potentially Enhance Bacterial Protein Capture of RDP

Before explaining the rationale for this expectation of a role for 2-hydroxy-4-(methylthio) butanoic acid (**HMB**) or its isopropyl ester (**HMBi**) to potentially stimulate bacterial protein synthesis, it is important to first explain why I would *not* expect a benefit from methionine directly. There are some studies showing a limiting role for methionine in the rumen (Demeyer and Fievez, 2004), but the more recent isotopomer studies are less supportive (Walker et al., 2005). In fact, as explained earlier, I don't expect that any single AA will be very useful because it will simply reduce bacterial biosynthesis of that AA transiently until that AA's concentration in rumen fluid declines from passage (a potential benefit because of increased bypass) or is degraded by the hyper-ammonia bacteria (a likely but variable possibility). Regarding methionine, SAM is the major "switch" to repress transcription of methionine biosynthesis or transporter genes in gram-negative (Zhang et al., 2003) and gram-positive (Tomsic et al., 2008) bacteria. It is important to

remember that bacteria tend to have a whole series of genes that are controlled simultaneously by the same molecule (Augustus et al., 2009). That is, the ”operon” (the series of genes) is repressed by the same repressor molecule (the ”regulon”).

Based on all this information, we have hypothesized that a bolus dose of methionine would simply down-regulate methionine biosynthesis, but a small but steady supply of methionine from some precursor that does not down-regulate methionine biosynthesis could help ”push” SAM for its major roles in cell growth while sparing important carbon skeletons that would then not be needed to synthesis methionine. Although little is known for rumen bacteria, non-rumen bacteria prioritize a branched chain amino transferase to convert 2-keto-4-methylthiobutyrate (KMTB, produced from HMB, as shown in Figure 1), and this enzyme is regulated independently of the methionine biosynthesis genes for a variety of bacterial species (Berger et al., 2003).

When either methionine or methionine hydroxy analog were radiolabeled, the addition of the analog did not decrease the disappearance of methionine’s label in the supernatant (excluding bacterial cells), whereas the addition of methionine decreased significantly the disappearance of the analog’s label in the supernatant (Patterson and Kung, Jr., 1988). The authors suggested that methionine was the preferred precursor. However, based on the previous discussion, I think differential regulation of methionine versus the analog is an appealing alternative explanation.

The results of Noftsger et al. (2003) demonstrate that a continuous supply of HMB or methionine did not increase microbial protein synthesis, probably because RDP was much higher than expected. Even so, HMB decreased ($P < 0.05$) the proportion of microbial N originating from ammonia by about 13%. If HMB were a simple carbon skeleton precursor for methionine, then it should increase the uptake of NH_3. The opposite result suggests that HMB could stimulate an overall improvement in incorporation of carbon from AA other than methionine into microbial protein. Compared with DL-methionine control, both HMB and especially the more slowly degraded HMBi seem to shift bacterial populations (Karnati et al., 2007).

Preliminary information from a second fermenter study at Ohio State assessed escape using ^{13}C-labeled HMBi (Fowler et al., 2010) by evaluating the elimination rate of the label after it was replaced with conventional HMBi. The HMBi escape was $58 \pm 5\%$ when only HMBi was dosed 3 times per day at the same rate as standard dosages for dairy cattle and $65 \pm 3\%$ when half of the HMBi was replaced with DL-methionine. Interestingly, the combination of DL-methionine with HMBi increased the accumulation of methionine in the fermenter contents. HMBi increased the concentration of isovalerate (the product of isoleucine deamination) compared with the DL-methionine control, supporting our expectation that a branched chain amino transferase is converting KMTB to methionine (Berger et al., 2003). In contrast with the study with HMB (Noftsger et al., 2003), HMBi increased the percentage of bacterial N derived from ammonia in the more recent study. HMBi decreased the total VFA production without a concomitant effect on organic matter digestibility. Thus, in this case, carbon was apparently diverted from VFA production toward AA biosynthesis when HMBi replaced methionine. A similar decrease in VFA production was noted for methionine hydroxy analog (Windschitl and Stern, 1988). My conclusion is that HMB directly or indirectly (after conversion from HMBi) influences the net of AA uptake and biosynthesis in ways that are not yet elucidated, but potential activity for rumen microbial protein synthesis and capture of RDP or BUN is likely.

Free Amino Acids for Dairy Cows?

What about feeding free AA and assuming that even a relatively low escape is still an economical way to increase metabolizable AA supply to the cow? Norwegian researchers (Velle et al., 1997; Volden et al., 1998) showed an accumulation of AA in the rumen and a resulting decline in the degradation rate associated with increasing dosage. In contrast, Robinson et al. (2005) explained why mixing with a TMR should decrease the escape of free lysine compared with bolus dosing (as in the Norwegian studies). However, when free lysine or HMB were mixed in feed alone or in combination, the combined treatments tended to increase milk production with some supportive evidence from mammary AA extraction data (Wang et al., 2010). Although appealing to confirm a combined effect from delivery of co-limiting AA, I question the expectation for a consistent metabolizable coefficient from free lysine for routine field application. Even if a fast passage rate of ruminal fluid from high producing cows washes out free AA, supplementing enough AA to have significant bypass would have to greatly exceed the ruminal capacity for bacterial needs for protein synthesis, not imbalance their convoluted regulatory mechanisms among AA pathways, and still simply not just feed an increased population of hyper-ammonia bacteria.

In contrast with free AA, I have provided information to support why I consider it unlikely that HMB or HMBi would imbalance AA availability by ruminal microbes while, in contrast, they might improve the conversion of AA other than methionine into microbial protein. Would HMB or HMBi that is converted to HMB in the rumen help to prevent toxicity from a single AA such as free lysine? For now, I think that precautions are very much in order for that next step without further data. In addition, the escape of free AA, if significant, is likely also highly variable. The transfer efficiency of absorbed AA into milk protein increases with decreasing MP supply (Rius et al., 2010). Therefore, efforts to lower RUP in the diet while improving the metabolizable AA profile in that RUP are complicated enough without adding more variability from supplementing high amounts of free AA. Regardless, to be successful in this strategy of achieving a more desirable metabolizable AA profile to the mammary gland, we also need to better match the RDP supply with its requirements. Besides more consistently improving microbial protein synthesis or DMI, a more consistent delivery of microbial protein relative to its prediction should allow MP models to work more consistently because of the fulcrum effect leveraging several key equations in those models. Better precision (less variability) leaves a reward that is hard to measure but still has economic value. Thus, higher quality RDP (more consistently available AA) should help prioritize the need for high quality RUP or bypass AA sources while retaining more nitrogen in microbial protein in the rumen rather than in the manure and ultimately the environment.

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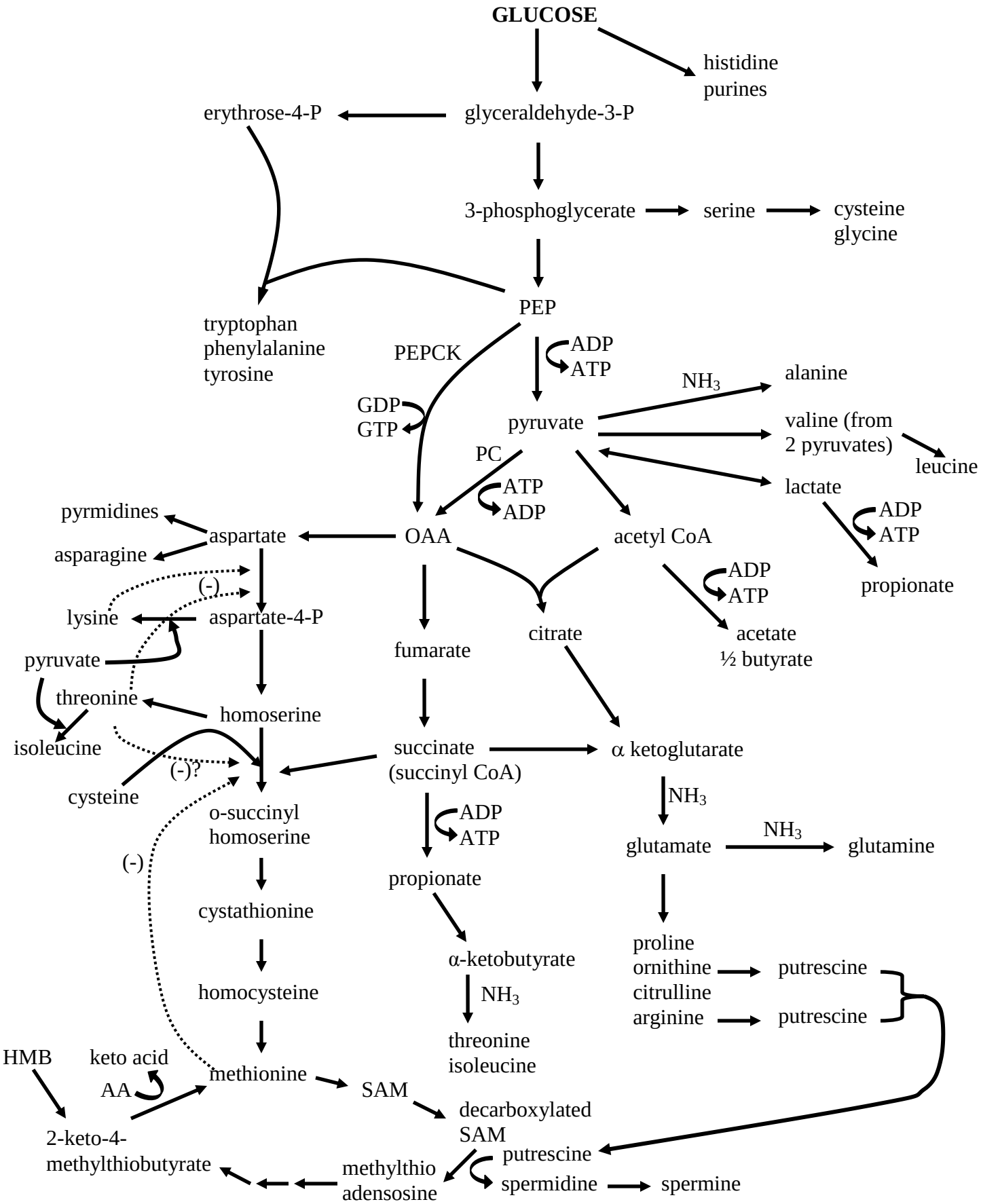
Figure 1. Amino acid (AA) biosynthesis in rumen bacteria, PEP = phosphoenolpyruvate, PEPCK = PEP carboxykinase, PC = pyruvate carboxylase, OAA = oxaloacetic acid, SAM = S-adenosyl methionine, HMB = 2-hydroxy-4-(methylthio) butanoic acid. Examples of feedback inhibition are indicated by dashed arrows, but most were omitted. Adenosine triphosphate (ATP) consuming reactions were also omitted.


GLUCOSE
histidine
purines
erythrose-4-P
glyceraldehyde-3-P
3-phosphoglycerate
serine
cysteine
glycine
PEP
tryptophan
phenylalanine
tyrosine
PEPCK
ADP
ATP
GDP
GTP
NH3
alanine
pyruvate
valine (from
2 pyruvates)
leucine
PC
ATP
ADP
lactate
ADP
ATP
propionate
pyrmidines
aspartate
asparagine
OAA
acetyl CoA
(-)
lysine
aspartate-4-P
ADP
ATP
acetate
½ butyrate
pyruvate
fumarate
citrate
threonine
homoserine
isoleucine
succinate
(succinyl CoA)
α ketoglutarate
(-)?
cysteine
NH3
o-succinyl
homoserine
ADP
ATP
glutamate
NH3
glutamine
(-)
propionate
cystathionine
proline
ornithine
citrulline
arginine
putrescine
putrescine
α-ketobutyrate
homocysteine
NH3
threonine
isoleucine
HMB
keto acid
AA
methionine
SAM
decarboxylated
SAM
2-keto-4-
methylthiobutyrate
methylthio
adensosine
putrescine
spermidine
spermine