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REVIEW ARTICLE

# Small-intestinal or colonic microbiota as a potential amino acid source in animals

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**Abstract** Factors affecting physiological impacts of the microbiome on protein nutrition are discussed for hind-gut fermenters (humans, pigs, rodents). The microbiome flourishes in all gastrointestinal organs, and is a major source of amino acids to fore-gut fermenting animals. In humans, rats and pigs the net effect of microbiome biomass synthesis on amino acid requirements is much less certain. Dietary proteins, amino acids, peptides, endogenous-secreted protein and recycled urea may all be utilized as nitrogen source by growing bacteria in the small intestine and colon. The inclusions of radiolabelled amino acid precursors will result in labeled bacteria which can be digested and absorbed in the ileum and to some degree in the colon. This does not necessarily indicate a significant nutritional role of the microbiome in humans, pigs and rodents. The physiological attributes required for small-intestinal and colon microbiome utilization are a vigorous proteolytic digestion with pancreatic or intestinal enzymes and the presence of amino acid transporters. Findings to date seem to suggest that these two physiological attributes for effective bacterial protein utilization are present in the small intestine; however, these attributes have a much lower capacity/impact in the colon. The gastrointestinal microbiome is likely a protein source of medium to high nutritional quality, but overall the microbiome is not an important amino acid source in humans and animals fed amino acids at requirement levels.

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## Introduction

The role of the intestinal microbiome in human health and disease has been extensively investigated over the last decade. In very rapid fashion the human microbiome and its multifunctional properties has become a major focus in biomedical research. This has included work on the role of the microbiome for example on interactions with small and large-intestinal mucosa, on the immune system, Crohn's disease, ulcerative colitis, amino acid metabolism, obesity and associated maladies and effects of diet and other factors on phylogenetic diversity (Redinbo 2014; Aidy et al. 2014; Sherman et al. 2014; Dai et al. 2011; Schnabl and Brenner 2014; Padmanabhan et al. 2013). Further, production of short-chained fatty acids (SCFA), particularly butyric acid, in the colon/cecum has been related to maintenance of colon epithelial cells and the process of colonic tumor genesis (Cho et al. 2014; Singh et al. 2014). To date, these studies have tended to enumerate organisms in intestinal contents and functional roles of the microbiome in the above medical issues (Ianiro et al. 2014). Earlier work on the role of intestinal microbiota (as far back as the nineteenth century) in comparative protein nutrition and metabolism in animals (from laboratory rodents to pigs to cattle) and in particular with pre-gastric fermenters (ruminants), had a more nutritional, physiological focus. (Hungate 1966; Bergen and Yokoyama 1977; Owens and Bergen 1983; Fuller and Reeds 1998; Scott et al. 2013). These efforts included extent of protein breakdown in the rumen, microbial cell production and quantifying the amount of salvage N from body

urea–ammonia recycling that can be used to meet the amino acid needs of such animals. Similar work was also initiated with pigs and horses which can be described as post-gastric fermenters. Work on pigs, in particular neonates, has focused on the possible contribution of microbiota amino acids to the protein nutriture of pigs and by inference humans (Fuller and Reeds 1998; Columbus et al. 2012; Fuller 2012). One general principle which may be broadly applied here is that in animals fed optimal or excess dietary protein, recycled nitrogen will not positively contribute to the requirement of nutritionally indispensable amino acids (Metges 2000).

Before assessing quantitative effects of gut microbiome anaerobes on filling amino acid needs in humans, the discussion must focus on the role of various microbial/digestive tract ecosystems and further the factors needed to insure that amino acid utilization from microbiota is possible. A vigorous fermentation based on carbohydrates is necessary for the production of acidic fermentation end products, gases and microbial biomass. These fermentation end products (SCFA) do not accumulate in gut contents but are readily absorbed (Bergen and Yokoyama 1977; Hintz and Schryver 1978). Diet-dependent extensive colonic fermentations and SCFA production may lower the pH of the colonic milieu and lower potential pathogen establishment (Scott et al. 2013). While the SCFA can materially add to the overall energy uptake in humans from lower gut fermentable substrates, an effect on quantitative aspects of amino acids (protein) nutrition depends on the fermentation-associated production of microbial biomass, subsequent enzymatic digestion and absorption from the colon. The production of microbial biomass is sizable (as evidenced from fecal output) but unequivocal, documented evidence for a net contribution of this biomass as an amino acid source for well-fed post-gastric fermenters is not available (Metges 2000; Torrallardona et al. 2003). Finally recent research showed that the microbial biomass level can vary in the human intestinal ecosystem (Lahti et al. 2014). These workers indicated many bacteria exhibit robust (Lahti et al. 2014) bi-stable abundance distributions by being altogether at low numbers or at high numbers during relatively short time periods. If these types of microbial density fluctuations turn out to be physiologically significant, this would lead to further questions about the microbiome to be a reliable/predictable net amino acid source. Taken all together, the small-intestinal microbiome, in part, breaks down proteins, utilizes amino acids directly, and/or uses ammonia ($NH_3$). There is also some deamination, but the $NH_3$ pool also arises from recycled urea hydrolysis, endogenous secretions (cells, enzymes) digestion (Bergen and Wu 2009; Dai et al. 2011). While amino acids arising from small-intestinal biomass production with recycled N are readily digested and absorbed in the lower ileum (Torrallardona et al. 2003), it is not at all clear whether the overall protein nutriture of well-fed animals will be positively affected (Bergen and Wu 2009; Metges 2000). In the colon, basically the microbiome digests and catabolizes proteins/amino acids from all sources arriving here.

To further complicate understanding of the role of intestinal bacteria are the adhesion properties of bacteria. As first shown in the bovine system by the Costerton Laboratory, bacteria will adhere to epithelial membranes and closely interact with these cells, other bacteria adhere to food particles while others are free floating (McCowan et al. 1978; Cheng et al. 1981). The rumen wall-adherent bacteria feed directly of the urea entering the rumen by recycling (Stewart 1997). Yang et al. (2014) discuss a similar distribution of intestinal bacteria in the non-ruminant small intestine. Here we have luminal (free) and tightly and loosely attached bacteria (Van den Abbeele et al. 2011). Thus, most likely some bacteria will adhere tightly to enterocytes and closely interact in a quasi-symbiotic process in N metabolism. In assessing the roles of the niche microhabitat and bacteria, Yang et al. (2014) showed that tightly adhered organisms preferred $NH_3$ for de novo amino acid synthesis while luminal (free) organisms preferred to utilize preformed amino acids. Thus, the intestinal microbiome cannot be thought of as homogeneous in terms of salvage N fixation or $NH_3$ utilization. Such a conclusion also complicates experimental designs trying to assess a net contribution of the intestinal commensal bacteria to amino acid nutrition of the host animal. In any case, there will be microbial synthesis as organisms are fermenting non-fiber and fiber carbohydrates to SCFA in the upper ileum and colon. As there seem to be minimal mammalian protease activity in the colon and despite the presence of many solute transporters, amino acid uptake does not seem to have a priority here.

## Comparative aspects of microbial species composition in the fore-stomach microbiome, small intestine microbiome, colon and fecal microbiome

While many studies have focused on the microbiological phylogenetic makeup in fore-stomachs of ruminants and fecal material in post-gastric fermenters, well-conducted, extensive work on complete phylogenetic makeup in the small-intestinal, colon and fecal microbiome under various dietary conditions in animals is not yet available. It is worth noting that the bacterial counts in the small-intestinal contents ($10^{4-6}$) are lower than in the colon. This is possibly a reflection of the high number of small-intestinal bacteria that adhere to epithelial cells/enterocytes (Metges 2000). Cell counts are often up to $10^{12}$ in the colon and fecal contents. The principal bacterial phyla are firmicutes (mostly clostridia and bacilli), bacteroidetes (spirochetes and prevotella), proteobacteria, and some critical archaeal

**Table 1** Amino acid compositions of various isolated rumen bacterial preparations

| Amino acid | Bacterial preparations (gm amino acid/100gm amino acids) | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | Casein |
| Aspartic acid | 12.1[a] | 11.1[b] | 11.1[c] | 11.3[d] | 12.0[d] | 9.5[b] |
| Threonine | 5.4 | 7.3 | 5.5 | 7.1 | 6.0 | 4.3 |
| Serine | 4.5 | 4.3 | 3.8 | 4.7 | 4.7 | 6.1 |
| Glutamic acid | 13.4 | 8.8 | 11.9 | 13.5 | 13.8 | 24.8 |
| Proline | 3.5 | 2.3 | 4.1 | 2.7 | 2.8 | – |
| Glycine | 5.0 | 4.0 | 6.1 | 7.6 | 5.9 | 1.8 |
| Alanine | 6.5 | 9.6 | 6.5 | 7.6 | 7.5 | 3.5 |
| Valine | 6.0 | 6.6 | 6.6 | 4.9 | 5.2 | 4.8 |
| Methionine | 2.4 | 3.6 | 2.6 | 2.2 | 2.0 | 2.6 |
| Isoleucine | 5.7 | 6.0 | 6.4 | 5.6 | 5.2 | 5.5 |
| Leucine | 7.6 | 8.1 | 7.3 | 7.8 | 7.7 | 10.0 |
| Tyrosine | 4.4 | 5.4 | 4.2 | 4.5 | 4.3 | 6.1 |
| Phenylalanine | 4.9 | 6.2 | 5.1 | 4.7 | 4.7 | 5.5 |
| Lysine | 8.5 | 8.0 | 9.3 | 8.3 | 8.8 | 8.7 |
| Histidine | 2.1 | 2.4 | 2.3 | 1.9 | 2.0 | 2.6 |
| Arginine | 5.2 | 5.2 | 5.4 | 5.2 | 5.3 | 3.7 |

[a] Storm and Orskov (1983)
[b] Bergen et al. (1968a)
[c] Purser and Buechler (1966)
[d] Bergen et al. (1968b)

genera (Eckburg et al. 2005; Dai et al. 2011). From various attempts to identify bacterial phyla distributions in bovine rumen, the case can be made that intestinal, colonic and fecal organism are qualitatively similar (Dai et al. 2011; Morrison 2013) but may vary in specific number/genera of organisms representing the whole microbiota (Ivarsson et al. 2014; No attempt is made here to detail a bacterial phyla and gastrointestinal site relationships in animals). Because of these similarities of gut anaerobic microbiota between fore-stomach fermenters and post-gastric fermenters, the nutritional characteristics (for the host animal) of bovine rumen microbiota may be used to characterize putative nutritional values of such organisms for humans (pigs) when microbial amino acids become available for absorption in the small intestine and colon.

## Nutritional (protein) quality of gut anaerobes

The fact that non-protein nitrogen can serve as a dietary protein source (i.e., absorption of amino acids from the small intestine) is undeniable in animals characterized as pre-gastric fermenters (Bergen and Yokoyama 1977). The anaerobic fermentation process results in degradation of energy sources (usually carbohydrates) with the production of acidic end products (SCFA), carbon dioxide, methane, reduced S gases and microbial biomass. Such net gains of microbial cells in the rumen microbiome have been evaluated as an amino acid source for ruminants. In as much as there are species similarities between firmicutes and bacteroidetes in human microbiomes and rumen ecosystem bacteria, reviewing the "protein quality" of rumen anaerobes will give us an insight of putative benefits of such protein sources to human protein nutrition.

The principal constituents of anaerobic microbial/bacterial biomass isolated from the rumen ecosystem are proteins and nucleic acids (Bucholtz and Bergen 1973; Storm and Orskov 1983), followed by ash, carbohydrates and lipids (Storm and Orskov 1983). The carbohydrate content may vary with the presence of highly fermentable dietary carbohydrates while the bacterial lipid content is fairly constant (Hungate 1966). Bacteria in the intestines and fore-stomachs are found as attached to gut epithelia, bound to particles and so called "free floating" in the digesta (Cheng et al. 1981). Bulk (total) amino acid compositions have been shown to be remarkably similar between mixed rumen microbial preparations and individually grown rumen anaerobes (Purser and Buechler 1966; Bergen et al. 1968a, b; Storm and Orskov 1983). On an amino acid content basis (essential amino acid index[EAA-I]), rat feeding trials (Biological Value [BV]), true digestibility [TD] and in vitro protein quality assessments (Net Protein Utilization [$NPU_{enzymatic}$], intestinal anaerobes are good quality protein sources (Tables 1, 2; Bergen et al. 1967, 1968a, b; Storm and Orskov 1983); however, various individual bacteria (pure culture bacteria)may differ in in vitro protein digestibility in these studies (Bergen et al. 1967) and hence a quality parameter based on amino acids liberated and digestibility of individual bacteria ($NPU_{enzymatic}$) varied from high protein quality to lower protein quality in these anaerobes (Bergen et al. 1967). Based on studies trying to relate the continuous profiles of amino acids arising during the digestion process, such profiles were found to not always reflect the amino acid profile of chemically assessed total amino acid contents of the bacteria

**Table 2** Protein quality values of various isolated rumen anaerobic bacteria preparations

| Preparation | (From animal nitrogen balance–digestibility assays) | | |
|---|---|---|---|
| | Biological value (BV) | True digestibility (TD) | Net protein utilization (NPU) |
| A[a] | 85 | 75 | 63.4 |
| Casein control[b] | 90 | 97 | 87 |
| | Estimated BV[f] | Pepsin–pancreatin digestibility[g] | NPU enzymatic[h] |
| B[c] | >100 | 70 | 77 |
| C[d] | >100 | 68 | 74 |
| Casein control[e] | 77 | 96 | 74 |

[a] Bergen et al. (1968a)

[b] Bergen et al. (1968b)

[c] Bergen et al. (1968b) high forage diet

[d] Bergen et al. (1968b) high corn-grain diet

[e] Bergen et al. (1967)

[f, g, h] From in vitro enzymatic digestion–protein quality evaluation Bergen et al. (1968b)

(Bergen 1969). Thus, the EEA-I (based on EAA content only) and $NPU_{enzymatic}$ may differ, but it has never been established that when complete digestion occurs in vivo microorganism markedly differ in protein quality (Bergen et al. 1968a, b; Storm and Orskov 1983). What implications could be drawn from these data as applied to the human microbiome? First most likely the protein quality of the small-intestinal microbiome will not be greatly affected by shifts in microbial species (usually in response to dietary factors, Scott et al. 2013); however, for the colonic microbiome potential quality differences between various species may exist as digestion (mammalian enzymes) is not vigorous in the colon. Hence in the colon, all organisms could potentially vary considerably as an effective amino acid source irrespective of similar amino acid content and net microbial N arising from salvage pathways.

## Defined roles and interactions of microbiota in various ecosystems and digestive tracts

Since the human microbiome has not been extensively studied in all regions of the gastrointestinal tract but mostly in fecal samples, we must depend on comparative microbial ecosystems studies to sharpen our understanding about the role of the microbiome in nutrition. Such studied ecosystems include bacteria in nutrient-rich waste processing facilities, in estuaries/ponds, in fore-stomachs and small intestine and colon in multiple animal species. Most certainly bacteria in waste processing plants and estuaries, ponds as well as other bodies of water bacteria serve the function of a nutrient sink via growth/accumulations of cellular organic materials and minerals. These bacteria can then be utilized by the next level of organisms in the food chain. Clearly then bacteria are protein and B complex vitamin sources. When considering animal species with a fermentative fore-stomach(s) here the microbiome grows and lives before the animal digestive organs and again the fore-stomach microbiome can be an excellent amino acid, B complex vitamin and possibly long-chain fatty acid sources for small intestine digestion–absorption (Hungate 1966). With respect to fore-stomach fermenters (most studied species are ruminants), these animals when fed low protein diets efficiently recycle limiting N (urea–ammonia) into microbial biomass protein for an amino acid source to be digested and absorbed in the small intestine. As these animals are fed higher protein diets as required for appropriate growth and development, any net effect of recycling, now with not limiting (salvage) N sources, is less effective (Calsamiglia et al. 2010). Feeding these animals excessive protein diets actually results in protein wasting without net N recapture. Little is known to date about putative nutritional contributions from the small-intestinal and colonic microbiome in ruminants. Hind-gut fermenters (i.e., humans, pigs, rodents, and equestrian species) need dietary amino acids to meet needs for typical growth rates and maintenance (Bender 2012). Rodents will also practice coprophagy and here fecal genome biomass will be a certain amino acid source. When utilizing rodents for microbiome nutritional studies coprophagy must be avoided. In situations of limiting dietary amino acid supplies, much attention has been directed at the microbiome for the reutilization of salvage N for amino acid synthesis (Fuller and Reeds 1998; Jackon 1995, 1998; Metges 2000). In various animal models, there was qualitative evidence that both essential/indispensible and non-essential amino/dispensable acids could be synthesized by the microbiome (NEAA in enterocytes; Bergen and Wu 2009) in the colon from salvage/recycled N, but quantitative impacts on meeting human amino acid requirements and functional significance have been difficult to ascertain (Jackson

1995; Metges 2000). Jackson (1995) from limited data indicated that colonic microbes may contribute about one to two times minimal amino acids needs based on nitrogen balance studies in humans as described by Rose (1968). Off and on over the years such data (i.e., amino acids available from salvage N) have been published particularly for humans/neonates on very minimal protein diets but functional relevance to long-term protein nutrition in normally fed animals/humans has not been in-disputatively documented (Metges 2000).

## Physiological attributes for utilization of the gut microbiome in the colon as a nutrient source

For a putative nutrient source to be utilized by the animal requires active digestion into small molecules and transport processes of these digestion products from the lumen across the intestinal epithelium to the blood supply. Obviously the most likely nutrient sources from microbial biomass are amino acids and B complex vitamins. These latter nutrients will not be discussed here. In the small intestine there are rich secretions of pancreatic proteolytic enzymes and a myriad of peptidases to hydrolyze peptide bonds of proteins. Likewise the enterocytes possess transporters to absorb the neutral, hydrophilic, hydrophobic and cationic amino acids via active, passive and facilitated transporters. As the digesta passes from the ileum into the colon, the material is initially rich in microorganisms, undigested carbohydrates and moderate to minimal levels of small nitrogenous compounds (Bergen and Wu 2009). Results from animal models here show that depending on the quantities of carbohydrates available, colonic/cecal fermentations will produce SCFA and more microbial biomass (Orskov et al. 1970). The SCFA will be absorbed from the colon for utilization in energy metabolism (Macfarlane and Macfarlane 2012). In the colon the presence of secreted digestive tract proteases (except those likely in the digesta flow from the ileum) has not been documented and hence breakdown of endogenous and microbial proteins will depend on colonic proteolytic organisms (Macfarlane and Macfarlane 2012). The documentation of amino acid transporters in colonocytes with an effective capacity for amino acid transport has not been achieved.

According to the classical review by Wiseman (1968), a very useful approach to study sites of amino acids absorption is by administration of radioactive amino acid sources at various regions in the small and large intestines (via intubation tubes of varying lengths). Absorption may then be detected by sampling the luminal contents at the various loci in the gastrointestinal tract. Other approaches include in vitro procedures with intestinal preparations from animal models, such as everted sacs (Wiseman 1968) or the use of mucosal epithelial preparations (Johns and Bergen 1973). Studies with humans (in vivo only), dogs and rodents showed that the principal absorption sites of amino acids were located in the jejunum and ileum (Wiseman 1968). When test meals including radioactive-human serum albumin and milk protein were given to human subjects, 90 % of amino acid uptake occurred in the upper 100 cm of the jejunum (Borgstrom et al. 1957). In addition amino acid uptake was examined after the deletions of various small-intestinal segments in animal models. Using this invasive approach, results showed that both the jejunum and ileum were able to absorb amino acids. In regard to amino acid uptake from the colon, Wiseman (1968) concluded that in "contrast to the small intestine, the human colon is probably unimportant in the absorption of amino acids" noting that some data had indicated the potential for colonic amino acid absorption. He then further concluded: "It is unlikely that the latter phenomenon is of nutritional value in the intact animal".

More recently the presence of solute carrier (SLC) families in various segments of the small intestine and colon has been identified utilizing q-(RT)-PCR analysis of transporters in RNA preparations from epithelial cells. In addition large-scale gut locations of numerous SLCs have been studied with micro-array techniques. While it is conceded that expression of SLC mRNA does not necessarily indicate synthesis/function of actual SLC, such studies emphasize the potential for amino acid SLCs in the colon. Assessing SLC mRNA in rats from the esophagus to distal colon, known AA-SLCs were expressed ubiquitously in the duodenum, jejunum, ileum and colon. Further overlap of specific SLC expressions was noted in different gut segments (Cedernaes et al. 2011). Likewise, Woodward et al. (2010) reported that cationic and neutral amino acid-transporter (4) mRNAs were differentially expressed in the equine intestinal tract. While all were expressed in the colon, amino acid-transporter mRNA abundance was lower in ileum, cecum, left ventral and left dorsal colon for SLC7A9; SLC7A, SLC7A8 than the jejunum and finally a modest increase was noted for SLC43A1 in the cecum. Woodward et al. (2012) also measured kinetic properties of L-lysine transport in porcine and equine jejunal (JBBV) and colonic brush border membrane vesicles (CBBV). These studies indicated that initial rates of total L-lysine uptake did not differ between the distal jejunum and proximal large colon in both species. Kinetic data analyses of active transport showed that while in pigs the $V_{max}$ did not differ between JBBV and CBBV, $K_m$ (less transporter affinity) tended to be greater in porcine CBBV. In the equine model, both $V_{max}$ and $K_m$ were greater in the CBBV. L-Lysine diffusion did not differ between jejunal and colonic BBV in both species; albeit a higher total intestinal diffusion was observed for the equine model. Woodward et al. (2012) concluded that the large colon may play a key role in L-Lysine absorption and homeostasis in hind-gut (post-gastric) fermenters.

Utilizing animal models, various other studies have been conducted to access the contribution of the large colon to

protein nutrition. One group showed that the N from cecal infusions of various protein sources in horses was absorbed, but in a form that was less efficiently utilized than the same protein source when fed (Reitnour and Salsbury 1972). The utilization of endogenous and dietary urea in the large intestine of horses was assessed by Martin et al. (1996). These workers showed that despite extensive urea recycling, the horses were in negative N balance when low protein- or urea-supplemented diets were fed; however, a positive N balance was achieved in horses fed soybean meal. These above studies appear to support the general conclusion that in a well understood hind-gut fermentation model, colon-generated microbial biomass is not a satisfactory N/protein source. Utilizing pigs feeding $^{15}NH_4Cl$ and measuring incorporation of $^{15}N$ into intestinal microbes both from $^{15}NH_4Cl$ and recycled $^{15}N$-urea, Torrallardona et al. (2003) showed that lysine synthesized by the total gastrointestinal microbiome is absorbed principally in the small intestine. As previously reviewed (Bergen and Wu 2009), there is extensive synthesis of bacteria from free amino acids arising from protein digestion, recycled urea/ammonia and subsequent digestion and absorption of microbial amino acids in the small intestine while such activity is likely only significant in the very proximal colon.

Taken all together, these studies in humans and animal models indicate that while SLC are found in the colon and N (urea) recycling into amino acids via microbial biomass production will occur, this source of amino acids is likely not nutritionally significant except possibly in humans fed either low or possible poor quality protein diets.

## Microbiome as a nutrient source: implications in certain animals

The best model for utilization of salvage urea for microbial biomass production is a pre-gastric (fore-stomach) fermenter. With these animals the actual amount of microbiome N can be measured and compared to protein intake (Stern and Satter 1982). Thus, the contribution to amino flow to the abomasum (stomach) from the microbiome can exceed protein intake (4–6 % dietary protein) while upon consumption of diets greater than 12 % dietary protein, total microbiome amino acid flow will be less than dietary N intake and the excess N is excreted as urea. At low protein intakes, ammonia arising from recycled urea, dietary proteins, breakdown peptides and amino acids in rumen and with favorable fermentation can support more microbiome protein production than the amount of dietary protein fed. The situation is quite different with post-gastric fermenters species. Focusing just on the human gastrointestinal tract, available N for microbial biomass growth arises from amino released from digested protein, endogenous protein, and $NH_3$ from recycled urea and amino acid deamination. Because gut enterocytes can also synthesize urea and return it back to the small intestine (Wu 1995; Bergen and Wu 2009), the net gain in microbiome N may not be too high. In addition some bacteria prefer de novo amino acid synthesis while others prefer available free amino acids as protein building blocks (Yang et al. 2014). Thus, upon extensive mixing of the ammonia pool with amino acids, small peptides, and as well as urea hydrolysis and synthesis in enterocytes, this recycling of labeled N or C will result in lower product-specific activities in the de novo-synthesized amino acids which when divided by the specific activity of the label source (but not the ever changing precursor pools specific activity) has the potential to overestimate net microbial amino acid synthesis.

Measuring net uptake of amino acids by portal-drained viscera (PDV) and the liver and, amino acid fluxes in animals at varying protein and energy intakes may provide an avenue to study changes in amino acid supply to the liver and peripheral tissues (Ahmed et al. 1983). Indeed in fore-gut fermenters these approaches have yielded important information about recycled N in the PDV, liver, peripheral tissues and the intestines. This experimental approach has been extensively used in ruminants and lactating ruminants (Calsamiglia et al. 2010; Huntington et al. 1989), but such studies have not been reported in human subjects as such experiments require extensive invasive blood vessel catheterizations (femoral artery, portal and hepatic veins) and both blood flow and plasma amino acid concentration analyses (Bloomgarden et al. 1981). Accessing the PubMed data base with "portal amino acid uptake" resulted in 357 citations (accessed November 4, 2014). A majority of these citations involved work with ruminants, intestinal glutamate and alanine metabolism in rats, PDV amino acid fluxes in rodents, dogs and pigs. Most of these studies did not focus specifically on net amino acid synthesis by the intestinal and colonic microbiome from recycled urea in animals and humans and the plethora of results provides little or no evidence for the proposition that in typically fed animals/humans gut microbiome N recycling is nutritionally important (Metges et al. 1999a, b).

## Implications

In many animal species, the recycling of N back into gastrointestinal organs may supply needed indispensable and dispensable amino acids via microbiome amino acid synthesis during periods of low protein intakes, but under typical feeding conditions this potential amino acid source especially in non-ruminants is likely physiologically insignificant.

**Conflict of interest** The author declares no conflict of interest.

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