

Balancing for Rumen Degradable Protein and Post-Ruminal Requirements for Lactating Cattle Using the CNCPS as a Basis for Evaluation

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Introduction

Improving the efficiency of use of feed nitrogen (**N**) has become a central component of the ration formulation process primarily to reduce feed costs and also for the desire to be more environmentally friendly. New data are available that better describe the characterization of feed fractionation and these data along with changes in available models suggest that more protein has been available to lactating cattle than previously considered. Evaluation of the current data are suggesting that how we currently characterize feed protein fractions and their associated degradation and passage rates might cause us to over-feed protein (Lanzas et al. 2007). Previous papers have discussed some of the relevant concepts and offsets (Chase and Stone, 2004; Van Amburgh et al., 2004; Recktenwald and Van Amburgh, 2008; Van Amburgh et al., 2010) and reviewed the data concerning the efficiency of dairy cattle to convert feed N into milk N (Chase et al., 2009). The objective of this paper is to examine current accounting for various fractions of N in feed, the behavior of these fractions in the rumen, and describe changes in the model library and framework that improve the sensitivity of predictions to changes in feed N fractions using the Cornell Net Carbohydrate and Protein System (**CNCPS**) approach. The outcome allows the user to formulate diets lower in overall crude protein if the appropriate feed inventory and forage quality are available.

Ruminal Nitrogen Metabolism and Urea Recycling

To better predict the amount of N available to the rumen microbes and then predict the post ruminal supply of protein, the ruminal supply of N must be estimated. This includes both N available from the feed and also the amount of N available from urea recycling and endogenous protein. The amount of urea recycled by the cow has been underestimated and subsequently undervalued which encourages nutritionists to over-formulate crude protein.

Protein entering the rumen has at least three fates: it is degraded to ammonia and is used for bacterial protein synthesis, leaves the rumen as ammonia and converted to urea in the liver, or escapes microbial action becomes metabolizable protein directly. Understanding or appreciating the level of urea recycling and accounting for this and the microbial utilization of the recycled N improves our ability to formulate more N efficient

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diets. All ruminants are obligate recyclers of N and the amount of urea N recycled is a function of N intake, the rate of degradation of the carbohydrate and protein and the associated microbial uptake of feed. There are other factors impacting urea N recycling, but N intake and the total pool size of N within the cow will have the largest impact (Recktenwald, 2010). Urea production ranges from approximately 40 to 70% of total N intake per day and this hepatic function does not require a significant amount of energy. In two studies, lactating cattle were fed diets ranging from 14 to 17% CP with N intakes ranging from 520 g to 736 g per day. In those cattle urea production ranged from 38% to 68% of the N intake and the range was related to several variables, primarily the level of N intake, the starch content of the diet, and the CNCPS/CPM Dairy predicted rumen N balance. The cattle fed diets predicted to be low in rumen N balance were not different in ureagenesis but did recycle significantly more of the daily urea production into the gastrointestinal tract, thus improving N efficiency. On average among the two studies, measured N intake by the cattle was 588 g and 618 g per day where urea production was 44% and 58% of N intake and urea recycling back to the gastrointestinal tract was 30% and 43% of N intake, respectively (Recktenwald, 2010; Van Amburgh et al., 2010). Thus, the cattle recycled on average 176 g and 265 g of urea nitrogen over the 24 hr period to help meet the needs of the rumen microbial activity. Prediction equations used in field based models such as the CNCPS, CPM Dairy and the NRC to predict recycling have underestimated the level by more than 50% or are not included in the estimates of rumen N availability. This potentially leads to diets that are formulated to meet the rumen N requirements through over-feeding of rumen degradable protein that most likely end up being excreted by the cow through the urine.

All rumen bacteria can use ammonia although data exists that indicates some of the starch and sugar fermenting bacteria prefer amino acids (**AA**) and peptides (Chen et al., 1987; Broderick and Wallace, 1988). The contribution of ammonia versus AA and peptides to microbial N varies under different dietary conditions. In mixed ruminal microbes, ammonia contributed only 26% of the microbial N in a medium with high concentrations of AA and peptides, and 100% when ammonia was the only available N source (Wallace et al., 1999). In a review by Bach et al., (2005), it was suggested that based on a variety of *in vitro* and *in vivo* work, on average, approximately 80% of bacterial N is derived from ammonia. Cellulolytic bacteria appear to be less able to use AA and peptides, but even they were able to derive up to 50% of their cell N from non-ammonia sources (Wallace et al., 1999). Data from Broderick and Wallace (1988) suggested that peptide uptake by the microbes was a rate limiting step versus peptide formation. Also, peptides from endogenous protein flow are used by the microbes with good efficiency and with ruminal microbial protein turnover there are many sources of peptides not considered in most ration formulation models (Ouellet et al. 2004; Marini et al., 2008). This suggests that feeding to supply peptides for ruminal requirements as has been done for many years causes us to overfeed protein and that the rumen is rarely short on peptides for microbial utilization. As modeled in the Cornell Net Carbohydrate and Protein System (**CNCPS**; Tylutki et al. 2008), nonstructural carbohydrate fermenting bacteria can obtain up to 67% of their N from AA or peptides

(Russell et al., 1992) but with emerging data, this value should be reconsidered as a constant and be considered for the structural carbohydrate bacteria.

CNCPS Feed Fractions, Pools and Passage and Digestion Rates

Multiple changes were made to expand the carbohydrate (**CHO**) pools to four A fractions (volatile fatty acids, lactic, other organic acids such as malate, and sugar) as well as adjusting CHO digestion rate (**Kd**) values downward based on gas production data from Molina (2002). Previous versions utilized a 200 to 300% per hour Kd for sugar. A 300% per hour Kd implies rumen retention time of 0.2 hours (12 min); a value greater than the mean growth rate of rumen bacteria. The original value of Kd for sugar came from *in vitro* fermentation studies from Jim Russell's laboratory using pure cultures of *Streptococcus bovis* grown on glucose. To update this, measurements were made using a mixed sugar fermentation with mixed rumen bacteria with gas production and the rates measured varied between 40 and 60% per hour (rumen retention time of 100 to 150 min) (Molina, 2002). Updates to the changes in degradation rates of the various fractions are found in Table 1.

Based on the changes in rates of degradation and passage there is a significant impact on soluble pool movement out of the rumen. As an example, the data in Table 2 demonstrates a 16% reduction in sugar (CHO A4) degradability. If a lactating dairy diet fed at 24 kg contains 5% sugar, this results in 192 g less sugar degraded. The 192 grams would equate to approximately 15 g lower MP flow, or approximately 1 liter lower MP allowable milk.

Further, it was assumed that protein (**PRO**) fraction A utilization was instantaneous with a Kd of 10,000 %/hr implying a rumen retention time of 0.6 min. This would imply that any addition of urea would be dissolved and captured by rumen bacteria in 36 seconds, an unrealistic expectation. This value was generated to represent the rate of solubilization and not necessarily microbial uptake. With these changes, rates for pools like PRO A Kd were reduced to 200%/hr. There were many other updates to the version including: new passage rate equations, maintenance requirements for heifers were updated, and error corrections to more appropriately account for microbial ash accumulation, rumen ammonia flow, and updating DM intake equations. These changes reduced predicted microbial protein flow approximately 5 to 7% compared with previous versions.

In CNCPS v6.1 the soluble pools, CHO A and PRO A and B1, have been re-assigned to the liquid passage rate equation to more appropriately reflect the biology of the cow. Both the solid and liquid passage rate equations were updated and account for a greater amount of variation in liquid turnover than the equation found in v5.0 (Seo et al. 2006). Prior to v6.1 the soluble pools were predicted to flow out of the rumen with the solids passage rate, thus with the high digestion rates and the slow passage rates, all of the soluble fractions were degraded in the rumen. This change in passage rate assignment increases the predicted outflow of soluble components, thus reducing microbial yield and estimated ammonia production and rumen N balance. These

changes improve the sensitivity of the model to changes in feeds high in soluble carbohydrates and protein and reduce, but don't eliminate, the under-prediction bias observed in a previous evaluation of the model (Tylutki et al. 2008).

The current CNCPS balances for AA using a factorial approach based on the AA content of the predicted metabolizable protein (**MP**) supply and the AA profile of the tissue and milk. The approach is identical to that described by O'Connor et al. (1993) with many upgrades and modifications to the prediction of MP supply (Fox et al., 2004; Seo et al., 2006; Lanzas et al., 2007a,b; Tylutki et al., 2008). In order to have confidence in the ability of the model to predict AA accurately, the model needs to be able to account for the MP allowable milk with reasonable accuracy and precision. During the development of CNCPS v6.1 (Tylutki et al., 2008; Van Amburgh et al. 2007; Van Amburgh et al. 2010), we have refined the model to be more sensitive to MP supply and thus more robust in evaluating the most limiting nutrient under field conditions. This has allowed current users to balance diets at crude protein levels below 16% and maintain milk yield to increase overall efficiency of use and in many cases enhance milk protein output.

Proteins, peptides and free AA in the soluble pool can be rapidly degraded, but because they are in the soluble pool, they move with the liquid phase from the rumen to the small intestine and supply the cow with AA. There are now several data sets that demonstrate that the soluble pool of feeds contributes between 5 and 15% of the total AA flow to the duodenum of the cow (Hristov et al. 2001; Volden et al., 2002; Choi et al. 2002a,b; Reynal et al. 2007). The pool sizes of the nonprotein N (**NPN**) and soluble true protein have been updated to reflect the presence of small peptides in what was previously considered the NPN fraction (Table 3) (Ross and Van Amburgh, unpublished). As the data illustrates, regardless of protein precipitating agent, as filter paper pore size is decreased, the amount of true protein recovered increases. Thus, what historically has been defined as PRO A was severely over-estimating true NPN supply.

Additionally, peptide length does not vary based upon pore size. Based upon these findings, NPN as a percent of soluble protein for forages has been adjusted and this will most likely occur for all of the remaining feeds in the feed library. In earlier versions of the model, the library described the soluble CP fraction of fermented forages as 95% NPN for feeds such as alfalfa silage, 45% has been implemented in the current version. This does not mean that all alfalfa silages fall into this range, but without a functional field applicable assay and given the values we derived, it was a reasonable compromise for this release. Feeds such as soybean meal have been reduced from 25 to 5% NPN as % of the soluble protein. This greatly impacts PRO A and B1 pool sizes (Table 4). These shifts in pool sizes, coupled with reduced microbial yield predictions, results in excessive peptide supply for the rumen. Therefore, reductions in dietary RDP requirements (and CP) are achievable. The soluble proteins and peptides move with the liquid phase from the rumen to the small intestine and supply the cow with AA (Choi et al. 2002; Volden et al., 2002; Hedvquist and Uden, 2006; Reynal et al. 2007), thus, to account for the AA profile of these peptides, we need to provide an AA profile for the

soluble pool and as the model moves forward we will be adopting whole feed AA values, not the insoluble residue (Sniffen et al. 1992). Thus, the CNCPS was adjusted so that CHO A1 to A4 and PRO A to B1 flow with the liquid phase and CHO B1 (starch) always flows with the concentrate solid phase. Table 4 provides an example of integrating the pool phase flow and Kd changes. This is currently being done by mathematical manipulation of the pools and rates but a more robust approach is needed to account for more variation in the predicted AA flow.

Further, relative to ruminal N requirements, the previously described peptide requirement was developed from in vitro data from Chen et al. (1987) and related papers. Data from Broderick and Wallace (1988) reported that peptide uptake by the microbes is a rate limiting step versus peptide formation. This, coupled with PRO B1 being a component of soluble protein, indicates that peptide supply is probably never limiting in the rumen as we have calculated. Also, peptides from endogenous protein flow (Ouellet et al. 2004) are used by the microbes with good efficiency and with ruminal microbial protein turnover there are many sources of peptides not considered when the model was developed. This suggests that feeding to supply peptides for ruminal requirements as has been done for many years causes us to overfeed protein and that the rumen is rarely short on peptides for microbial utilization.

This version of the CNCPS uses an overall efficiency of use of MP to net protein (NP) of 0.67, the same value utilized in the 2001 Dairy NRC (Tylutki et al., 2008; National Research Council, 2001). In addition each AA has individual efficiencies for maintenance, growth and lactation and the efficiencies are currently static. Data from recent studies in lactating cattle call into question the use of static efficiencies for either overall MP or specific AA and this makes sense given the possible roles certain AA have in metabolism (Doepel et al., 2004; Pacheco et al., 2006; Wang et al. 2007; Metcalf et al., 2008).

Metcalf et al. (2008) challenged the use of a static efficiency and observed a range in efficiency of use of 0.77 to 0.50 as MP supply was increased. They further determined using a best fit of data that the optimal efficiency of use of MP to NP was between 0.62 and 0.64 for the average cow. This is quite a bit lower than our current value but is consistent with the data of Doepel et al. (2004). Taking the simple mean of the efficiencies from the Doepel et al. (2004) publication, the average efficiency of use of the essential AA is 62.2%, again lower than the value we are currently using in the model but consistent with the data of Metcalf et al. (2008). Most likely, any change in efficiency of use of MP or AA will be associated in a change in ME utilization, thus the absolute differences within one nutrient will be hard to detect or manipulate.

Additional changes have been made to the calculations for metabolic fecal nitrogen. This was a double-accounting error that resulted in under-estimating endogenous protein losses. As this directly impacts maintenance protein requirements, MP maintenance has increased slightly.

Prediction Outcome

An evaluation of most limiting (metabolizable energy or protein) milk is found in Figure 1. Studies and actual farm data are contained in these comparisons and demonstrate that the model is doing a reasonable job in predicting the most limiting nutrient supply, thus this provides us with a reasonable platform from which to start making changes. The evaluation was made from both research and on-farm datasets for lactating dairy cows. The dataset represents cows producing 21 to 52 kg of milk per day fed diets ranging from 12.7 to 17.4% crude protein. Model predicted milk reported is the lower of metabolizable energy (**ME**) or MP allowable milk. The intercept was not different from zero and the mean prediction bias was less than 1%. As an example, the CPM ver.3 100 lb cow session file was inputted into CNCPSv6.1. Table 5 lists selected output variables from the two programs. In almost all cases, MP allowable production (milk or gain) will be predicted to be higher in CNCPSv6.1 and ME allowable milk reduced. In this case, MP allowable milk is 10.8% greater than in CPM v3 while ME allowable milk is decreased 6.2%. This example in CPMv3 is perfectly balanced for ME and MP while v6.1 suggests opportunity for reformulation. MP from bacterial sources was reduced 6.8% while MP from feed increased 23.8%. This shift changes MP from bacteria from 52% of total MP supply to 44%. As can be expected, these shifts impact AA flows and ratios. Microbial protein has a near perfect AA pattern for milk protein production. Thus, reducing microbial yield introduces altered ratios and potentially more variability in ratios as RUP LYS from feed is more variable in composition.

Flows of all AA changed as represented by the AA balances illustrated in Table 5. Leucine (**LEU**) and isoleucine (**ILE**) balances changed over 100% whereas those of methionine (**MET**) and lysine (**LYS**) increased nearly 50%. These, coupled with the MP balance, suggest reformulation to decrease MP supply, while maintaining AA balance (and ratio) is possible. The LYS ratio (% of MP) dropped from 6.9 to 6.6% (a 10% reduction) whereas the LYS:MET ratio shifted from 3.1:1 to 3.3:1. In general, we have found that LYS as % of MP has a larger shift in going from CPM v3 to CNCPS v6.1.

Evaluation Diets with CNCPS V6.1

Given that the evaluation guidelines nutritionists routinely use when formulating with CPM v3 have changed, the following is an updated list for evaluating diets with CNCPSv6.1:

1. Dry matter intake: Inputted DM intake should be within the range of CNCPS and NRC predictions. If it is not, review inputs for body weight, environment, and feed amounts.
2. Rumen ammonia should be between 100 and 150% of requirements established by the model. Diets high in hay silage, or given ingredient availability limitations might be as high as 200% and, although unacceptable from an efficiency perspective, they are realistic depending on the total forage availability.

3. Peptide balance can be ignored.
4. The considerations given to urea cost can be minimized. However, you can target a urea cost of less than 0.25 Mcal/d.
5. Nonfibrous carbohydrates for lactating dairy cow diets can vary between 30 and 42% depending upon sources.
 - a. Sugar versus starch versus soluble fiber is user preference in our opinion. Given that cattle require fermentable CHO, sources of fermentable CHO should rely upon local availability and pricing.
6. The ME and MP allowable milk should be within 1 kg of each other and should match the observed milk before any ration changes are made. For growing cattle, MP allowable gain should be 0 to 250 grams greater than ME allowable gain.
 - a. For replacement heifers, keep lactic acid less than 3% DM. Data from the 1980s suggests a direct link between lactic acid intake and empty body fat composition in growing cattle.
7. Physically effective NDF should be greater than 22% DM for lactating dairy cows (8 to 10% for feedlot cattle).
8. Lysine should be greater than 6.5% MP and Methionine greater than 2.2% MP
9. LYS:MET ratio to maximize milk protein yield should be between 2.80-2.95:1
10. Total unsaturated fatty acid intake should be monitored. Values greater than 500 g/d are a risk factor coupled with quantity and quality of forage NDF (lower quality forages and/or lower quantities of forage NDF fed increase the risk of milk fat depression).
11. Minerals and vitamins. Given that CNCPS v6.1 has implemented the Dairy NRC recommendations for minerals and vitamins (as a dietary supply including bioavailability), we suggest following NRC recommendations.

Summary

Nutritional models are evolutionary. The CNCPS v6.1 is the latest evolutionary generation in the CNCPS/CPM path. Between analytical improvements, error corrections, and new research being implemented within the CNCPS framework, model accuracy has been improved. These changes allow the nutrition professional to reduce dietary crude protein levels while maintaining or improving production and profitability.

Take-home messages

- Nutritional models are evolutionary and should be expected to change with improved understanding of and continue to change as new research is published;
- The current version of CNCPS has improved passage rates, feed chemistry and error corrections and will predict greater MP supply from feed protein;
- Evaluations of herd level nutritional management, when the actual feed chemistry and inputs are used and all other factors are properly characterized, the CNCPS v6.1 is more accurate and precise in estimating ME and MP allowable milk with a lower prediction bias; and
- These changes allow the user to formulate diets with reduced CP and still meet the MP requirements of the cow and to maintain milk yield and components, provided the cattle, forages and feeds are properly characterized.

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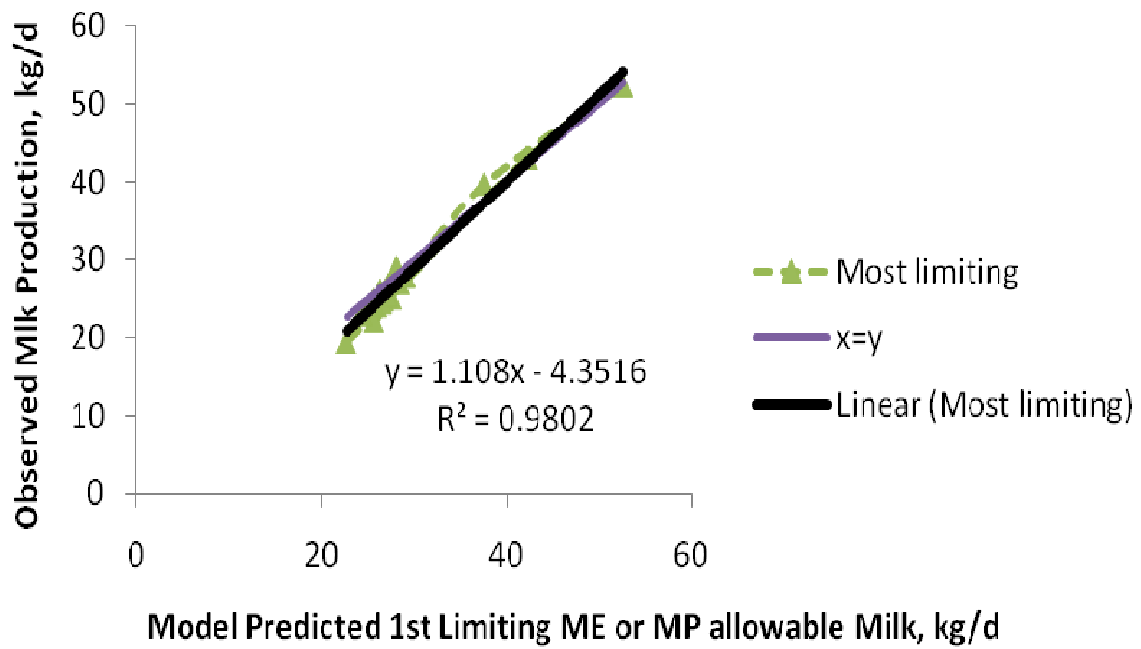


Figure 1. Observed versus predicted milk production as predicted by CNCPSv6.1. Diets range in crude protein from 12.7 to 17.4% DM with milk yields ranging from 21 to 52 kg per day.

Table 1. Feed degradation rates (%/hr) used for carbohydrate (CHO) and protein (PRO) pools in CNCPS v6 and prior to version 6.1

Component	Prior to v6	V6.1
CHO A1 (volatile fatty acids)	Not modeled	0%
CHO A2 (lactic acid)	Not modeled	7%
CHO A3 (other organic acids)	Not modeled	5%
CHO A4 (sugar)	300-500%	40-60%
CHO B1 (starch)	20-40%	20-40%
CHO B2 (soluble fiber)	20-40%	20-40%
CHO B3 (available neutral detergent fiber)	4-9%	4-9%
CHO C (unavailable neutral detergent fiber)	0%	0%
Pro A (nonprotein N)	10,000%	200%
Pro B1 (soluble true protein)	130-300%	10-40%
Pro B2 (moderately degraded protein)	3-20%	3-20%
Pro B3 (slowly degraded protein, bound in NDF)	0.05-2.0%	For forages, same as the CHO B3
Pro C (unavailable protein)	0%	0%

Table 2. Calculated rumen degradability of several pools using previous and current digestion (Kd) and passage (Kp) rates

Pool ¹	Prior to v6			V6.1		
	Kd, %/hr	Kp, %/hr	% degraded	Kd, %/hr	Kp, %/hr	% degraded
CHO A4	500	4	99	60	12	83
CHO B1	20	4	83	20	6	77
PRO A	10,000	4	100	200	12	94

¹ CHO = carbohydrate; PRO = protein.

Table 3. Precipitable true protein of trypticase with varying protein precipitating agents and filter paper pore size. The 20 μm pore size represents Whatman 54 filter paper

PPT agent	Filter pore, μm	True protein	Filtrate peptide chain length	True protein, % of largest pore
Tungstic acid	1	34.4	3.0	1,911
	6	23.1	4.3	1,283
	20	1.8	4.2	
Stabilized TA	1	31	3.3	705
	6	28.5	3.4	648
	20	4.4	3.6	
TCA	1	2.57	3.4	612
	6	0.78	4.3	186
	20	0.42	5.0	

Table 4. Calculated protein A and B1 pool sizes using original and updated nonprotein N % and soluble protein values using an alfalfa silage as an example

Component	prior to v6	v6.1
Crude protein, % DM	20.0	20.0
Soluble protein, % CP	55.0	55.0
NPN, % soluble protein	95.0	45.0
PRO A + B1, % DM	11.0	11.0
PRO A, % DM	10.45	4.95
PRO B1, % DM	0.55	6.05

Table 5. Selected outputs from 100 lb cow session file as predicted by CPM ver. 3.0.10 and CNCPS v6.1

Component	CPM ver 3	CNCPS v6.1	% change
Predicted DM intake	24.5 kg	24.6 to 27.6 kg	0 to 12%
Metabolizable energy			
Supply, Mcal	69.2	64.9	-6.2%
Required, Mcal	66.8	66.3	-0.7%
Allowable milk, kg	47.6	44.1	-7.4%
Metabolizable protein			
Supply, g	2,887	3,093	7.1%
Required, g	2,887	2,875	-0.4%
Allowable milk, kg	45.4	50.3	10.8%
Bacteria, g	1,499	1,374	-8.3%
From RUP, g	1,388	1,719	23.8%
From bacteria, % total MP	52%	44%	-14.4%
Ammonia balance (g)	122	100	-18.0%
RDP, % DM	11.5	10.0	-13.1%
Amino acids			
Metabolizable LYS, g	199.3	204.1	2.4%
LYS, % MP	6.90	6.60	-4.3%
Metabolizable MET, g	63.5	62.7	-1.3%
MET, % MP	2.20	2.03	-7.7%
LYS:MET	3.1	3.3	3.7%
LYS balance, g	32.2	48.0	49.1%
MET balance, g	10.7	15.6	45.8%
ARG balance, g	26.3	25.9	-1.5%
THR balance, g	39.7	48.2	21.4%
LEU balance, g	2.4	28.1	1,070.8%
ILE balance, g	-15.8	3.4	121.5%
VAL balance, g	20.4	18.2	-10.8%
HIS balance, g	22.2	33.3	50.0%
PHE balance, g	52.8	66.3	25.6%
TRP balance, g	15.8	14.9	-5.7%
Nonfibrous carbohydrates, %	40.0	38.4	-4.0%
Diet ME, Mcal/kg	2.82	2.65	-6.0%

SESSION NOTES