Canadian Science Publishing

ABSTRACTS

Proceedings of the 2021 Meeting of the Animal Science Modelling Group

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The Animal Science Modelling Group meets approximately yearly for one-day meetings. The 2021 meeting was sponsored by Trouw Nutrition (Guelph, ON, Canada), Lallemand Specialties, Inc. (Milwaukee, WI, USA), and Adisseo (Alpharetta, GA, USA). It was held on July 9 as a virtual meeting prior to the virtual ADSA Annual Meeting. Summaries of the papers presented follow. Each summary has been peer reviewed and edited for clarity. The lead author of the summary is the person who presented the paper.

Multivariate time series classification for prediction of clinical mastitis

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The aim of this study was to investigate the application of time series classification techniques to detect clinical mastitis (CM; binary variable) one-step ahead of time for dairy cattle. Quarter level milking time data from 249 cows were collected daily from automatic milking system (AMS) using DelPro software (DeLaval, Tumba, Sweden) over 12 wk in the summer of 2020. We tested multiple classification algorithms including Decision Tree, Random Forest, Logistic Regression, Naïve Bayes, Gradient Boosting. We tested 16 features (variables) recorded by the AMS unit at each milking event for initial model development: teats not found (binary), blood in milk (binary), kick-offs (binary), occurrence of incompletely milked quarter (binary), previous mastitis history (previous mastitis incident before the milking event; binary), surface plasmon resonance (SPR; 4 levels), milking interval between two successive milking events (hour), milk yield (kg), lactation number, days in milk (day), milk duration (second), electrical conductivity (mS/cm), mean milk flow rate (kg/min), peak milk flow rate (kg/min), milking frequency, and milking order per day. We selected features for inclusion with two methods: backward stepwise logistic regression (Wang et al. 2007) and mutual information (MI) (Vergara and Estévez 2014). We used the Augmented Dickey Fuller (ADF) test (Mushtaq 2011) to check if the numerical features are stationary. We normalized all features and transformed the time element into a maximum of 9 time-lags plus the current milking time (t) to predict CM one-step ahead (t + 1). We split the data into training and testing subsets containing 80% of the observations for training and the remaining 20% for testing. Due to the small number of CM (positive) records (accounting for 0.04% of all the records), we utilized several oversampling methods (e.g., random oversampling, synthetic minority oversampling technique (SMOTE)) (Cateni et al. 2014) and undersampling methods (e.g., Tomek link, and edited nearest neighbors) (Bach et al. 2019) to improve balance in the dataset. We evaluated model performance with a 5-fold cross validation in which the data were folded by cows with a CM incident and assessed model performance with metrics of specificity, sensitivity, and the area under the curve of the receiver operating characteristic (AUC-ROC). The Naïve Bayes algorithm with all features included performed robustly and gave the best predictions, resulting in 60% sensitivity and 88% specificity with AUC (72%). Thus, this machine learning algorithm could be a method for early detection of cows at-risk for CM and help farmers take timely actions to reduce the negative impacts of CM but a larger dataset with more incidents of CM mastitis is likely needed to improve classification sensitivity and specificity.

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Mathematical approach to fitting fermentation kinetic parameters of multiple carbohydrate fractions from in vitro gas production data

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In vitro gas production (IVGP) methods may be advantageous in comparison to aNDFom and starch digestibility methods for

Received 13 January 2022. Accepted 13 January 2022.

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determining carbohydrate fermentation kinetic parameters due to the nearly continuous measurements, which allow for more accurate and precise parameter estimates, plus ease of the methodology and reduced labor. In fitting a multiple carbohydrate fraction fermentation model to IVGP data, a Monte Carlo approach was employed in proof-of-concept testing followed by an evaluation of fitting observed IVGP data from corn silages. In the Monte Carlo analysis, a set of 10 corn silage samples was randomly generated based on known population variance in nutrient composition and fermentation parameters. Gas fermentation curves were generated for each sample using the exponential growth function (y = digestible pool × exp(-Kd(time - lag)) for each carbohydrate fraction (water soluble carbohydrates, starch, aNDFom) and summed together. Further randomness was added to model the within-sample variation in gas production. The model was fit to the generated gas curves using SAS non-linear fitting procedures. Parameters for lag time and degradation rates (Kd) of starch and aNDFom fractions were fit with good success ($p > 0.20$) as evidenced by paired t-tests between original and estimated parameters. Results highlighted that independent measures of carbohydrate fraction size and the digestible aNDFom pool were critical to the approach and that parameters for starch and aNDFom fermentation could be determined with good confidence. The Monte Carlo analysis also demonstrated that the approach was sufficiently robust to handle the range of nutrient variation observed in "real world" corn silage. The subsequent evaluation used a set of 12 corn silage samples with known variation in starch and aNDFom fermentation as determined by *in vitro* starch (IVSD) and aNDFom digestibility (IVNDFD) assays. Samples were incubated in an Ankom RF system in duplicate and gas production data compiled at 2 min intervals for 48 hrs. The 3-fraction carbohydrate model was fit to the gas production data. Comparing IVGP-estimated parameters to those from IVSD and IVNDFD, both slope and mean bias was evident, likely due to fixed lag assumptions in IVSD and IVNDFD. IVGP-estimated Kd's for starch and aNDFom were less than IVSD and IVNDFD estimates ($p < 0.001$) and more precise. Parameter estimates deduced from the non-linear fitting of IVGP data were statistically significant, biologically reasonable, correlated to parameters determined in independent assays, and more precise. Overall, the mathematical modelling approach of fitting kinetic parameters to multiple carbohydrate fractions with known pool sizes was satisfactorily achieved.

Empirical and mechanistic modelling of B-vitamin synthesis and use across the rumen in dairy cows

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It has long been accepted that the B-vitamin (BVit) needs of ruminants are generally met by the rumen microbiome's ability to synthesize them, without their requirements ever being clearly defined (see NRC 2001). While symptoms of deficiency are rarely observed, positive responses have been seen in milk production, reproduction and overall health resulting from supplementation with rumen protected BVit (Evans et al. 2006). Such results indicate the occurrence of subclinical deficiencies which impact the animal's overall metabolic efficiency. The objective of this study was to create empirical models of thiamin, riboflavin, niacin, pyridoxine, folate and cobalamin apparent ruminal synthesis in dairy cows (ARS, mg/d) (representing the summation of BVit synthesis and degradation by the rumen microbiota) through a meta-analysis approach to detect the major drivers of ARS variation. Further, the knowledge gained will be used to develop a theoretical mechanistic framework of BVit ARS across the rumen. Data utilized were from 340 individual lactating cows from 16 published studies. Potential model driving variables considered in the meta-analysis included the diet chemical composition and rumen parameters. A suite of potential models (with study treated as a random effect) were developed using PROC GLIMMIX of SAS. Dry matter intake (kg/d) was a major driver for all BVit, with varying responses to digestible starch (g/kg of DM) and digestible NDF (g/kg of DM) being major drivers for all BVit studied other than cobalamin. The respective dietary BVit concentrations (mg/kg of DM) were also driving variables for the ARS of thiamin, riboflavin, niacin, and pyridoxine. The major driving variables identified for cobalamin ARS included dietary starch (g/kg of DM), dietary NDF (g/kg of DM), total VFA concentration (mM) and propionate molar proportion (% of total VFA). The heterogeneity of these responses to the major drivers identified through the empirical models highlight the importance of considering these vitamins individually. Based on this knowledge, a theoretical framework was developed to explain cobalamin ARS (mg/d), which suggests that amylolytic and fibrolytic microbial pools are responsible for both the synthesis and use of BVit. Empirical models describe a negative association between propionate molar proportion and cobalamin ARS, supported by our knowledge of the use of cobalamin in the propionate synthesis pathway as a co-enzyme for methylmalonyl-CoA mutase (Elliot 1980). In conclusion, the empirical models suggest different microbial groups to be represented in mechanistic approaches of BVit ARS while highlighting the need for further research on rumen microbiome conditions influencing BVit ARS.

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Relationship between milk urea nitrogen and urea recycling in lactating dairy cows

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Milk urea nitrogen (MUN) and blood urea nitrogen (BUN) are correlated with nitrogen balance and nitrogen excretion MUN (Broderick and Clayton 1997); however there is also a genetic component to MUN concentrations (Stoop et al. 2007) which could be associated with differences in urea transport. It was hypothesized that a portion of the variation in MUN concentrations among cows is caused by variation in gastrointestinal and kidney urea clearance rates. Eight lactating cows varying MUN concentrations while fed a common diet were infused with [$^{15}N^{15}N$] urea to determine urea-N entry rate (UER), gastrointestinal entry rate (GER), returned to ornithine cycle (ROC), urea-N used for anabolism (UUA), urea-N excretion in feces (UFE) and urine (UUE). Urea clearance rates by the kidneys and gastrointestinal tract were calculated from isotopic enrichment of urea excretion in urine and gut entry rate, respectively, and plasma urea N concentrations (PUN). Over the course of the experiment, animals weighed an average of 506 ± 62 kg and produced 26.3 ± 4.39 kg of milk/d, with MUN concentrations ranging from 11.6 to 17.3 mg/dL (average of 14.9 ± 2.1 mg/dL). Plasma urea N was positively correlated with UER, UUE, and UUA ($p \leq 0.05$). Kidney clearance rates, which correct for the effect of PUN concentration and reflect transporter activity, only

tended ($p = 0.13$) to be related to PUN. These results would indicate that kidney transport activity was not significantly different across animals, and thus would not likely be the cause of the observed range in MUN across animals. Plasma urea N and MUN were negatively correlated with gut clearance rates and GER:UER ratio ($p \leq 0.06$). This relationship supports the hypothesis that differences in gut urea transport activity among animals causes variation in PUN and MUN concentrations, and that cows with high PUN and MUN are less efficient at recycling PUN to the gastrointestinal tract (GIT) and thus may be more susceptible to ruminal N deficiencies when fed low rumen degradable protein diets. If so, the relationship between a reference MUN concentration and overall N efficiency will be variable. Such biological variation in urea metabolism necessitates an adequate safety margin when setting regulations for maximal MUN levels as an indicator of herd N efficiency.

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Adaptation of the Hill model to describe phosphorus retention and excretion in calves fed milk replacer and solid feed

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In an effort to describe phosphorus retention and excretion in calves, the dairy cow model of Hill et al. (2008) was adapted for use in 'SIMON', a growth simulation model for calves fed milk (replacer) and solid feed (Ellis et al. 2019). Phosphorus (P) enters the model as phytate bound P (Pp), organic bound P (Po), and inorganic P (Pi). Phosphorus inputs to the rumen enter their respective pools viz. Pp, Po and Pi. Using mass action kinetics equations, flows occur between the Pp, Po and Pi pools representing degradation of phytate bound P to its lower inositols. Flows occur between the organic, inorganic and microbial P pools in response to the growth and turnover of the rumen microbial mass. Flow of Pp, Pi and Po from the rumen into the small intestine is based upon concentration of respective pools and a fractional fluid passage rate. Absorption of Pi from the small intestine into the blood pool is governed by a Michaelis-Menten like equation whereby concentration of blood P has an inhibitory effect on absorption. Remaining unabsorbed Pi and undegraded Po and Pp enter the hindgut. The microbial hindgut population is capable of further degrading Pp and Po. Pi can enter the hindgut microbial P pool and exit the hindgut via microbial passage. In the post-absorptive environment, P in the blood pool has four potential destinations. Based upon bone and body organ growth, P is pulled from the blood pool and deposited in bone and soft tissue pools, with mobilization from the bone pool occurring. Ruminal recycling of blood P, via saliva, in addition to excretion of blood P to urine is dependent on blood P concentration. Preliminary simulations of the study of Berends et al. (2012) whereby 108 and 164 kg calves were fed four levels of solid feed with constant level of milk replacer displayed good agreement between predicted and observed values (g/d) based upon fecal P (CCC = 0.837) and to a lesser extent retained P (CCC = 0.583), with poorer agreement for urinary P (CCC = 0.363). Authors hypothesize poor prediction of urinary P may be attributed to asynchronous supply of P from the pulse dose milk replacer feeding and the constant demand of P for deposition in bone and body. Additionally, it is possible that the model represents the rumen recycling of P poorly, affecting urinary P excretion, as limited data exists regarding P recycling in calves.

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Development and implementation of a dairy calf model in NDS Professional

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A quality dairy replacement program starts from birth and it is an investment in the future of a dairy. NDS Professional has recently used data from various studies, while using the same structure of the NRC (2001) calf model, to develop a new calf model. A maintenance requirement of 0.100 Mcal/kg $BW^{0.75}$ was maintained, while, as the birth weight decreases, maintenance requirement increases accordingly, as the surface area to BW relationship will cause larger loss. A lower efficiency of ME to NE was implemented (0.85 to 0.77) based on works by Drackley and Van Amburgh (2005). A second adjustment was to account for environmental conditions so that requirements would increase outside of the thermoneutral zone (i.e.,: 15 to 25 °C or 5 to 25 °C for calves, respectively younger or older than 21 d). Relative to growth requirements, for calves fed milk or milk replacer only, the NRC model set the efficiency of use of ME to NE at 0.69 based on older data. Re-evaluation using more recent data suggests 0.60 is consistent with less mature calves accreting less fat and more protein tissue than the original data used from the NRC. The cumulative intake of NFC from calf starter appears to be a critical factor in the development of the digestibility of NDF and NFC of the calf and, thus, the energy content in the starter. We therefore implemented an equation from Quigley et al. (2019) reflecting changing digestibility and starter ME contribution. A trial with 80 Holstein calves was used to validate the model. Records included birth body weight (BBW) and, from 5 to 82 d of age, weekly body weights (BW), daily milk replacer (MR) and starter intakes. The model simulated the 80 calves weekly from day 5, using the true MR and starter intakes. The expected weekly BWs obtained from the model for either energy or protein allowable gain were compared with the actual BWs. Regression of observed on predicted BWs resulted in a very high R^2 of 0.98 for both energy and protein predicted values. However, while slopes of the regressions were not different than 1 ($p = 0.44$), intercepts were different than 0 ($p < 0.01$), namely −4.41 and −1.91 for energy and protein, respectively. Residuals were not apparently uniformly distributed, with more positive residuals above 50 kg, showing an over prediction for both energy and protein, of on average 1.16 and 0.56 kg, respectively. However, regressions of residuals on predicted resulted in a slope not different than 0 ($p = 0.23$), therefore showing an unbiased model. The Nash-Sutcliffe error was larger than 0.85 for both predictions and therefore shows a very good fit. The NDS Professional model thus represents at the moment a valid alternative to the NRC (2001) model, which was previously shown to be less accurate and precise.

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Development of a model to predict dietary metabolizable energy from digestible energy in beef cattle

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Understanding the utilization of feed energy is essential for precision feeding in beef cattle production. We aimed to assess whether predicting the metabolizable energy (**ME**) to digestible energy (**DE**) ratio (**MDR**), rather than a prediction of ME with DE, is feasible and to develop a model equation to predict MDR in beef cattle. We constructed a literature database based on published data. A meta-analysis was conducted with 306 means from 69 studies, containing both dietary DE and ME concentrations measured by calorimetry to test whether no y-intercept is adequate in the linear relationship between DE and ME. A random coefficient model with the study as the random variable was used to develop equations to predict MDR in growing and finishing beef cattle. Routinely measured or calculated variables in the field (body weight, age, daily gain, intake, and dietary nutrient components) were chosen as explanatory variables. The developed equations were evaluated and compared with other published equations. The no-intercept linear equation was found to represent the relationship between DE and ME more appropriately. The y-intercept (-0.025 ± 0.0525) was not different from 0 ($p = 0.638$), and Akaike and Bayesian information criteria of the no-intercept model were significantly smaller than those with the y-intercept. Within the data of growing and finishing cattle data, the physiological stage was not a significant variable after accounting for the study effect ($p = 0.213$). The mean (±SE) of MDR was 0.849 (±0.0063). The best prediction model for MDR ($n = 106$ from 28 studies) was $0.9410(\pm 0.02160) + 0.0042(\pm 0.00186) \times DMI(kg) - 0.0017(\pm 0.00024) \times NDF(\%DM) - 0.0022(\pm 0.00084) \times CP(\%DM)$ We also presented a model with a positive coefficient for the ether extract ($n = 80$ from 22 studies). When using these equations, the observed ME was predicted with high precision ($R^2 = 0.92$). The model accuracy was also high, as shown by the high concordance correlation coefficient (> 0.95) and small root mean square error of prediction (RMSEP), less than 5% of the observed mean. Moreover, a significant portion of the RMSEP was due to random bias (> 93%), without mean or slope bias ($p > 0.05$). We concluded that dietary ME in beef cattle could be accurately estimated from dietary DE and its conversion factor, MDR, which can be predicted by the dry matter intake and concentration of several dietary nutrients, using the two prediction equations developed in this study.

Prediction of body condition score in dairy cows in CNCPS and NDS Professional

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The body condition score (BCS) prediction allows to fine tune dairy cows' diets according to the objectives and the predicted animal responses. In the CNCPS, BCS is a function of the animal's energy reserves (NRC 2001) presenting the following limitations: peak milk yield (MY) is a breed set value; nutrient partitioning across lactation is not considered; intake, MY and composition, are assumed to be constant. The aim of this study was to develop and validate alternative BCS prediction models (BCS_{NDS}) to overcome the CNCPS BCS (BCS_{CNCPS}) limitations. Two BCS_{NDS} were developed: BCS_{NDSv1} included in the BCS_{CNCPS} the "cows' actual potential", calculated as predicted peak MY over breed-specific peak, and the fat catabolism equation by Johnson et al. (2016); BCS_{NDSv2}, in addition, accounts for dynamic milk production (Wood 1967) and intake (Allen et al. 2019). Model predictions after 30 d in milk (DIM) were validated by using the following data, obtained at 25, 50 and 80 DIM from 50 Holstein Friesian: diet composition and characterization, parity, age, BW, BCS, milk yield and composition, and intake. BCS was predicted at 55, 80 and 110 DIM. The observed values were regressed on the predicted ones and RMSPE calculated. At 55 DIM, the BCS_{CNCPS} and BCS_{NDSv1} had a similar R^2 (0.48), compared with the BCS_{NDSv2} ($R^2 = 0.035$), but the BCS_{NDSv1} had the lowest RMSPE (0.312 vs 0.330 and 0.509 of the BCS_{CNCPS} and BCS_{NDSv2}) and the best NSE (−0.13 vs −2.02 and −0.78 of the BCS_{CNCPS} and BCS_{NDSv2}). The accuracy of all the models was dramatically reduced with prediction at 80 DIM ($R^2 \leq 0.12$). At 110 DIM, the BCS_{NDSv2} was the most accurate model with a R^2 of 0.535, a RMSPE of 0.156 and a NSE of 0.52 vs. a R^2 of 0.166 and 0.214, RMSPE of 0.545 and 0.278, and NSE of -0.45 and 0.18 for the BCS_{CNCPS} and BCS_{NDSv1}, respectively. All the models presented a very low slope indicating a high variability of the predicted BCS over the observed ones. Very interestingly, except for the prediction at 110 DIM with BCS_{NDSv2}, for which the residuals were evenly distributed, and for the prediction at 55 DIM with BCS_{NDSv1}, for which the residuals showed the opposite trend, all the other models and predictions had increased residuals with low BCS observed. In conclusion, although a more dynamic model seemed to be more accurate, prediction accuracy becomes more reliable after peak lactation.

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MANU, a mathematical nutrition modeling framework to assist on predictive and non-predictive multiple regression applied to model biological data

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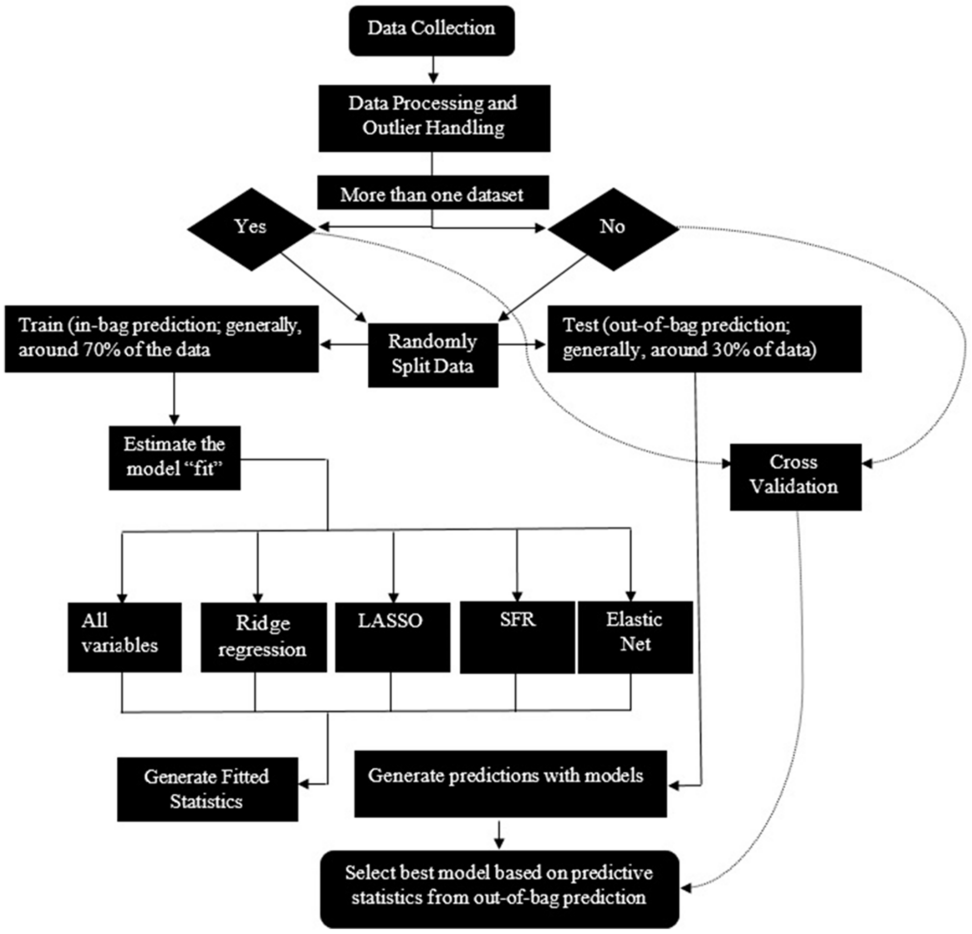
Accurate prediction of carcass (CA) and empty body (EBW) composition are fundamental for assessing nutrient requirements. Current predictive CA and EBW models rely on stepwise feature selection (SFR). Smith (2018) describes that, though SFR-models may fit data well, they perform poorly in out-of-sample predictions. The goals herein involve the development of a mathematical nutrition modeling framework (MANU; Figure 1) designed to assist in the decision making of animal scientists using predictive and non-predictive

Fig. 1. Flow chart of proposed MANU framework algorithm for generation of empirically adequate and correct predictive mathematical models.



multiple regression while modeling biological phenomena. In a regression model such as $\hat{Y} = \hat{\beta}_0 + X_1\hat{\beta}_1 + \cdots + X_p\hat{\beta}_p$, $\hat{Y}$ represents the predicted response, $\hat{\beta}_0$ the intercept, X_p the predictor variables, and $\hat{\beta}_p$ the corresponding coefficients of the predictors. Generating estimates for $\hat{\beta}_p$ is troublesome when many predictors are available and are highly correlated. Statistical significance represents one of the largest flaws of SFR at the expense of excluding biologically relevant parameters. The MANU-framework proposes three alternatives: first the ridge regression (RR) works conserving all features with regularization/penalization on coefficients: $\hat{\beta}_{i;RR} = (X^TX + \lambda I_P)^{-1}X^Ty$, where I_P is the identity matrix and λ a penalization coefficient; second, the least absolute shrinkage and selection operator (LASSO) also penalizes coefficients: $\hat{\beta}_{i;LASSO} = \arg\min(||Y - X\beta||_2^2 + \lambda \sum_{j=1}^{y} |\beta_j|)$ where $Y \in \Re^n$ is the continuous response, X is the $n \times p$ design matrix, $\beta \in \Re^p$ the parameter vector, and λ a penalty parameter that defines inclusion criteria within a given model (Meier et al. 2008); lastly, the net elastic regression (NET) combines the benefits of L1-LASSO and L2-RIDGE penalizations, being: $\hat{\beta}_{i;NET} = (1 + \frac{\lambda_2}{n})\{argmin||Y - X\beta||^2 + \lambda_2||\beta||^2 + \lambda_1||\beta||_1\}$where $Y \in \Re^n$ is the continuous response, X the nxp design matrix, $\beta \in \Re^p$ the parameter vector, and λ_1 and λ_2 are the penalization coefficients (Hoerl and Kennard 1970; Tibshirani 1996; Zou and Hastie 2003). The MANU-framework was evaluated on 19 currently published predictive models to highlight the poor out-of-sample prediction of the models generated with SFR; these results are compared with MANU models. A total of 121 predictive equations were generated for CA and EBW chemical and physical fat composition. The MANU-framework models generated better predictions for all models evaluated; Table 1 provides an example for CA and EBW physical fat data. Features selected from RR, LASSO, and NET highlight not only the betterment in predictive ability, but also inclusion of additional significant biological features. Overall, this work will assist researchers in generation of more parsimonious, precise and accurate models and improve biological understanding of current CA and EBW predictions.

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A proposed methodology for modeling gut health additives in monogastrics

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The proposed methodology assumes an existing process that quantifies the impacts of health on tissue growth and maintenance.

Table 1. Evaluation and assessment of currently published predictive equations for carcass and empty body physical fat measures compared with newly generated predictive equations with the MANU framework.

No.[a]	Method[b]	Equations[c]	R[d]	RMSE[e]	MAE[f]	CCC[g]	Cb[h]
Carcass physical fat							
Reparametrized	BID	= −31.522 (4.387) + 0.173 (0.010) * SBW	0.825	11.223	8.979	0.838	0.923
MANU	LASSO	= −95.719 + 0.076* (SBW) + 1.265 * (HBW) + 0.147 * (PBW) + 0.024 * (PGL) + 0.592* (RiDe) −0.503* (Sc) + 0.273 * (DIAG)	0.838	7.366	6.151	0.902	0.985
Empty body physical fat							
Reparametrized	BID	= −190.333 (17.219) + 2.661 (0.770) * HBW + 1.982 (0.478) * RiDe	0.861	28.043	24.287	0.672	0.724
MANU	NET	= −140.975 + 0.217 * (SBW) + 1.953 * (HBW) + 0.220 * (RuHe) + 0.267 * (PGL) – 0.714 * (Sc) + 0.300 * (DIAG) + 0.106 * (AbWi)	0.859	11.020	9.953	0.924	0.997

[a]Reparametrized = represents currently published models reparametrized with our data for prediction; MANU = best three equations generated with the proposed MANU framework.
[b]Feature selection method: BID = bidirectional stepwise selection, LASSO = least absolute shrinkage and selector operator, NET = net elastic regression.
[c]parentheses with numbers in equations represent standard error.
[d]R2 = goodness of fit.
[e]RMSE = root mean squared error.
[f]MAE = mean absolute error.
[g]CCC = concordance correlation coefficient.
[h]Cb = correction bias.

The foundation was based on three key aspects of gastro-intestinal tract (GIT) functionality: (1) microbial population; (2) integrity of the gut epithelium and barrier; and (3) immune-modulation. Any modification of these GIT characteristics will impact the health status of the animal, nutrient digestibility, and maintenance requirements. The framework for incorporating feed additives into a mechanistic growth model combines (1) quantifying the mode of action of the additive, and (2) the metabolic consequences within the animal. Firstly, to quantify the ability of an additive to influence the microbial population, GIT integrity and immune-modulation capacity, a metabolic index defining its influence relative to an objective standard is required, with a maximum, for each of the 3 possible modes of action. The index values and their effects will be influenced by their dose (or efficacy), probability of success based on existing health status, a predefined maximum beneficial effect, age of the animal and in the case of immunomodulation, the potential to over stimulate the immune system. The second component of the framework is to define the consequences on animal metabolism namely (1) improve health status; (2) improve nutrient digestibility; and (3) improve post absorption nutrient utilization. Improving general health status will improve the ability of the individual to express its genetic potential, and reduce maintenance costs, resulting in an increase in protein growth rate and possibly an increase in feed intake. With improved gut health nutrient digestibility and absorption will also increase resulting in a higher efficiency of nutrient utilization. Providing optimum immune-stimulation can also improve efficiency of nutrient utilization post absorption by reducing maintenance. The total beneficial effects of additives on animal performance will be from the sum of the benefits from the microbial, GIT integrity and immunomodulation functions. As metabolic effects of gut health additives are likely to be partially additive, the proposed method will allow some synergistic effect between additives, because of their different modes of action. However, over-estimating the cumulative effect of multiple additives will be prevented because there is a maximum level for each mode of action. Model predictions were validated against swine data from trials and published literature for a commercial gut modifier, in-feed acidifier, zinc oxide and copper sulphate and all results showed the predicted results were within 1 s.e.m of the trial or published data means.

Modeling amino acid transport in bovine mammary epithelial cells.

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Transport of amino acids (AA) by bovine mammary epithelial cells is subject to competition from other AA for common transporters and exchange activity. For example, System L works by exchanging non-essential AA (NEAA) for larger essential AA (EAA). In a meta-analysis, valine (Val) was negatively correlated with milk protein, potentially indicating transport competition (Hanigan et al. 2018). Further, Jackson et al. (2000) observed that Val influx was inhibited by high concentrations of lysine and leucine in porcine mammary tissue. Thus, the objective of this study was to assess the impact of varied NEAA and Val concentrations on EAA influx and efflux rates. Treatments were low (70% of *in vivo,* LV) or high (200% of *in vivo;* HV) Val; and low (LNEAA) or high glutamine, glycine, and alanine as a group (HNEAA) arranged in a 2×2 factorial design. Mammary cells were obtained from the State Key Laboratory of Animal Nutrition, Beijing, China (Hu et al. 2009). Nearly confluent cells were cultured for 24 h in a medium with AA profile and concentrations of lactating dairy cow plasma. Twenty-four plates were randomly assigned to treatment (3 plates per treatment) and time point (0, 0.5, 1, 5, 15, 60, and 240 min). Cells were preloaded with ^{15}N-AA followed by ^{13}C-AA as described by Yoder et al. (2020). Enrichments in media, cell cytosol, and cell protein were assessed

at each time point. Flux parameters (per (min × protein volume)) were fitted to unlabeled, ^{13}C, and ^{15}N pools and isotope ratios by replicate using FME. Parameter and flux estimates were accessed for treatment effects by ANOVA. Statistical significance and tendencies were declared at $p < 0.05$ and $p < 0.1$, respectively. High Val resulted in lower influx rate constants (K_i) for Val and leucine, probably due to feedback and competitive inhibition, respectively, and increased K_i for threonine. High NEAA tended to increase K_i for isoleucine and increased K_i for leucine, phenylalanine, threonine, and Val. There were treatment interactions for isoleucine and leucine; also, the K_i for Val tended to be highest with HNEAA and LV. In addition, HNEAA increased efflux rate constants (K_e) for isoleucine, leucine, and Val with tendencies to increase K_e for phenylalanine and threonine. In summary, relationships among AA can affect both influx and efflux in cells. If the responses are the same *in vivo*, nutritionists may have to also consider some NEAA when balancing for EAA.

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