Technical note: Assessment of the oxygen pulse and heart rate method using respiration chambers and comparative slaughter for measuring heat production of cattle

D. B. Oss,*† M. I. Marcondes,*¹ F. S. Machado,‡ T. R. Tomich,‡ M. L. Chizzotti,* M. M. Campos,‡ and L. G. R. Pereira‡

*Universidade Federal de Viçosa, Viçosa, MG, Brazil, 36570-900

†CAPES, Ministério da Educação, Brasília, DF, Brazil, 70040-020

‡Empresa Brasileira de Pesquisa Agropecuária, Embrapa Gado de Leite, Juiz de Fora, MG, Brazil, 36038-330

ABSTRACT

The objective of this study was to assess the oxygen pulse and heart rate (O₂P-HR) technique using the respiration chamber (RC) and comparative slaughter (CS) methods for measuring the heat production (HP) of crossbred (Holstein × Gyr) yearling bulls. Twenty-four bulls were used. Six bulls were slaughtered at the beginning of the experiment as a reference group to estimate the initial empty body weight (BW) and energy content of the remaining animals. The remaining bulls were assigned to a completely randomized design with 3 levels of dry matter intake, with 6 replicates. The levels of dry matter intake were 1.2% of BW, 1.8% of BW and ad libitum, with target orts of 5%. The bulls were fed a diet consisting of 59.6% corn silage and 40.4% concentrate on a dry matter basis. The HP (kcal/BW^{0.75}) was measured using 3 techniques, first using O₂P-HR, followed by the RC and CS methods. The HP did not differ among assessed techniques, averaging 162.7 kcal/BW^{0.75}. The intercepts of the linear regressions (mean ± SE) were 64.82 ± 25.515 (H₀: intercept = 0; *P* = 0.024), 33.77 ± 13.418 (H₀: intercept = 0), and 50.02 ± 27.495 (H₀: intercept = 0) for O₂P-HR versus RC, CS versus RC, and O₂P-HR versus CS, respectively. The slopes of the linear regressions were 0.59 ± 0.153 (H₀: slope = 1), 0.88 ± 0.081 (H₀: slope = 1), and 0.62 ± 0.155 (H₀: slope = 1) for O₂P-HR versus RC, CS versus RC, and O₂P-HR versus CS, respectively. The coefficients of determination were 0.52, 0.90, and 0.52 for O₂P-HR versus RC, CS versus RC, and O₂P-HR versus CS, respectively. The concordance correlation coefficients, 0.70 and 0.68, were moderate for O₂P-HR versus RC and O₂P-HR versus CS, respectively, but high, 0.90, for CS versus RC. The between-animal coef-

ficient of variation was greater for the O₂P-HR method (16.6%) compared with RC (7.7%) or CS (6.7%). We conclude that there was an agreement among the HP measurements detected using the assessed methods and that O₂P-HR is able to predict HP in cattle with great accuracy but only moderate precision. Therefore, the O₂P-HR method may have limitations in terms of assessing HP in low numbers of replications due to greater between-animal coefficient of variation than either the RC or CS methods.

Key words: heart rate, heat production, oxygen pulse

Technical Note

Heat production (HP) of cattle can be determined under controlled and confined conditions by using the respiration chamber (RC) method; however, these conditions do not reflect free-ranging animals or commercial cattle in feedlots or pastures. Also, capital investment for RC is high, training of animals is required, and the behavior of animal may be altered from that which occurs under most production settings (e.g., low activity and reductions in DMI). In an attempt to overcome the limitations of measuring HP in the environment of cattle, HP can also be measured using the comparative slaughter method (CS), as this technique allows the evaluation of animals that are being raised in several production conditions. However, CS is terminal, laborious, and requires accurate estimates of DMI and metabolizable energy intake (MEI) and thus can introduce significant errors; besides, it is able to provide only one HP value on average for all of the experiment.

The O₂P-HR method is an alternative technique for measuring HP that is based on long-term measurements (24-h periods) of the heart rate (HR) of free-range animals and on short-term measurements of oxygen pulse (O₂P; mL of O₂ consumed/heart beat), which are measured by attaching a face mask (FM) to the animal's nose (Brosh, 2007).

Received March 10, 2016.

Accepted July 12, 2016.

¹Corresponding author: marcosinaciomarcondes@gmail.com

Few experiments have been conducted to examine the reliability of the O₂P-HR method in measuring the HP of cows (Arieli et al., 2002; Brosh et al., 2002). However, to our knowledge, no studies have compared O₂P-HR with the more traditional techniques (RC and CS) of determining HP in cattle energetics research (Arieli et al., 2002; compared O₂P-HR with CS in sheep rather than cattle). The use of O₂P-HR method as an alternative method can be useful in studies including genetic and efficiency selection, evaluation of physiological stage or growth stage and also different feeding paradigms.

Therefore, the objective of this study was to compare the O₂P-HR, RC, and CS methods of measuring and assessing variation in HP in cattle. We hypothesized that the O₂P-HR method would predict HP comparable to the RC and CS techniques for these same growing bulls.

The present study followed the guidelines of the Ethics Committee in Animal Use of the Universidade Federal de Viçosa (process number 44/2012). The experiment was conducted at the Multi-Use Complex on Livestock Bioefficiency and Sustainability at Embrapa Gado de Leite, in Coronel Pacheco, MG, Brazil, from August 2013 to February 2014.

Twenty-four Holstein × Gyr crossbred 10-mo-old bulls (initial BW = 155 ± 24.6 kg) were used. All bulls were adapted to the experimental diet before the initial slaughter over 30 d being fed the same level of DM: 2.0% of BW. After the adaptation period, bulls (184 ± 23.4 kg) were randomly subdivided into 4 groups of 6 animals. One of those groups was designated as a baseline reference group and was slaughtered at the beginning of the experiment to measure the initial body energy content in their empty BW (**EBW**) to facilitate the CS method. The 3 remaining groups were fed at 3 different DMI: (1) restricted to 1.2% of BW, (2) restricted to 1.8% of BW, or (3) ad libitum with target 5% orts. These treatments were selected to introduce the necessary variation in MEI to assess the validity of the method. One bull from the ad libitum group had to be removed from the experiment due to health issues. Throughout the experiment, bulls were housed in a tie stall barn with free access to water and fed a diet consisting of corn silage and concentrate (59.6: 40.4 DM basis) once daily (0830 h). The concentrate was composed of soybean meal (24.8%), ground corn (67.9%), urea (2.4%), mineral mix (3.5%), and limestone (1.4%). The DM feed offered and refused was weighed to determine total daily DMI. The estimated ME content of the diet was 2.4 Mcal/kg of DM on average. The ME content of the diet was determined by multiplying digestible energy by 0.82 (NRC, 1996)

taking into account the DM digestibility coefficients of each animal. Although the fixed ratio ME:digestible energy (**DE**) of 0.82 (NRC, 1996) has been accepted in many publications, this ratio might change as MEI increases [i.e., Chaokaur et al., 2015, who determined energy balance of Brahman bulls raised on increasing dietary allocations observed ME:DE ratio of 0.82, 0.86, 0.87, and 0.88 for maintenance (**M**), 1.4 × M, 1.8 × M, and ad libitum groups, respectively]. The digestibility coefficients in our study were obtained from 2 digestibility trials conducted at 2 points throughout the experiment: 2 mo after the reference slaughter and 2 mo before the final slaughter. The DM digestibility coefficients did not differ between trials. The digestibility trial consisted of 3 d of total feces and urine collection as described by Costa e Silva et al. (2015).

Treated bulls were slaughtered at the end of the experiment to measure the final body energy content in their final EBW for CS calculations. The experiment lasted 173, 171, and 168 d for the 1.2% of BW, 1.8% of BW and ad libitum groups, respectively, after which the animals were slaughtered. The slaughters followed the same procedures described by Costa e Silva et al. (2015).

The bulls were accustomed to the FM for a period of 2 wk before measurements. Bulls were placed in a squeeze chute and the FM was fitted for two 20-min periods (morning and afternoon). Following the training period, 3 O₂P (mL/heart beat) measurements were collected over a 3-d period with measurements made about 6 h after feeding, separated by 3 d of HR (beats/min) measurements at the tie stall.

The O₂ consumption data were recorded using a Sable System (Sable Systems International, Las Vegas, NV) attached to the FM. Details about the gas measurements were described by Oss et al. (2016). Samples from the FM were collected at 20-s intervals and recorded at 1-min intervals over 20 min, with ambient air collected 5 min before and after the 20-min measurements to establish baseline gas levels. All data were recorded using an automated data acquisition program (Expedata, Sable Systems International). The O₂ consumption (VO₂; mL/min) was calculated from the product of mass flow measurements of the atmospheric air corrected for standard temperature (273.5 K) and pressure (101.325 kPa) conditions (STP) and difference in average from FM [O₂fm, % and baseline (O₂b; %), O₂ concentration measurements over 30 min] as follows:

$$VO_2 = [STP \times (O_{2fm} - O_{2b})].$$

The HR was recorded using a Polar equine transmitter and monitor (model RS800CX, Polar Electro Inc.,

Kempele, Finland). The transmitters were embedded in a 10-cm-wide girth strap with a Velcro latch placed around the bulls' girth behind the shoulders. The negative electrode was positioned on the right side and the positive electrode on the opposite side of the bulls, parallel to the left elbow. The area around the electrodes was shaved and a conductivity gel was applied to increase conductance.

The HR measurements during the O₂ consumption measurement and the 3 d of HR at the tie stall were averaged and recorded every 60 s. To obtain a representative daily average HR for each measurement day, a deviation was calculated for each 60 s of HR recorded, taking into account the average of 5 min before and 5 min after each minute recorded. Outliers were identified when this deviation was over 30%. The HR days were then overlapped and an average was calculated.

The O₂P (mL/heart beat) was determined by the average O₂ consumption per min over the average HR per min during the same 20-min period. Total daily O₂ consumption (L/d) was calculated from the average of O₂P and average daily HR. Daily HP was then calculated as the product of total daily O₂ consumption and the constant 20.47 KJ per liter of O₂ (McLean, 1972).

Two weeks after performing the O₂P-HR method, the HP of the bulls was again estimated but using indirect open-circuit RC. Two RC were used. The chamber's design and operation were described by Machado et al. (2016). Bulls were housed in the chambers for two 24-h periods for HP measurement. Bulls were placed in the chamber, feed was delivered, the chamber was closed, and measurements were initiated. After 24 h the measurements were interrupted and the bulls were removed to clean the chambers. The daily O₂ consumption and CO₂ and CH₄ production were measured over 24 h with correction for the CO₂ and CH₄ recovery levels in each chamber, which were 99.0 and 98.0%, respectively. The HP was calculated based on Brouwer (1965). To compute the energy loss in urine as N, the urinary N was determined from samples obtained from the digestibility trials.

For the comparison of HP among the 3 techniques assessed, we assumed that HP remained the same in terms of kilocalories per BW^{0.75} along the experimental period once bulls were under very low performance due to the intake levels under which they were. This assumption made us able determine HP by RC and O₂P-HR methods only once during the experimental period. Therefore, we suggest that in future studies, HP by RC or O₂P-HR methods in growing animals with greater performance be determined at least 2 times during the growing period, one during each half of the experimental period.

With regard to CS, the HP was calculated as the difference between the MEI and retained energy. The retained energy was calculated as the difference between the final energy content and the initial energy content in the EBW of bulls.

All HP were expressed as kilocalories per BW^{0.75}. Data were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). Significance among HP means was declared at $P \leq 0.05$. The agreement between HP using O₂P-HR versus RC, CS versus RC, and O₂P-HR versus CS methods was analyzed using the Model Evaluation System (Tedeschi, 2006; <http://nutritionmodels.tamu.edu/models/mes/>) in which predicted values (RC or CS) and observed (O₂P-HR or CS) values were analyzed for accuracy and precision using several variables. These variables included linear regression of observed and predicted values, *t*-tests to identify the significance of parameters (intercept = 0 and slope = 1; Neter et al., 1996), coefficients of determination, concordance correlation coefficients (CCC; Lin, 1989), accuracy (Cb; Liao, 2003), the mean square error of prediction, and its decomposition into mean bias, systematic bias, and random errors (Bibby and Toutenburg, 1977). The estimated means and standard deviations were used to compute between-animal coefficients of variation in HP (kcal/BW^{0.75}).

The relationships between HP (kcal/BW^{0.75}) measured using O₂P-HR versus RC and CS versus RC methods are illustrated in Figure 1, whereas the comparison between O₂P-HR versus CS is illustrated in Figure 2. Table 1 contains the description of the database of HP

Figure 1. Relationships between heat production (HP; kcal/BW^{0.75}) measured using oxygen pulse and heart rate (O₂P-HR) versus respiration chamber (RC) and comparative slaughter (CS) versus RC methods of individual crossbred (Holstein × Gyr) yearling bulls fed at different intake levels. Open symbols and dotted line are related to O₂P-HR versus RC. Solid symbols and dashed line are related to CS versus RC. The solid line is the line $y = x$.

Table 1. Description of the database of heat production (HP; kcal/BW^{0.75}) measurements used to assess the oxygen pulse and heart rate (O₂P-HR) in comparison with respiration chamber (RC) and comparative slaughter (CS) methods

Method	Mean	Minimum	Maximum	SD	<i>P</i> -value	CV (%)
O ₂ P-HR	157.1	94.6	232.5	40.03	0.076	16.6
RC	159.4	95.8	238.9	49.00		7.6
CS	171.6	118.2	266.1	46.23		6.7

measurements for each method used. Related descriptive statistics are presented in Table 2.

For comparisons of O₂P-HR versus RC and O₂P-HR versus CS, the regression analysis indicated that the slopes were different from unity ($P < 0.050$); however, for comparison CS versus RC slope did not differ from 1 ($P = 0.173$). The slopes of the linear regressions (mean $\pm$ SE) were 0.59 ± 0.153 (H_0 : slope = 1; $P = 0.018$), 0.88 ± 0.081 (H_0 : slope = 1; $P = 0.173$), and 0.62 ± 0.155 (H_0 : slope = 1; $P = 0.028$) for O₂P-HR versus RC, CS versus RC, and O₂P-HR versus CS, respectively (Table 2).

The intercept of the linear regression was different from zero ($P < 0.050$) for O₂P-HR versus RC and CS versus RC but did not differ from zero for O₂P-HR versus CS ($P = 0.089$). The intercepts of the linear regressions (mean $\pm$ SE) were 64.82 ± 25.515 (H_0 : intercept = 0; $P = 0.024$), 33.77 ± 13.418 (H_0 : intercept = 0; $P = 0.025$), and 50.02 ± 27.495 (H_0 : intercept = 0; $P = 0.089$) for O₂P-HR versus RC, CS versus RC, and O₂P-HR versus CS, respectively (Table 2). In a study in which a comparison between O₂P-HR versus CS was performed using sheep, a slope of 0.86 (closer

to unity than our finding) and an intercept of 135.29 were found (Arieli et al., 2002). Slopes and intercepts different from unity and zero, respectively, are in this case indicative of low accuracy of the O₂P-HR method compared with RC. However, the Cb values, another accuracy index, indicate great accuracy of the O₂P-HR method compared with RC (0.97) and CS (0.94); similarly, a great accuracy was observed for CS compared with RC (0.95).

The regression estimates of coefficient of determination for O₂P-HR versus RC was 0.52, a moderate value compared with the coefficient of determination for CS versus RC (Table 2). The third comparison, O₂P-HR versus CS, also presented a moderate coefficient of determination, 0.52. The coefficient of determination is a good indicator of precision; the greater the coefficient of determination, the greater the precision (Tedeschi, 2006). Precision measures how close individual O₂P-HR and CS values are within each condition (treatment), whereas accuracy measures how close O₂P-HR and CS values are to the RC values, and also how close O₂P-HR values are to CS values. Given these definitions of accuracy and precision, the O₂P-HR and CS methods

Table 2. Descriptive statistics of relationship between heat production (kcal/BW^{0.75}) using oxygen pulse and heart rate (O₂P-HR) versus respiration chamber (RC) and O₂P-HR versus comparative slaughter (CS) methods of individual crossbred (Holstein $\times$ Gyr) yearling bulls fed at different intake levels¹

Item	Method comparison		
	O ₂ P-HR vs. RC	CS vs. RC	O ₂ P-HR vs. CS
Regression			
Intercept			
Estimate	64.82	33.77	50.02
SE	25.515	13.418	27.495
<i>P</i> -value	0.024	0.025	0.089
Slope			
Estimate	0.59	0.88	0.62
SE	0.153	0.081	0.155
<i>P</i> -value	0.018	0.173	0.028
R ²	0.52	0.90	0.52
CCC	0.70	0.90	0.68
Cb	0.97	0.95	0.94
MSEP	1,116.92	469.17	1,219.63
MB	0.09 (0.01%)	233.92 (49.86%)	209.99 (17.22%)
SB	375.47 (33.62%)	30.21 (6.44%)	284.35 (23.31%)
RE	741.36 (66.38%)	205.03 (43.70%)	725.29 (59.47%)

¹CCC = correlation and concordance coefficient. H_0 : intercept = 0. H_0 : slope = 1. Cb = bias correction factor. MSEP = mean square error of prediction. MB = mean bias. SB = systematic bias. RE = random errors.

Figure 2. Relationship between heat production (HP; kcal/BW^{0.75}) measured using oxygen pulse and heart rate (O₂P-HR) versus comparative slaughter (CS) methods of individual crossbred (Holstein × Gyr) yearling bulls fed at different intake levels. The solid line is the line $y = x$.

have the ability to estimate the “correct” values because they presented great accuracy (Cb) compared with RC; however, the O₂P-HR is not as sensible as CS to measure values consistently because O₂P-HR method presented moderate precision whereas CS presented great precision (R^2). The moderate precision presented by the O₂P-HR method was also confirmed by the greater between-animal coefficient of variation (16.6%) compared with RC (7.7%) and CS (6.7%).

It is difficult to point out which is better, accuracy or precision. According to Tedeschi (2006), it is easy to understand why accuracy could be argued as the most important measure as the true mean can be detected using an imprecise method simply by averaging a large number of data points. However, measurements using an accurate but imprecise method are unrealistic as they may interfere in the variation of means and consequently make it difficult to find differences between treatments using this technique.

Based on the discussion above, the O₂P-HR method has great accuracy and will provide good estimates of HP; however, to identify treatment differences in an experiment the sample size (n) must be increased. The implication of a greater between-animal coefficient of variation is that to detect a treatment difference with a certain power, more animals (replicates) per treatment would be required with the O₂P-HR method compared with the RC or CS techniques; however, benefits of the technique are significant and it can complement a program to assess energy use as affected by many factors. Although more animals are required and multiple sampling is needed, the O₂P-HR could be used more readily than RC and CS.

The CCC, also known as the reproducibility index, indicates if the O₂P-HR and the CS measurements are precise and accurate at the same time. The closer to 1 the better. The CCC values were moderate, 0.70 for O₂P-HR versus RC and 0.68 for O₂P-HR versus CS, which was expected as the technique presented moderate precision. This analysis confirmed the great accuracy (Cb) of the estimates, as the accuracy had to be greater than the coefficient of determination to get a moderate CCC value. In contrast, CCC was greater, 0.90, for CS versus RC.

The mean differences between observed (O₂P-HR or CS) and predicted values (RC or CS) were 2.3 kcal/BW^{0.75} for O₂P-HR versus RC, 12.2 kcal/BW^{0.75} for CS versus RC, and 20.5 kcal/BW^{0.75} for O₂P-HR versus CS, indicating that O₂P-HR measurements were under-predicted relative to RC and to CS and CS was over-predicted relative to RC. However, HP means did not differ among the 3 assessed techniques (Table 1). The mean square error of prediction calculation confirmed that most of the error associated with the O₂P-HR method was random error (66.4% compared with RC and 59.5% compared with CS; Table 2).

The O₂P-HR method generated HP estimates that were comparable to HP estimated using RC and CS across a range of DMI with great accuracy and moderate precision. The CS estimated HP compared with RC with great accuracy and also great precision. The O₂P-HR method had a greater between-animal coefficient of variation, which has a negative effect on the power of an experiment using the O₂P-HR method. However, despite this loss in statistical power, it is an alternative technique for those that do not have respiration chambers or who wish to avoid slaughtering animals and estimating DMI and MEI (needed to estimate HP by using CS), especially under grazing conditions. In general, this method can be useful for the study of energy metabolism in cattle. Further studies should be performed to investigate ways to minimize errors associated with the O₂P-HR method to increase the precision and the statistical power of experiments using this technique.

ACKNOWLEDGMENTS

The results from this study are part of the results of the project “Nutrição de Precisão” of EMBRAPA. The authors gratefully acknowledge the funding support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do estado de Minas Gerais (FAPEMIG), and Empresa Brasileira de Pesquisa Ag-

ropecuária (EMBRAPA) of Brazil. The author D.B.O. gratefully acknowledges CAPES/EMBRAPA for the doctoral scholarship.

REFERENCES

- Arieli, A., A. Kalouti, Y. Aharoni, and A. Brosh. 2002. Assessment of energy expenditure by daily heart rate measurement—Validation with energy accretion in sheep. *Livest. Prod. Sci.* 78:99–105. [http://dx.doi.org/10.1016/S0301-6226\(02\)00094-5](http://dx.doi.org/10.1016/S0301-6226(02)00094-5).
- Bibby, J., and H. Toutenburg. 1977. *Prediction and Improved Estimation in Linear Models*. Wiley, Berlin, Germany.
- Brosh, A. 2007. Heart rate measurements as an index of energy expenditure and energy balance in ruminants: A review. *J. Anim. Sci.* 85:1213–1227. <http://dx.doi.org/10.2527/jas.2006-298>.
- Brosh, A., Y. Aharoni, and Z. Holzer. 2002. Energy expenditure estimation from heart rate: Validation by long-term energy balance measurement in cows. *Livest. Prod. Sci.* 77:287–299. [http://dx.doi.org/10.1016/S0301-6226\(02\)00033-7](http://dx.doi.org/10.1016/S0301-6226(02)00033-7).
- Brouwer, E. 1965. Report of sub-committee on constants and factors. Pages 441–443 in *Energy Metabolism of Farm Animals*, Academic Press, London, UK.
- Chaokaur, A., T. Nishida, I. Phaowphaisal, and K. Sommart. 2015. Effects of feeding level on methane emissions and energy utilization of Brahman cattle in the tropics. *Agric. Ecosyst. Environ.* 199:225–230. <http://dx.doi.org/10.1016/j.agee.2014.09.014>.
- Costa e Silva, L. F., S. C. Valadares Filho, T. E. Engle, P. P. Rotta, M. I. Marcondes, F. A. S. Silva, E. C. Martins, and A. T. Tokunaga. 2015. Macrominerals and trace element requirements for beef cattle. *PLoS ONE* 10:e0144464. <http://dx.doi.org/10.1371/journal.pone.0144464>.
- Liao, J. J. Z. 2003. An improved concordance correlation coefficient. *Pharm. Stat.* 2:253–261. <http://dx.doi.org/10.1002/pst.52>.
- Lin, L. I.-K. 1989. A concordance correlation coefficient to evaluate reproducibility. *Biometrics* 45:255–268.
- Machado, F. S., T. R. Tomich, A. L. Ferreira, L. F. L. Cavalcanti, M. M. Campos, C. A. V. Paiva, M. N. Ribas, and L. G. R. Pereira. 2016. Technical note: A facility for respiration measurements in cattle. *J. Dairy Sci.* 99:4899–4906. <http://dx.doi.org/10.3168/jds.2015-10298>.
- McLean, J. A. 1972. On the calculation of heat production from open-circuit calorimetric measurements. *Br. J. Nutr.* 27:597–600.
- Neter, J., M. H. Kutner, C. J. Nachtsheim, and W. Wasserman. 1996. *Applied Linear Statistical Models*, 5th ed. McGraw-Hill, Boston, MA.
- NRC. 1996. *Nutrient Requirements of Beef Cattle*. National Academy Press, Washington, DC.
- Oss, D. B., M. I. Marcondes, F. S. Machado, L. G. R. Pereira, T. R. Tomich, G. O. Ribeiro Jr., M. L. Chizzotti, A. L. Ferreira, M. M. Campos, R. M. Mauricio, A. V. Chaves, and T. A. McAllister. 2016. An evaluation of the face mask system based on short-term measurements compared with the sulfur hexafluoride (SF₆) tracer, and respiration chamber techniques for measuring CH₄ emissions. *Anim. Feed Sci. Technol.* 216:49–57. <http://dx.doi.org/10.1016/j.anifeeds.2016.03.008>.
- Tedeschi, L. O. 2006. Assessment of the adequacy of mathematical models. *Agric. Syst.* 89:225–247. <http://dx.doi.org/10.1016/j.agry.2005.11.004>.