Growth and Accretion of Energy and Protein in the Gravid Uterus During Late Pregnancy in Holstein Cows

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ABSTRACT

Multiparous Holstein cows (n = 18) were bred artificially to the same bull and then slaughtered at times ranging from 190 to 270 d postconception to assess accretion of energy, protein, fat, and ash by the conceptus. Wet weights, dry weights, and concentrations of energy, CP, crude fat, and ash were obtained for the following: fetus, combined amniotic and allantoic fluids, fetal membranes, cotyledons, caruncles, and uterine tissues. Rates of accumulation of these components in the gravid uterus (sum of all uterine contents) and fetus were described by linear or quadratic equations. Estimated rates of accretion of energy in the gravid uterus (i.e., conceptus) increased from 567 kcal/d at 190 d of gestation to 821 kcal/d at 270 d of gestation; corresponding rates of accretion of CP were 62 and 117 g/d. These daily rates represent net energy and protein requirements for conceptus growth during late pregnancy in mature Holstein cows. Conversion of predicted net energy to metabolizable energy requirements for conceptus growth, using the accepted efficiency factor of .14, yielded estimates that were consistent with current NRC recommendations. Factorial estimation of absorbed protein requirements is hampered by lack of precise information on the efficiency with which absorbed AA are deposited in conceptus tissues.

(Key words: energy and protein accretion, gravid uterus, fetus, Holstein cows)

INTRODUCTION

The estimation of nutrient requirements during late pregnancy requires accurate values for rates of nutrient accretion in conceptus tissues, calculated from measurements of growth rate and chemical composition. Widely used tables for prediction of energy and protein requirements of dairy cows in late pregnancy (1, 24) are based almost exclusively on a single study involving the serial slaughter of Red Danish cows (17, 18). Applicability of these data to modern Holsteins is limited by breed, inadequate description of the cows used, uncertainty about breeding date, and most importantly, the relatively small number of observations in late pregnancy, when conceptus growth and nutrient demands are greatest. Ellenberger et al. (9) measured some developmental changes in fetal body composition (but not energy or protein) in Holstein cows; however, most late gestation fetuses were aborted, and nonfetal components of the conceptus were not measured.

There is a particular need for accurate definition of the pattern of fetal and total conceptus growth during late pregnancy in mature Holstein cows. Two of three studies (8, 11, 25) of bovine pregnancy with reasonable sample sizes suggest that fetal growth rate peaks at about 230 d of pregnancy and then declines rapidly to about 50% of maximum by term. One of these studies (8) used a variety of dairy breeds, more than half of which were Jerseys, and did not measure tissue composition; a second study used beef heifers of mixed breed that were bred to bulls of large frame size (25). The third study (11) used Hereford heifers, and a peak in fetal growth rate during late pregnancy was not readily discernible. No comparable studies have been done on modern Holstein cows; therefore, the objective of this study was to measure rates of growth and chemical composition of conceptus tissues in mature, multiparous Holstein cows that were serially slaughtered from 190 to 270 d of pregnancy.
MATERIALS AND METHODS

Cows and Diets

Multiparous Holstein cows (n = 18) in the herd at the Cornell University Animal Science Teaching and Research Center were bred artificially to the same Holstein bull (Jamaica, NAAB (National Association of Animal Breeders) number 29-H-4710; calving ease score, 11%). Prior to breeding, cows had completed 2.7 ± .3 (X ± SE) lactations and had a mean 305-d yield of 8789 ± 356 kg in the previous lactation. Pregnancy was confirmed by rectal palpation 35 to 40 d after breeding, and the cows were slaughtered at various times during late pregnancy. At slaughter, live BW (fed) ranged from 579 to 814 kg (X ± SE, 714 ± 16 kg). All cows carried a single fetus.

Cows were fed and managed according to usual practice at the Center for cows in late lactation and the dry period. All cows were dried off by 220 d of pregnancy, except for a few that were dried off earlier. Each cow consumed daily 10 to 12 kg of DM of a TMR containing 1.4 Mcal of NEL/kg of DM and 130 g of CP/kg of DM. After ~250 d of pregnancy, the ration fed contained 2.2 Mcal of NEL/kg of DM and 140 g of CP/kg of DM. A mixed alfalfa grass hay (900 g/kg of DM, 1.2 Mcal of NEL/kg of DM, and 117 g of CP/kg of DM) was also available to cows during the early dry period, but hay consumption was not measured.

Tissue Collection

Cows were slaughtered at 190 to 270 d of pregnancy after transport to a USDA-inspected facility, using a captive-bolt pistol and exsanguination. The pregnant uterus was severed at the cervix, removed, and weighed; the fetus was immediately euthanatized by intravascular injection with pentobarbitone sodium (Beuthanasia; Schering-Plough, Kenilworth, NJ).

The pregnant uterus was separated into the following components: fetus, fetal fluids (amniotic and allantoic fluids combined), fetal membranes (including umbilical cord and blood vessels), placentomes (cotyledons plus caruncles), and uterine tissues (myometrium and endometrium). Except for fetal fluids, the fetus and adnexa were ground separately, and duplicate 300-g samples of each were frozen, stored at -20°C, and then freeze-dried before chemical analysis. Amniotic and allantoic fluids were mixed and weighed, and two 50-ml samples were stored at -20°C until analysis. The fetus was weighed, dismembered, ground in a grinder (model 801B; Autio Co., Astoria, OR) with a 9-mm end-plate screen, mixed, and reground using the same screen. The ground fetus was then reground twice more in a grinder (model 4432; Hobart Corp., Troy, OH) with a 3-mm end-plate before samples were collected. Fetal membranes and uterine tissues were weighed and ground with a food chopper (model 8181D; Hobart Corp.), and duplicate samples were prepared. Placentomes were dissected into cotyledonary (fetal) and caruncular (maternal) parts, which were weighed and homogenized separately in a Waring high speed blender (Waring Products Division, Dynamic Corp. of America, New Hartford, CT); samples of each were then collected and stored at -20°C.

Chemical Analyses

Frozen tissue samples were pulverized and ground more finely in the Waring blender with liquid N2. Duplicate samples were dried to constant weight at 60°C to determine residual water content. Dried samples were extracted for 120 h in a Soxhlet apparatus (Corning Glass Works, Corning, NY) with chloroform to determine crude fat concentration (13). Ash was determined on the chloroform-extracted samples as the residue remaining after 12 h in a muffle furnace at 550°C. Crude protein was determined by the macro-Kjeldahl method using N x 6.25 (3). Energy content was measured in an adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL) using standard methodology (20).

Calculations and Statistics

Conceptus concentration and content of chemical components and energy were calculated as weighted means and aggregates, respectively, of individual values for fetus, fetal fluids, fetal membranes, cotyledons, caruncles, and uterine tissues. These calculated values and those for fetal concentration and content were related to gestational age by linear and polynomial regression analysis. Poly-

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nominal equations for nonlinear relationships were derived with a program (Sigma Plot™; Jandel Scientific, Corte Madera, CA) that used a least squares, iterative procedure. The simplest function that was not statistically inferior to more complex expressions, as judged by $R^2$ values and residual plot analysis, was differentiated by day of gestation to obtain estimates of daily accretion rates of wet matter, DM, crude fat, CP, ash, and energy in the gravid uterus and fetus.

RESULTS AND DISCUSSION

Uterine and Fetal Growth

Growth of the gravid uterus and fetus, as judged by fresh and dry weights, are depicted in Figure 1. In essence, uterine tissue accretion represents conceptus growth because it is composed almost entirely of fetus, amniotic and allantoic fluids, fetal membranes, placentomes, and new uterine tissues (mostly endometrium), all of which are specific products of pregnancy. The relationships between fresh weight and gestational age were adequately fit by linear functions. House and Bell (15) published a polynomial function for description of fetal growth in these cows, but later found that this function did not offer statistically significant improvement over the simpler expressions, coefficients of which describe rates of total uterine and fetal growth during late pregnancy (Table 1).

Previous studies of cows (7, 25) and sheep (22) suggest that fetal growth rate usually declines toward parturition, even in very well-fed animals. Nevertheless, in the present study, fetal growth rate did not decline appreciably after 230 to 240 d, in contrast to results of at least two previous studies. In one of those (25), the dams were mixed breed beef heifers that had been bred to bulls of large frame size, and fetal growth was likely constrained in late pregnancy by maternal factors related to size, as demonstrated by Ferrell (10). In the other study (8), the dams were mainly Jerseys, presumably bred to Jersey sires. In the present study, all cows were multiparous, and most were relatively large (mean BW 714 ± 16 kg). This was associated with a projected mean birth weight of 46 kg, which was heavier than average for the Holstein breed, as discussed previously (15), possibly because of minimal maternal restriction of fetal growth in late pregnancy.

Relative growth of the fetus between d 190 and 270 was greater than that of the nonfetal components of the uterus, especially when expressed in terms of dry weight. Thus, the fetus accounted for only 45% of uterine dry weight at 190 d, but almost 80% at 270 d. In aggregate, the nonfetal tissues of the conceptus accounted for only 18 g/d of the mean rate of accretion of dry tissues, 138 g/d, in the whole gravid uterus over this period. A more detailed description of the separate patterns of growth in the nonfetal components of the gravid uterus is presented elsewhere (15).

Fetal dry weight increased exponentially at a relatively faster rate than that of fetal wet weight (Figure 1) because of the progressive dehydration of fetal tissues during late gestation (Figure 2). Thus, fetal DM concentration increased from about 180 g/kg at d 190 to about 260 g/kg at d 270, a value similar to that
for newborn calves (9). Most of the prenatal changes in tissue water content were assumed to have occurred in the extracellular fluid space, as shown for other species (14).

Composition of Uterine and Fetal Growth

The proximate composition of total uterine and fetal DM did not significantly change between d 190 and 270 of pregnancy, except for ash. Thus, gestational increases in CP, fat, and energy concentrations on a fresh weight basis generally resembled those in DM concentration (Figure 2). Ash concentration in fetal DM was constant between d 190 and 235, then increased sharply between d 240 and 250, before declining, between d 260 and 270, to concentrations that were similar to those observed before d 240 (Figure 3). This transient increase is difficult to explain because none of the individual macrominerals in fetal DM showed a similar pattern, although the DM concentrations of calcium and sulfur tended to increase with gestational age (15, 16). However, we think that the transient increase is real because it followed a plausible time course in the four animals slaughtered between d 240 and 250 of pregnancy (Figure 3).

Using conventional values for the energy content of fat and protein (7), these chemical components were estimated to account for about 85% of the measured energy concentration of fetal tissues. The discrepancy between calculated and measured values is almost certainly due to the rapid accumulation of glycogen in fetal tissues during late gestation (27). Assumption of a mean glycogen concentration of about 30 mg/g of wet weight in soft tissues, with a caloric value of 4.2 kcal/g (7), almost completely explains the difference between energy concentration that was estimated from the sum of protein and fat and energy concentration that was measured by bomb calorimetry.

Energy and Nutrient Accretion
In Conceptus and Fetus

Relationships between gestational age and the total uterine and fetal contents of energy and CP were best fit by simple quadratic equations (Table 1, Figure 4), implying an accelerating rate of increase in accretion of these components during the last trimester of conceptus growth. The similarity between patterns of energy and protein deposition are to be expected because protein is the most abundant organic constituent of conceptus tissues. The much slower rates of total uterine and fetal accretion of fat and ash were adequately described by linear functions (Table 1, Figure 4).

Differentiation of the equations relating total uterine and fetal contents of protein and
TABLE 1. Relationships of wet weight; dry weight; weights of CP, crude fat, and ash; and energy content of the gravid uterus and fetus to day of gestation during late pregnancy in Holstein cows.

<table>
<thead>
<tr>
<th>Item</th>
<th>Gravid uterus</th>
<th>R²</th>
<th>Equation</th>
<th>Fetus</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet weight, kg</td>
<td>$y = 0.664x - 102$</td>
<td>0.93</td>
<td>$y = 0.418x - 70$</td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>Dry weight, kg</td>
<td>$y = 0.000476x^2 - 0.0796x + 1.07$</td>
<td>0.96</td>
<td>$y = 0.000558x^2 - 0.136x + 7.55$</td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>CP, g</td>
<td>$y = 0.345x^2 - 0.69.2x + 2818$</td>
<td>0.96</td>
<td>$y = 0.461x^2 - 138.8x + 10.895$</td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>Crude fat, g</td>
<td>$y = 15.2x - 2687$</td>
<td>0.88</td>
<td>$y = 13.5x - 2484$</td>
<td></td>
<td>0.92</td>
</tr>
<tr>
<td>Ash, g</td>
<td>$y = 23.0x - 4084$</td>
<td>0.94</td>
<td>$y = 21.6x - 3901$</td>
<td></td>
<td>0.93</td>
</tr>
<tr>
<td>Energy, Mcal</td>
<td>$y = 0.00159x^2 - 0.0352x - 35.4$</td>
<td>0.96</td>
<td>$y = 0.00254x^2 - 0.567x + 24.9$</td>
<td></td>
<td>0.95</td>
</tr>
</tbody>
</table>

1$ y = \text{Value of trait indicated}; x = \text{day of gestation. Equations describe changes during the third trimester of pregnancy and should not be used to predict weight or composition of the gravid uterus or fetus prior to 190 d of gestation.}$

Figure 4. Relationships of CP (upper left panel), crude fat (lower left panel), energy (upper right panel), and ash (lower right panel) contents of the gravid uterus (○) and fetus (●) to gestational age in Holstein cows during late pregnancy.

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CONCEPTUS ENERGY AND PROTEIN ACCRETION

TABLE 2. Predicted rates of deposition of energy and CP in the gravid uterus and fetus at different stages of late pregnancy in Holstein cows.1

<table>
<thead>
<tr>
<th>Gestation (d)</th>
<th>Energy</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gravid uterus</td>
<td>Fetus</td>
</tr>
<tr>
<td>190</td>
<td>567 kcal/d</td>
<td>398 g/d</td>
</tr>
<tr>
<td>210</td>
<td>631 kcal/d</td>
<td>500 g/d</td>
</tr>
<tr>
<td>230</td>
<td>694 kcal/d</td>
<td>601 g/d</td>
</tr>
<tr>
<td>250</td>
<td>757 kcal/d</td>
<td>703 g/d</td>
</tr>
<tr>
<td>270</td>
<td>821 kcal/d</td>
<td>805 g/d</td>
</tr>
</tbody>
</table>

1Values were derived by differentiation of equations relating gravid uterine and fetal contents of energy and CP to day of gestation.

energy to gestational age (Table 1) allowed prediction of gestational changes in rates of deposition of protein and energy in conceptus tissues (Table 2). Comparison of the slopes of these predicted relationships showed that the fetal contribution to protein and energy deposition in the gravid uterus was increasing during late pregnancy, which is not surprising, considering the previously discussed patterns of growth of fetal and nonfetal conceptus tissues. Thus, the fetus accounted for only 58 and 70% of uterine protein and energy accretion rates at 190 d of pregnancy, but these values had increased to 94 and 98%, respectively, at 270 d.

Predicted rates of uterine accretion of energy and protein in the Holstein cows of the present study were considerably higher at 190 d, but lower at 270 d, than rates predicted for Hereford heifers (12). Genetic differences in growth potential must account for at least part of the discrepancy at 190 d because, at this stage, Holstein fetuses were almost 40% heavier than their Hereford counterparts (12). The later reversal of this trend for energy might be explained partially by the greater fatness of Hereford fetuses at 260 to 270 d (12); however, a much more important consideration is the different ranges of gestational ages and, therefore, different mathematical models used to describe the primary data in the two studies. The exponential model used to describe the Hereford data (12) was influenced strongly by its need to encompass a greater span of gestation, and especially by the very small size of the conceptus at the earliest time of measurement (~134 d). This model satisfactorily described the entire data, but may have overestimated rates of nutrient accretion during late pregnancy when the pattern of conceptus growth approaches linearity. We think that the simpler quadratic model adequately describes gravid uterine accretion of energy and CP in Holstein cows during this phase; however, it must be stressed that the equations in Table 1 are for predictive purposes in late pregnancy (>190 d) only.

Not surprisingly, fetal accretion also accounted for most of the relatively meager rates of deposition of fat and ash during late pregnancy (Figure 4). When compared with the exponential relationship between fetal protein and gestational age, the linear relationship between fetal fat content and stage of gestation implies that the relative contribution of fat to fetal energy deposition actually decreased toward term. This relationship is consistent with that for sheep, in which the capacity for de novo fatty acid synthesis in fetal adipose tissue is markedly reduced after about 120 d of pregnancy (29), the equivalent of about 230 d in the cow. Also, Alexander (2) reported that, in well-fed pregnant ewes, fetal fat content was actually greater 5 to 6 wk before term than at term.

Energy and Protein Requirements for Conceptus Growth in Late Pregnancy

Few quantitative data are available on energy and protein requirements for gestation of dairy cows. For example, the NRC publication on nutrient requirements of dairy cattle (24) cited only two references dealing specifically with energy requirements, and one with
protein requirements, the most recent of which was published in 1972. Therefore, the major purpose of this study was to define the net requirements of energy and protein for conceptus growth in mature Holstein cows. These values, which were derived from estimated rates of accretion of energy and CP in the gravid uterus, can be converted to units of metabolizable energy and protein after application of appropriate factors for efficiency of tissue utilization.

Ferrell et al. (12) derived a mean estimate of about 14% for the efficiency of utilization of metabolizable energy for conceptus growth in beef heifers using a factorial method, multiple regression analysis, and indirect calorimetry. Their estimate agrees with a similar estimate for pregnant ewes (26). From Table 2, the mean net energy requirement for conceptus growth between d 240 and 270 of pregnancy was predicted to be 773 kcal/d. Division of this value by an efficiency factor of .14 yields an estimated metabolizable energy requirement of 5523 kcal/d, or 40 kcal/kg of BW per d. This value is identical to that obtained by less direct means from the data summarized by NRC (24), which adds considerably to confidence in the present recommendations.

The appropriate basis for calculating metabolizable or absorbed protein requirements for conceptus growth is less clear. In NRC (24), net requirements after 210 d of pregnancy are predicted from the data of Jakobsen (17) as 1.136 g/kg of BW75 per d. For cows in the present study, the NRC prediction (24) would yield an estimate of 99 g/d, which is reasonably similar to values that were directly obtained (Table 2). Other estimates, based on studies of pregnant beef heifers, vary considerably from approximately 55 g/d (25) to well in excess of 100 g/d (11), mainly because of apparent differences in patterns of conceptus growth during late pregnancy. To convert net protein to metabolizable (absorbed) protein units, NRC (23, 24) recommends using an efficiency factor of .50; however, the source of this factor is not specified (23). It is now well established that the sheep fetus catabolizes almost 50% of the AA it acquires from the placenta (4) and that there is a significant additional net metabolism of AA in the nonfetal tissues of the conceptus (19). Together, these observations suggest that, in sheep, the efficiency with which AA taken up by the pregnant uterus are used for conceptus protein accretion may be about .4. Recent analysis of the much smaller amount of similar data from beef cattle suggested that in pregnant cows, this factor may be as low as .33 (5). If so, the estimated requirement of metabolizable protein for conceptus growth in the present study would be about 300 g/d.

Factorial estimation of the total absorbed protein requirement during late pregnancy requires addition of the requirement for conceptus growth to those for maintenance and metabolic fecal N, plus any additional pregnancy-related requirement, such as that for mamno-genesis and dry secretions during late pregnancy. Even with a generous estimated allowance for the latter, the sum of these additional predicted requirements is not more than 480 g/d. Thus, we predict that during the last 2 mo of pregnancy a mature, multiparous cow with a live weight of 714 kg should need at most 780 g/d of absorbed protein. This conservatively translates to a CP requirement of about 1100 g/d, assuming a ratio of .7 for absorbed protein to CP intake, which is about 300 g/d less than the tabulated prediction of NRC (24).

Given the uncertainty of several assumptions underlying this series of calculations and the field experience that feeding dry cows less protein than recommended by NRC (24) can lead to decreased production and metabolic problems during early lactation (6, 28), it would seem prudent to adhere to the present recommendations. Also, NRC (24) and other extant feeding systems for dairy cows assume that body protein reserves are static during the dry period. We have recently found that in ewes fed a diet adequate in energy and containing 12% CP in late pregnancy, whole body N balance was slightly positive, but carcass tissue N balance was substantially negative. When similar ewes were fed a diet containing 16% CP, much of the additional dietary N was retained and carcass tissue N balance became substantially positive (21). This result suggests that mature, pregnant ruminants have a greater capacity for protein storage in muscle and other carcass tissues than hitherto recognized. These tissues are presumably the site of much of the labile protein reserve, which must be an extremely important source of AA for sustaining milk protein synthesis and hepatic gluconeogenesis during the severe N deficit suffered by most cows during the 1st or 2nd wk after calving (5).
CONCLUSIONS

The present paper provides a foundation for accurately defining the organic nutrient requirements for conceptus growth in Holstein cows during late pregnancy. Because our multiparous cows were well fed and weights of dam and conceptus were somewhat heavier than those of many Holsteins, we think that recommendations based on our data are unlikely to underestimate the needs of modern Holstein cows. It is reassuring to find that predicted values for requirements of metabolizable energy and total CP, derived from our measured values for net energy and protein requirements, are reasonably close to those predicted by NRC (23). In addition to improving the accuracy of predicted relationships between ingested and absorbed nutrients, especially AA, future work should seek to define the efficiency with which absorbed AA are deposited in conceptus tissues. Such studies can confidently build on the data presented in this paper.

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References