EFFECTS OF A PARENTERAL SUPPLEMENT OF FOLIC ACID AND ITS INTERACTION WITH LEVEL OF FEED INTAKE ON HEPATIC TISSUES AND GROWTH PERFORMANCE OF YOUNG DAIRY HEIFERS

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ABSTRACT

Forty-seven dairy heifers of approximately 10 d of age were assigned to a factorial experiment in which a supplement of folic acid (0 or 40 mg) administered weekly by i.m. injection and level of feed intake were the two factors studied. The heifers were weaned after 5 wk of experimentation. Following weaning, and until the end of the experiment, 11 wk later, they had ad libitum access to grass hay and concentrates at two different levels, ad libitum or restricted, to allow a body weight gain of 700 g/d. A supplement of folic acid (P < .05) and ad libitum access to feed (P < .05) increased the mean concentration of serum folates. Blood hemoglobin and packed cell volume were not affected by the level of feed intake. However, they were both increased (P < .05) by the supplement of folic acid.

Average daily gain was analyzed over three different periods: 0 to 5 wk (before weaning), 5 to 10 wk, and 10 to 16 wk. Average daily gain was increased by the supplement of folic acid during the second period (P < .05) and by ad libitum access to feed during the last two periods (P < .05). Ad libitum access to feed increased (P < .05) weight of the liver, decreased the (P < .05) concentrations of RNA and DNA, and increased (P < .05) the ratios of protein/DNA and RNA/DNA. The supplement of folic acid decreased (P < .05) weight of the liver and increased the ratio RNA/DNA (P < .05). These effects of supplement of folic acid on growth performance and on hematological cells may reflect a lack of folic acid during the weeks after weaning.

Key Words: Folic Acid Supplements, Dairy Cows, Growth


Introduction

Folic acid is necessary for the synthesis of RNA, DNA (Herbert and Das, 1976), and protein (Chang and Kaiser, 1972). Therefore, it is essential for all tissues with a high rate of cellular division and growth. The ruminant is generally considered independent of an exogenous supply of folates because their synthesis by ruminal microorganisms makes their inclusion in the diet unnecessary (ARC, 1980; NRC, 1988). However, some doubts persist about the efficiency of vitamin synthesis by rumen microflora during weaning and later during the stabilization of rumen function (McDowell, 1989). Fluctuations in the concentration of serum folates observed during the first months of life of young ruminants may be an indication that synthesis of folates by ruminal microflora is not sufficient to meet requirements during weaning (Girard et al., 1989a).
The present experiment was undertaken to determine the effects of a supplement of folic acid given by i.m. injections and its interaction with level of feed intake on the evolution of serum folates and growth performances as well as hepatic folates of young dairy heifers during their first 4 mo of life.

Materials and Methods

Animals. Forty-eight black and white heifers of approximately 10 d of age were randomly distributed by weight in 12 blocks of four heifers each. Within each block, the following factorial treatments were used: supplement of folic acid (0 or 40 mg) and level of feed intake (ad libitum or restricted). A level of 40 mg of folic acid was used according to Girard et al. (1989a). Before the transport to the farm, the heifers received vaccines against viral diarrhoea and parainfluenza and one injection of antibiotic. At their arrival, 600 mg of vitamin A (retinol), 3.75 mg of vitamin D, 146 mg of vitamin E (d-alpha tocopheryl acetate), and 3 mg of Se were administered intramuscularly. Ten millimeters of Amigex, a solution containing amino acids, electrolytes, dextrose, and B-complex vitamins except folic acid was given subcutaneously. The heifers received also one i.m. injection of 5 ml of a solution of dextran iron (100 mg/ml) at their arrival and another 7 d later. The heifers were housed together under 14 h of light twice a day during the first 2 wk of the experiment and once a day for the subsequent 2 wk. Thereafter and until the end of the experiment, these observations were made only if an unusual behavior was observed. Antibiotic treatments were given when necessary, under veterinarian supervision. Data from one heifer with ad libitum intake and injected with saline were eliminated from the statistical analysis because bovine viral diarrhoea was diagnosed.

Diet. Before weaning, the heifers received whole milk (54.01 ng/ml folates) collected during milking of the dairy herd, twice a day at 0715 and 1615. Milk intake was fixed at 10% BW per day until the 3rd wk of the experiment and thereafter decreased gradually (.5 kg/d) until weaning, when the animals were able to eat 1 kg of concentrates per day. They had free access to concentrates (Table 1) and long grass hay containing, on a DM basis, 8.73% CP, 18.99 kJ/g GE, 33.13% ADF, 65.32% NDF, and .78 mg folates/kg. The heifers were weaned after 5 wk of experimentation and given ad libitum access to grass and concentrates at two different levels, ad libitum or restricted, to allow an ADG of 700 g. These two levels of feeding were chosen according to suggestions of Petitclerc et al. (1984). Milk was analyzed for DM (method #16.032, AOAC, 1975), N (method #16.036, AOAC, 1975), and fat (method #16.055, AOAC, 1975). Grass hay and concentrates were analyzed for DM (method #7.003, AOAC, 1975), and fat (method #9.001, AOAC, 1975).

### TABLE 1. COMPOSITION OF CONCENTRATES

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<tr>
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Composition, DM basis

Calculated composition

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Chemical analysis

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*Supplied per kilogram of total diet; 198 mg vitamin A; .08 mg vitamin D3; 65.2 mg vitamin E; 195.8 mg Zn; 130.6 mg Mn; 65.85 mg Cu; 1.3 mg I; .65 mg Co; .33 mg Se; 41 mg antioxidants.

"Amigex", Syntex (Canada), Mississauga, ON.
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1975), N (method #7.016, AOAC, 1975), Ca, P, and Fe (method #7.077, AOAC, 1975) according to standard procedures. Energy was determined with an adiabatic bomb calorimeter; ADF and NDF were determined by Van Soest's techniques (Dorleans, 1985).

Folic Acid Administration. During the 16 wk of experimentation, one-half of the heifers received saline weekly, at 1100, one i.m. injection of a solution containing a minimum of 20 mg folic acid/ml (24.28 ± 2.14 mg/ml) was administered to the other half of the animals according to the same schedule.

Feed Intake and Growth Performance. Feed intake and BW were measured weekly. At the end of the experiment, withers height was measured and heifers were slaughtered. Length and weight of carcasses were measured. Livers were removed and weighed.

Blood Sampling. Blood samples were taken by jugular venipuncture between 1000 and 1100, before the weekly injection of saline or folic acid. Blood hemoglobin and packed cell volume were measured in duplicate on freshly drawn blood every 4 wk. Hemoglobin was determined according to the method of Drabkin (Manet, 1969). Serum folates were measured every 2 wk. The blood was allowed to clot at 4°C in darkness for approximately 2 h. Serum was separated by centrifugation at 1,854 × g for 15 min, transferred into polypropylene tubes, and stored at -20°C until it was assayed.

Determination of Serum and Milk Folates. Serum folates were measured in duplicate by radioassay as described by Girard et al. (1989a). Preparation of milk samples and determination of folates in milk were made as described by Girard et al. (1989b).

Determination of Folic Acid in Concentrates and Hay. Samples were prepared before the assay following a method adapted from Cerna and Kas (1983). A sample of 1 g, ground at 1 mm, was homogenized in a grinder tube with 12 ml of McIlvain buffer (for 100 ml: .2 M Na₂HPO₄, 50 mg ascorbic acid, add distilled water, adjust the pH to 4.6 with 3.3 N NaOH, and complete with distilled water up to 100 ml). The homogenate was transferred to a conical tube and placed in autoclave for 10 min at 121°C. The pH was adjusted to 7.0 with 3.3 N NaOH and the volume completed to 20 ml with distilled water. The solution was vortexed and centrifuged at 1,854 × g for 10 min. Supernatants were diluted 1:2 with bovine albumin (7%) and folates were measured in duplicate by radioassay. The effect of chicken pancreas conjugase on concentration of folates was tested using the method described by Cerna and Kas (1983). No effect of conjugase was noted on concentrations of dietary folates, and, subsequently, all assays were run without pretreatment with conjugase. Parallelism was good between 1 and 5 ng/ml (CV = 14.6%) and recovery tests were 94.2% for concentrates and 87.9% for grass hay. The mean coefficients of variation between duplicates made in two different assays were 9.1 and 2.74% for concentrates and grass hay, respectively.

Determination of Folates, DNA, RNA, and Protein in Liver. Folates in liver were analyzed according to a method adapted from Zemp et al. (1976). A sample of 1 g of liver was homogenized, in a grinder tube, at 4°C in 4 ml of .5 mM citric acid (monohydrate) and 1 mM disodium phosphate buffer, with a pH of 5.5. A 3-ml aliquot of homogenate was added to 4 ml of ascorbic acid solution (10 mg/ml, pH 6.0) freshly prepared in a 15-ml polycarbonate tube for centrifugation, vortexed, and covered with aluminium foil. The samples were heated in a 75°C water bath for 30 min and centrifuged for 10 min at 51,520 × g. The supernatants were diluted 1:100 with a solution of ascorbic acid (10 mg/ml, pH 6.0) and folates were measured in duplicate by radioassay. The effect of chicken pancreas conjugase on concentrations of folates was tested using the method described by Zemp et al. (1976). There was no effect of conjugase on concentrations of liver folates, and, subsequently, all assays were run without pretreatment with conjugase. Concentrations of folates were not affected by the site of sampling in liver. Parallelism was good between 1 and 20 ng/ml (CV = 5%) and recovery tests were 105.9%. The mean coefficient of variation between duplicates made in two different assays was 3.87%.

Liver content of protein, RNA, and DNA was analyzed according to methods adapted from Mainz et al. (1973) by Morisset (1980, 1984). Samples of 1 g of each lobe of liver...
were pooled in one analysis. Protein, RNA, and DNA were expressed as milligrams per gram of wet liver.

**Carcass Composition.** Because the effect of folic acid on ADG was observed mainly in heifers given ad libitum access to feed, carcass composition was studied only in this group. After slaughter, carcasses were chilled for approximately 24 h at 4°C. The 9-10-11th rib section of heifers with ad libitum intake was cut from the right side of the chilled carcass according to the method of Hankins and Howe (1946), boned, ground, and stored at -20°C for subsequent analysis. Fat and protein contents were determined on dry samples with a Goldfisch-ether extraction (method #7.044, AOAC, 1975) and a micro-Kjeldahl apparatus (method #7.016, AOAC, 1975).

**Statistical Analysis.** Values were analyzed as a completely randomized block design using the GLM procedure of SAS (1985). The following model was used for variables whose evolution in time was not studied: $Y_{ijkl} = \mu + B_i + D_j + F_k + DF_{jk} + T_l + DT_{jl} + FF_{kl} + DF_{kl} + e_{ijkl}$, where $Y$ indicated the dependent variable (serum folates, hemoglobin, or packed cell volume). The overall mean was $\mu$, $B_i$ was the block effect, $D_j$ was the effect of dose of folic acid injected, $F_k$ was the effect of level of feed intake, and $T_l$ was the effect of age. The two error terms were $DF_{ijk}$ used to test main effects, and $e_{ijkl}$, the residual error, decomposed according to the method of Rowell and Walters (1976). The effects of doses, feed intake, age, and their interactions on concentrations of serum folates, hemoglobin, and packed cell volume were split up into linear, quadratic, and other effects by orthogonal contrasts when appropriate (Snedecor and Cochran, 1971). The analysis of repeated measurements were made according to procedures described by Rowell and Walters (1976).

The following model was used for variables whose evolution in time was not studied: $Y_{ijkl} = \mu + B_i + D_j + F_k + DF_{jk} + e_{ijk}$, where $Y$ indicated the following dependent variables: ADG, feed intake, feed conversion, liver folates, weight of liver, protein, DNA and RNA in liver, bone, fat, protein, DM, ash in meat of rib section, withers height or length, and weight of carcass. The overall mean was $\mu$, $B_i$ was the block effect, $D_j$ was the effect of dose of folic acid injected, $F_k$ was the effect of level of feed intake, and $e_{ijk}$ was the error term. Means were compared by orthogonal contrasts when appropriate. Average daily gain was analyzed separately for the following three periods: 0 to 5 wk (before weaning), 5 to 10 wk (immediately after weaning), and 10 to 16 wk. Feed intake and feed conversion were analyzed only for the last two periods. The weight of liver was analyzed with body weight at slaughter as a covariable.

Unless indicated in the test, at a level of significance superior to 5%, effects of treatments were not considered different.

**Results and Discussion**

The supplement of folic acid increased the mean concentration of serum folates from 17.30 ng/ml to 22.58 ± 0.60 ng/ml in unsupplemented animals ($P < .01$). Girard et al. (1989a) observed previously that the concentration of serum folates increased during the first 4 mo of life of dairy heifers. Moreover, one i.m. injection of 20 mg of folic acid administered to animals aged 18.2 d increased by 23% the concentration of serum folates, which agrees with the results of the present experiment; no marked effect of the injection was, however, noted in heifers aged 4 mo (Girard et al. 1989a). The pattern of evolution of serum folates (Figure 1) was different for unsupplemented and supplemented animals (interaction folic acid × cubic effect of time, $P < .01$). As shown in Figure 1, serum folates increased dramatically during the first 4 to 6 wk of the experiment in heifers supplemented or not supplemented with folic acid. The development of ruminal function after weaning could contribute, at least partly, to an augmentation of serum folates. The low concentration of folates observed during the first weeks of life in calves could be due to an increase of the metabolic usage of this vitamin. Such a phenomenon was reported in newborn infants (Shojania and Homady, 1970). Although a rise in serum folates was measured in both treatments, the effect was more pronounced after a supplement of folic acid. The level of feed intake also affected the concentrations of serum folates during the experiment (interaction of level of feed intake × linear effect of time, $P < .01$); feeding for ad libitum intake compared with restricted feeding increased ($P < .05$) the mean concentration of serum folates from 22.70 ng/ml to 25.73 ± .60 ng/ml. This result might be due to the influence of the diet on availability of folates; however, the information on such an effect is
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ambiguous. Indeed, Kon and Porter (1954) reported that the concentration of folates in the rumen is not affected by the diet because ruminal synthesis of folates decreases with diets high in folic acid. Dong and Oace (1975), however, reported that high concentrations of folates were measured in milk from herds grazing on green pastures and that low concentrations were found in milk from herds fed dry feeds. A study on absorption of a supplement of folic acid given in concentrates to ruminant heifers (149.5 ± 11.4 kg) demonstrated that a supplement of .5 mg of folic acid per kilogram of BW did not increase the concentration of serum folates, whereas a dose of 1.0 mg/kg BW increased it (Girard et al., 1990). Therefore, in the present experiment the difference in quantities of folates ingested by heifers, 1.60 ± .04 mg/d for animals fed for ad libitum access compared with 1.19 ± .02 mg/d for animals with restricted feed intake, was probably not sufficient to explain the effect on serum folates. The rise in serum folates of heifers fed for ad libitum intake (high level of concentrates) during the last month of the experiment could probably be explained by the development of a ruminal microflora able to synthesize more folates than that of animals with restricted feed intake. This fact would be in accordance with the work of Allison (1965) and Blanchart et al. (1989), who suggested that bacterial digestion of fiber requires folic acid.

The supplement of folic acid increased blood hemoglobin (P < .01) and packed cell volume (P < .01) from 11.67 g/liter and .347, respectively, for unsupplemented heifers to 12.22 ± .12 g/liter and .363 ± .004 for heifers injected with folic acid. The level of feed intake modified the evolution of blood hemoglobin and packed cell volume during the experiment (interaction level of feed intake x quartic effect of time, P < .01, Figure 2). The general rise of hemoglobin and packed cell volume observed during the first month was partly attributable to the 2 i.m. injections of iron administered to the calves at the beginning of the experiment. The administration of a supplement of folic acid also increased hemoglobin and packed cell volume. It is known that folic acid is essential for synthesis of red blood cells in different species (Stokstad, 1968). The effect of the supplement of folic acid on hemoglobin and packed cell volume observed in the present experiment suggests that the supply in folates by the diet and the rumen microorganisms might not be optimum to maximize the hematological status of calves near weaning. Moreover, during the last month of the experiment, hemoglobin and packed cell volume were higher for the heifers with ad libitum access to feed than for those with restricted feed intake; this difference coincided with an augmentation of the concentration of serum folates, suggesting again a relationship between the supply of folates and these hematological variables.

Body weight ± SE of heifers (43.68 ± .46 kg) at the beginning of the experiment was similar between treatments. There was no effect of treatments on ADG before weaning (mean = 442 ± 19.2 g/d, Figure 3). From the 5th to the 10th wk, ADG was higher in animals with ad libitum access to feed (1,080 g/d) than in those with restricted feed intake (668 ± 22 g/d) (P < .01); the supplement of folic acid increased ADG by 7.6% (P < .05) (Figure 3). From the 10th to 16th wk, level of

Figure 1. Concentration of serum folates of heifers with ad libitum access to feed (●,■) or restricted to an ADG of 700 g (○,□) and weekly injected with 0 (●,○) or 40 (■,□) mg of folic acid during the 16 wk of experimentation (bars are SE).
feed intake affected ADG; 1,273 g/d and 720.5 ± 24.5 g/d, respectively, for heifers with ad libitum intake and restricted animals (P < .01). The supplement of folic acid had no effect on ADG during this period (Figure 3). Whatever the period studied, there was no interaction between level of feed intake and supplement of folic acid on growth performance.

The supplement of folic acid affected ADG during the 5 wk after weaning. After this period, the animals receiving the supplement did not increase their advantage over the

![Figure 2. Evolution of blood hemoglobin (●, ○) and packed cell volume (■, □) of heifers with ad libitum access to feed (●, ■) or restricted to an ADG of 700 g (○, □) during the 16 wk of experimentation (vertical bars represent SE).](image)

![Figure 3. Average daily gain of heifers receiving an intramuscular injection of 0 (●) or 40 (□) mg of folic acid every week during the 1st to 5th, 5th to 10th and 10th to 16th wk of experimentation (bars represent SE).](image)
TABLE 2. INTAKE OF CONCENTRATES AND GRASS HAY (EXPRESSED AS PERCENTAGE OF BODY WEIGHT) AND FEED CONVERSION OF HEIFERS DURING THE 5TH TO 10TH AND 10TH TO 16TH WEEKS OF EXPERIMENTATION

<table>
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<th>10 to 16 wk</th>
<th>Feed conversion*</th>
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*Expressed as kilograms of feed per kilogram of gain.

unsupplemented animals. Establishment and stabilization of the ruminal function some weeks after weaning through an increase of the quantity of folates synthesized by ruminal microflora may have provided sufficient amounts of folates during the last weeks of the experiment to attenuate the effects of the supplement on ADG from 10 to 16 wk of age.

From the 1st to the 5th wk, milk intake was not different among treatments. After weaning, feed intake was analyzed as a ratio, kilograms of DM ingested per kilogram of BW (DMI/BW), for two different periods: 5th to 10th and 10th to 16th wk (Table 2). Due to the nature of the treatments, for the two periods studied, intake of concentrates was higher (P < .01) for heifers with ad libitum access to feed than for those with restricted intake (2.99 vs 1.73 ± .04). However, the intake of grass hay was higher (P < .01) when intake of concentrates was restricted (.89 vs .23 ± .03). In an attempt to maintain body weight gain of heifers at 700 g/d, access to concentrates was restricted while hay was offered for ad libitum intake. Consequently, heifers in this treatment consumed less concentrates and more hay than heifers with ad libitum access to feed during the two periods after weaning. From the 5th to the 10th wk, in heifers with ad libitum access to feed, those supplemented with 40 mg of folic acid consumed more concentrates than their corresponding controls. This augmentation of feed intake was related to an increase of ADG. However, when animals were restricted to a body weight gain of 700 g/d, heifers supplemented with folic acid ingested a smaller quantity of concentrates than their controls (interaction of folic acid x level of feed intake, P < .05). The opposite tendency in the group of heifers supplemented with folic acid and submitted to feed restriction is likely to be a consequence of the nature of treatments. Indeed, because heifers in this group tended to have an ADG superior to 700 g, their access to concentrates, adjusted every week, was more limited than in the unsupplemented group. From the 10th to the 16th wk, there was no interaction between the level of intake and the supplement of folic acid for the ingestion of concentrates; however, the supplement of folic acid increased the quantity of grass hay ingested by heifers with restricted intake (interaction folic acid x level of intake, P < .05). Heifers restricted to an ADG of 700 g and supplemented with folic acid increased their ingestion of grass hay during this period, maybe as a rebound effect to the severe restriction imposed on the quantity of concentrates available.

During the two postweaning periods (5 to 10 wk and 10 to 16 wk), feed conversion was improved by ad libitum (P < .01) compared with restricted intake. There was no effect (P > .05) on feed conversion of the supplement of folic acid or of the interaction of folic acid x level of intake (Table 2). Feed conversion of animals with ad libitum access to feed was better than that of heifers restricted to an ADG of 700 g, probably because the proportion of concentrates in the diet of animals with ad
restricted feed intake. The same effect of the heifers with restricted feed intake were heavier than those of reported a marked increased in DM digestibility. The livers of heifers with ad libitum access were heavier at slaughter than animals restricted to a level of hepatic folates is a function of the feeding; however, there was no effect on concentration of hepatic folates. The supplement of folic acid increased the content of folates in liver of heifers, which agrees with results observed in rats (Steinberg et al., 1978; Fuller et al., 1988) and lactating (Fuller et al., 1988) rats reported variable effects of a supplement of folic acid on liver weights. The storage of folates in liver increased by 38.32% with the supplement of folic acid (Table 3). However, the level of feed intake and its interaction with the supplement of folic acid did not affect the concentration of hepatic folates. The supplement of folic acid increased the content of folates in liver of heifers, which agrees with results observed in rats (Steinberg et al., 1979) and in mice (Schreiber et al., 1973). According to Lakshmaiah (1987), the level of hepatic folates is a function of the intake of folic acid.

Concentrations of DNA and RNA decreased by 14.74% and 8.48%, respectively, with ad libitum access to feed compared with restricted feeding; however, there was no effect on concentration of protein (Table 3). Total amounts of protein, RNA, and DNA of the liver were lower in heifers with restricted feed intake ($P < 0.01$). Ad libitum access to feed increased ($P < 0.01$) the ratios of protein/DNA and RNA/DNA; the supplement of folic acid increased ($P < 0.05$) only the ratio of RNA/DNA in liver. In the present experiment, the overall ratio of protein/DNA in livers of

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<td>1.77</td>
<td>51.05</td>
</tr>
<tr>
<td>Restricted</td>
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<tr>
<td>0</td>
<td>2.27</td>
<td>96.02</td>
<td>3.46</td>
<td>2.21</td>
<td>43.87</td>
<td>1.58</td>
<td>32.23</td>
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<td>40</td>
<td>2.14</td>
<td>99.49</td>
<td>3.70</td>
<td>2.24</td>
<td>44.47</td>
<td>1.66</td>
<td>55.07</td>
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<tr>
<td>Probabilities</td>
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<tr>
<td>Feeding regimen</td>
<td>0.0001</td>
<td>0.11</td>
<td>0.0003</td>
<td>0.0001</td>
<td>0.0016</td>
<td>0.55</td>
<td></td>
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<tr>
<td>Injection</td>
<td>0.0093</td>
<td>0.13</td>
<td>0.12</td>
<td>0.75</td>
<td>0.31</td>
<td>0.057</td>
<td>0.0001</td>
</tr>
<tr>
<td>Feeding × injection</td>
<td>0.56</td>
<td>0.28</td>
<td>0.11</td>
<td>0.32</td>
<td>0.62</td>
<td>0.81</td>
<td>0.41</td>
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<tr>
<td>SE</td>
<td>0.086</td>
<td>1.285</td>
<td>0.072</td>
<td>0.055</td>
<td>1.144</td>
<td>0.034</td>
<td>2.758</td>
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</table>

*All variables were expressed on a wet basis.

Corrected means, following a covariance analysis for body weight.
heifers was similar to the values reported by Munro and Downie (1964) for cows. However, the ratio of RNA/DNA was higher in heifers (1.67) than in cows (1.29), whereas the concentrations of protein (97 vs 190 mg/g), RNA (3.43 vs 5.3 mg/g), and DNA (2.1 vs 4.1 mg/g) were lower. Ferrell (1988) observed that a restriction of feed intake increases concentrations of DNA in livers of growing lambs and decreases hepatocyte size. In the present experiment, concentrations of DNA and RNA were also increased by a reduction of feed intake, whereas the ratios of protein/DNA and RNA/DNA, indicators of cell hypertrophy, were decreased. According to Munro (1970), a reduction of the ratio of RNA/DNA is also related to a decrease of the number of ribosomes and, consequently, to an alteration of protein synthesis. Moreover, RNA and protein contents of liver change in parallel (Munro, 1968). Similarly, in the present experiment, total amounts of protein, DNA, and RNA in the liver decreased in animals with restricted feed intake. Folic acid is essential for cell multiplication because it is involved in purine and pyrimidine synthesis for DNA and RNA formation (Herbert and Das, 1976) and in protein synthesis (Chang and Kaiser, 1972). In the present experiment, the supplement of folic acid increased the ratio of RNA/DNA in liver of heifers with ad libitum access to feed. The low ratio of RNA/DNA observed in heifers receiving no supplement of folic acid could also be related to a decrease of the number of ribosomes per cell.

The effects of intramuscular injections of folic acid on growth performance, hemoglobin, and packed cell volume as well as metabolism of cellular division in liver might reflect a lack of folic acid at weaning and during the weeks following weaning. These results are in accordance with those of Zinn et al. (1987), who observed that folic acid supply in steer calves was less than 10% of the requirements estimated, on a body weight basis, from the needs of growing pigs. The increase of serum folates, packed cell volume, and hemoglobin observed in heifers with ad libitum access to feed compared with animals restricted to a body weight gain of 700 g/d might indicate that the nature and the quantity of feed ingested affected the folates supply of young ruminants, probably through an alteration of the rumen microflora.

**Implications**

During the 5 wk after weaning, approximately 7 to 12 wk of age, a supplement of folic acid administered by intramuscular injection increased the average daily gain of dairy heifers by 7.6%. This supplement also augmented serum and hepatic folates, as well as blood hemoglobin and packed cell volume. These observations seem to confirm that, during the weeks preceding and following weaning, the supply in folates by the diet and the rumen microorganisms might not be optimum for dairy heifers. The consequences of this suboptimal supply of folates during the first months of life on metabolism of dairy heifers remain to be investigated.

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