ABSTRACT Absorption at the level of the intestine is likely a primary regulatory mechanism for the deposition of dietary supplemented folic acid into the chicken egg. Therefore, factors affecting the intestinal transport of folic acid in the laying hen may influence the level of egg folate concentrations. To this end, a series of experiments using intestinal everted sacs were conducted to characterize intestinal folic acid absorption processes in laying hens. Effects of naturally occurring folate derivatives (5-methyl and 10-formyltetrahydrofolate) as well as heme on folic acid absorption were also investigated. Folic acid absorption was measured based on the rate of uptake of $^{3}$H-labeled folic acid in the everted sac from various segments of the small and large intestines. Folic acid concentration, incubation length, and pH condition were optimized before the performance of uptake experiments. The distribution profile of folic acid transport along the intestine was highest in the upper half of the small intestine. Maximum uptake rate (nmol $\cdot$ 100 g tissue$^{-1}$·min$^{-1}$) was observed in the duodenum (20.6 ± 1.9) and jejunum (22.3 ± 2.0) and decreased significantly in the ileum (15.3 ± 1.1) and cecum (9.3 ± 0.9). Transport increased proportionately ($P < 0.05$) between 0.0001 and 0.1 μM folic acid. Above 0.1 μM, the slope of the regression line was not significantly different from zero ($P < 0.137$). Folic acid uptake in the jejunum showed a maximum rate of transport at pH 6.0, but was lowest at pH 7.5. The presence of 5-methyl and 10-formyltetrahydrofolate as well as heme impeded folic acid uptake, reducing intestinal folic acid absorption when added at concentrations ranging from 0 to 100 μM. Overall, these data indicated the presence of a folic acid transport system in the entire intestine of the laying hen. Uptake of folic acid in the cecum raises the likelihood of absorption of bacterial-derived folate.

INTRODUCTION

The supplementation of folic acid (FA) in the laying hen diet leads to the production of folate-enriched eggs with an approximately 3-fold increase in egg folate concentrations relative to regular commercial eggs (House et al., 2002). However, increases in egg folate concentration do not go beyond an achieved maximum level (45 to 50 μg of folate/egg) when FA is further increased in the diet (above 4 mg of FA/kg of diet), demonstrating a pattern of saturation (Sherwood et al., 1993; House et al., 2002; Hebert et al., 2005). These observations led to the hypothesis that factors limiting the absorption of dietary FA, its biochemical conversion, and its eventual transfer into the blood circulation explain the observed saturation profile. In previous studies (House et al., 2002; Hebert et al., 2005), a pattern of parallel responses between blood and egg folate concentrations when FA was supplemented in the diet was established, indicating an efficient transport mechanism of absorbed FA in the blood and into the egg. It was also demonstrated that supplemental FA in the laying hen diet is equally effective as dietary 5-methyltetrahydrofolate (5-MTHF) in terms of enhancing egg folate concentrations (Tactacan et al., 2010), thereby dismissing any views of inadequate conversion of absorbed FA to 5-MTHF, the major form of folate in the egg. However, the role of the intestine as a potential control point in regulating egg folate concentration has yet to be determined.

It is generally accepted that the rate of absorption in the intestine primarily determines the availability of nutrients derived from the diet. With respect to folate absorption, its transportation in the intestine is believed to be mediated by a highly specific folate transporter, which transports folate optimally at an acidic pH condition (Steinberg, 1984; Sirotnak and Tolner,
FA uptake in everted intestinal sacs were also determined. naturally occurring folate derivatives and heme on FA developed by Inoue et al. (2008) in rats. The effects of an in vitro absorption model of everted intestinal sacs absorption of FA in the intestine of the laying hen using of experiments were conducted to characterize the ab-
underlying mechanisms by which it is absorbed may provide insights into how the intestine plays a part in the expression implies functional capability for absorption of dietary folate remains to be known. Because FA is the most commonly used supplemental dietary source of folate for egg fortification, an understanding of the underlying mechanisms by which it is absorbed may provide insights into how the intestine plays a part in regulating egg folate concentrations. Therefore, a series of experiments were conducted to characterize the absorption of FA in the intestine of the laying hen using an in vitro absorption model of everted intestinal sacs developed by Inoue et al. (2008) in rats. The effects of naturally occurring folate derivatives and heme on FA absorption were also determined.

MATERIALS AND METHODS

General

Shaver White laying hens (Manitoba Perfect Pullets, Winnipeg, Manitoba, Canada) confined under semicon-
trolled environmental conditions were used in 6 experi-
ments. A laying hen diet based on wheat and soybean meal (2900 kcal/kg, 19.0% CP, 4.0% calcium, 0.4% available phosphorus) and water were made available to provide ad libitum consumption. Because basal dietary ingredients provided sufficient folate to meet the needs of the laying hen, no additional FA was included in the diets. Representative birds at 40 to 50 wk of age were selected for intestinal tissue isolation. The laying hens weighed (means ± SD) 1,498 ± 128.0 g, 1,545 ± 151.5 g, 1,706 ± 132.9 g, 1,655 ± 121.9 g, 1,602 ± 171.7 g, and 1,640 ± 132.1 g in experiments 1, 2, 3, 4, 5, and 6, respectively.

FA Uptake in Everted Intestinal Sac

Folic acid uptake experiments were conducted using everted sacs from the small and large intestine of lay-
ing hens following the procedure of Inoue et al. (2008) with slight modifications. The experiments were con-
ducted with the approval of the University of Mani-
toba's Animal Care Protocol Review Committee and in accordance with the Canadian Council on Animal Care (1984). In brief, hens were fasted overnight and were killed by cervical dislocation. From their abdomi-
nal cavity, duodenal, jejunal, ileal, and cecal segments were immediately isolated and rinsed free of digestive contents using oxygenated physiological saline. Intesti-
nal segments of approximately 4 cm in length from each region were cut and everted by a glass rod and infused with previously oxygenated 1 mL of Krebs-Ringer-bicarbonate buffer (118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 25 mM NaHCO3) and added with 20 mM HEPES (pH 6.0), 10 mM glucose, and 15 mM sodium ascorbate using a tuberculin sy-
ringe. Each length of the everted segment was tied off on both ends by a thread ligature to form the intestinal sac. Sacs were transferred to a 50-mL conical flask and incubated in 20 mL of Krebs-Ringer-bicarbonate buffer solution (mucosal medium) at 41°C and a shaking rate of 100 strokes/min. Throughout the incubation period, the intestinal sac was continuously oxygenated with 95% O2-5% CO2 gas. Trace amounts of 100,000 cpm/50 μL of [3H]FA (37 MBq/mL; Moravek Biochemicals Inc., Brea, CA), 20,000 cpm/50 μL of [1,2-14C]polyethylene glycol 4000 (1,850 MBq/mL; American Radiolabeled Chemicals, St. Louis, MO) as a nonabsorbable marker, and also unlabeled FA (to adjust FA concentration; Shircks Laboratories, Jona, Switzerland) were added to the mucosal medium. An intestinal sac was incubated without adding labeled FA in the mucosal medium for each measurement to deduct background FA uptake. At the end of incubation, sacs were removed from the conical flask and briefly washed with ice-cold saline to stop uptake. Sac content (serosal fluid) was drained into individual scintillation vials and counted for radioactivity. Uptake in the mucosal tissue of the sac was evaluated by determining the radioactivity after solubilization of the tissue sample using 2 mL of Solu-
en-e-350 (Perkin-Elmer Inc., Waltham, MA) as a tissue solubilizer. Mucosal-to-serosal uptake of FA, which con-
stitutes the overall transport processes (apical transport, intracellular processing, basolateral transport) of FA across the intestinal wall, was calculated by adding uptake in the mucosal tissue and the serosal transfer (Smyth, 1974) and was expressed per 100 g of wet tissue weight of intestinal segment.

Experimental Approach

Experiment 1 was conducted to determine an optimal incubation time of everted gut sacs according to the change in FA uptake when time varied. The incubation times of the 5 treatments were 1, 2, 5, 10, and 20 min, respectively. A FA concentration of 0.1 μM and an acidic medium of pH 6.0 were used. The optimal incubation time (5 min) determined in experiment 1 was adopted and used in the succeeding experiments. For experiment 2, FA uptake at pH 6.0 was evaluated with increasing FA concentrations (0.0001, 0.001, 0.01, 0.1, 1, 10, and 100 μM). To investigate the effect of pH on FA uptake at 0.1 μM FA, pH conditions of 4.5, 5.0,
5.5, 6.0, 6.5, 7.0, and 7.5 were used as treatments in experiment 3. For treatments with pH 5.5 and below, 20 mM 2-(N-morpholino)ethanesulfonic acid was used, whereas 20 mM HEPES was used for treatments with pH 6.0 and above. Either 1 N HCl or 1 N NaOH was used to adjust pH levels for each treatment. Experiment 4 was conducted to investigate FA uptake in the different segments of the intestine. Duodenal, jejunal, ileal, and cecal sections of the intestine were used to measure uptake at 0.1 μM FA concentration, pH 6.0, and 5 min of incubation. Because the rate of FA uptake in experiment 3 was found to be at a maximum at an acidic pH of 6.0, a transport mechanism requiring a proton gradient might facilitate movement of FA across the intestinal cell membrane. Therefore, to determine the effect of eliminating the transmembrane proton gradient on intestinal transport of FA, the presence of carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP; Sigma-Aldrich, Oakville, Ontario, Canada), an ionophore that dissipates the H+ gradient across the cell membrane, was used in experiment 5. Folic acid uptake in the jejunal everted sac containing 0.1 μM FA was measured after incubation for 5 min at pH 6.0 in the presence of serial FCCP concentrations (0, 10, 20, 30, 40, and 50 μM). Last, experiment 6 was designed to determine the effect of 5-MTHF [6S-5-methyl-5,6,7,8-tetrahydrofolic acid (calcium salt), Shircks Laboratories], 10-formyltetrahydrofolate (10-FTHF; Shircks Laboratories), and heme (Sigma-Aldrich) on FA transport in the jejunum. Uptake of FA at 0.1 μM was measured in the jejunal everted sac after incubation for 5 min at pH 6.0 and in the absence and presence of varied concentrations of 5-MTHF, 10-FTHF, and heme (0, 0.01, 0.1, 1, 10, 100 μM).

The FCCP and heme were predissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) because both compounds are poorly soluble in aqueous solutions. The final concentration of DMSO in the mucosal medium was 1%. To account for the amount of DMSO added, controls for FCCP and heme were run in the presence of the same concentration of DMSO without added inhibitors.

The jejunal was used in all uptake studies except when FA uptake in the other segments of the intestine was evaluated (experiment 4). Each bird was used as a replicate of each treatment.

Cell Viability

Samples of the intestinal sac used in the FA uptake study were subjected to cell viability test using the trypan blue exclusion assay (Strober, 1997). The effects of incubation time, region of the intestine, and FCCP concentration on percentage of cell viability were assessed. In brief, preparations of intestinal everted sacs were scraped with a glass slide to harvest mucosal cells. Fifty microliters of harvested mucosal cells were suspended in 1 mL of Dulbecco’s Ca2+ and Mg2+-free PBS solution (Sigma-Aldrich) to obtain a cell suspension containing approximately 1 x 10^6 cells/mL. A 15-μL aliquot of the cell suspension was mixed with an equivalent volume of 0.4% trypan blue (Sigma-Aldrich) and loaded in a Bright-Line hemocytometer (Sigma-Aldrich) for cell counting via the aid of a fluorescence microscope (Olympus, Tokyo, Japan). Viable cells were determined by their ability to exclude trypan blue. When viewed under a microscope, viable cells had a clear cytoplasm, whereas nonviable cells possessed a blue cytoplasm.

Statistical Analysis

Following a completely randomized design, all transport studies were the results of multiple separate determinations using everted sac preparations isolated from laying hens on different occasions. Intestinal sections from the laying hens were cut into small parts (depending on the number of treatments for each experiment) and were assigned to each treatment in a random manner (experiment 1 = 5 parts, n = 8 hens, n = 8 replications for each measurement; experiment 2 = 7 parts, n = 10 hens, n = 10 replications for each measurement; experiment 3 = 7 parts, n = 7 hens, n = 7 replications for each measurement; experiment 4 = 5 parts, n = 6 hens for each intestinal section, n = 6 replications for each measurement; experiment 5 = 6 parts, n = 8 hens for each inhibitor, n = 8 replications for each measurement; experiment 6 = 6 parts, n = 8 hens, n = 8 replications for each measurement). Using SAS software (SAS Institute, 1998), data from experiments 1, 2, 3, 5, and 6 were analyzed using regression analysis, whereas data from experiment 4 were subjected to ANOVA using the GLM procedure of SAS. Differences between means for experiment 4 were determined using Tukey’s honestly significance difference test. Data from experiments 1, 2, and 6 were log_{10} transformed before analysis. Data were expressed as means ± SEM in nanomoles per 100 g of wet tissue weight per minute. Differences with an α-level of P < 0.05 were considered statistically significant.

The apparent kinetic constants for FA transport (K_m, V_max) were determined by fitting initial transport rates to the Michaelis-Menten equation [v = (V_max[S])/(K_m + [S]), where v is the uptake rate, V_max is the maximum transport rate, [S] is the substrate concentration, and K_m is the Michaelis constant] using nonlinear regression analysis (Prism 4.0, Graphpad Software Inc., San Diego, CA).

RESULTS

The mucosal-to-serosal uptake of 0.1 μM FA as a function of time was examined at pH 6.0 in the jejunal everted sacs of the laying hens to determine the linear phase of uptake (initial uptake rate; Figure 1). Uptake of 0.1 μM FA increased linearly (P < 0.001) from 1 to 5 min and proceeded slowly after 5 min of incubation. Although mucosal-to-serosal uptake of FA appeared to proceed in a more linear fashion between 1 and 2 min.
than between 2 and 5 min, the 5-min incubation time was chosen as the optimal time of incubation to balance the need to have sufficient FA transport activity present in the jejunum to allow the measurement of inhibition by 5-MTHF, 10-FTHF, and heme on FA transport rates. Although mucosal-to-serosal uptake of FA was linear in the first 5 min of incubation, the overall change in tendencies for uptake of 0.1 μM FA with time was best fitted to a quadratic model \[Y \text{ (mucosal-to-serosal uptake)} = 0.6394 + 1.5183X \text{ (time)} - 0.6702X^2 \] (\(P < 0.001\), \(R^2 = 0.8452\)), showing a decreasing rate of mucosal-to-serosal uptake as incubation time is increased.

With respect to the influence of FA concentration, mucosal-to-serosal rates of FA uptake are given in Figure 2. The transport of FA in the jejunal mucosa increased (\(P < 0.05\)) in a linear proportion in relation to FA concentrations from 0.0001 to 0.1 μM. Above 0.1 μM, the slope of the regression line was not significantly different from zero (\(P = 0.137\)). On the basis of these results, the trace concentration of 0.1 μM FA was chosen as the test substrate concentration for all the performed uptake experiments, to standardize subsequent experiments. The change in tendencies for mucosal-to-serosal uptake over the full range of FA concentrations was best fit to a quadratic model \[Y \text{ (mucosal-to-serosal uptake)} = 1.3153 + 0.1252X \text{ (concentration)} - 0.0378X^2 \] (\(P < 0.001\), \(R^2 = 0.9258\)), demonstrating a nonlinear response when FA concentrations in the mucosal medium were increased. With the evidence of a significant quadratic relationship, the data were also fit to the Michaelis-Menten equation, to calculate estimates of transport kinetics. The calculated \(K_m\) constant was 0.03 ± 0.004 μM, and the maximum velocity (\(V_{max}\)) was 22.7 ± 0.9, nmol × 100 g of tissue−1 × min−1 (\(R^2 = 0.9824\)).

With respect to the effect of pH, the change in tendencies for mucosal-to-serosal uptake of FA at varying pH conditions was best fit to a quadratic model \[Y \text{ (mucosal-to-serosal uptake)} = -74.115 + 31.994X \text{ (pH)} - 2.7483X^2 \] (\(P < 0.002\), \(R^2 = 0.8198\); Figure 3). Therefore, by substituting the range of pH values to the best fitted model, we deduced that the mucosal-to-serosal uptake of 0.1 μM FA was maximum at pH 6.0. For this reason, succeeding transport assays were then carried out at this pH.

The distribution of FA transport along the small and large intestine, mucosal-to-serosal uptake of FA was increased in the upper half of the small intestine and decreased in the remaining lower half. Mucosal-to-serosal uptake of FA was highest in the jejunum, followed by the duodenum, and decreased (\(P < 0.001\)) toward the ileum and cecum (Figure 4). Mucosal-to-serosal uptake of FA was also increased in the ileum (\(P < 0.001\)) relative to the cecum.

The addition of increasing concentrations of FCCP in the mucosal medium decreased the mucosal-to-serosal uptake of 0.1 μM FA in the jejunal everted sac of the laying hen. The extent of inhibition on mucosal-to-serosal uptake of FA was greatly manifested at higher FCCP concentrations (30, 40, and 50 μM FCCP) and
best fit to a quadratic model: $Y (\text{mucosal-to-serosal uptake}) = -2.1454 + 24.659X (\text{FCCP concentration}) - 12.357X^2$ ($P < 0.05$, $R^2 = 0.6602$).

Similarly, the mucosal-to-serosal transport of 0.1 μM FA in the jejunal everted sac of the laying hen was linearly decreased by the presence of 5-MTHF ($P < 0.009$) and 10-FTHF ($P < 0.05$); however, the mucosal-to-serosal transport of 0.1 μM FA was not significantly affected by heme ($P < 0.189$; Figure 6). When inhibitors were added to the mucosal medium at 100 μM concentration, the percentage inhibition in the mucosal-to-serosal uptake of 0.1 μM FA was greatest with 5-MTHF, with a maximal decreased transport rate of 28% relative to control rates. The corresponding maximal decreased rates with 10-FTHF and heme were 22.2 and 16.5%, respectively.

The trypan blue exclusion test was conducted to determine the viability of the intestinal everted sac preparations used in the uptake experiments. Isolated everted sacs taken from the section of the jejunum did not show differences in the percentage of viable cells when incubated for time periods of 0, 5, 10, and 20 min. Percentage viability of the cells after incubations of 0, 5, 10, and 20 min were 70.4, 68.7, 67.5, and 67.7%, respectively. Percentage viability of the cells in the jejunum

**Figure 3.** The pH dependence of the uptakes of folic acid (FA) in the everted sacs of the jejunum of laying hens (experiment 3). Data represent means ± SEM (n = 7 hens, n = 7 replications for each measurement). The uptakes of 0.1 μM FA were evaluated at 41°C for 5 min. The change in mucosal-to-serosal uptake of FA at varying pH conditions was described by the quadratic equation: $Y (\text{mucosal-to-serosal uptake}) = -74.115 + 31.994X (\text{pH}) - 2.7483X^2$ ($P < 0.002$, $R^2 = 0.8198$).

**Figure 4.** Uptakes of folic acid (FA) by everted sacs from various regions of the small and large intestine of laying hens (experiment 4). Data represent means ± SEM (n = 6 hens, n = 6 replications for each measurement). The uptakes of 0.1 μM FA were evaluated at 41°C and pH 6.0 for 5 min. a,b,cData that do not share superscripts are significantly different ($P < 0.05$).

**Figure 5.** Effect of carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) on the uptake of folic acid (FA) in the everted sacs of the jejunum of laying hens (experiment 5). Data represent means ± SEM (n = 8 hens, n = 8 replications for each measurement). The uptakes of 0.1 μM FA were evaluated in the presence and absence of FCCP at 41°C and pH 6.0 for 5 min. Regression analysis of the data revealed a quadratic relationship between the mucosal-to-serosal uptake of 0.1 μM FA and FCCP concentration as described by the following equation: $Y (\text{mucosal-to-serosal uptake}) = -2.1454 + 24.659X (\text{FCCP concentration}) - 12.357X^2$ ($P < 0.05$, $R^2 = 0.6602$).
(69.8%) and ileum (71.0%) section of the intestine were similar but decreased \((P < 0.05)\) when compared with the duodenum (62.1%) and cecum (63.2%). Everted jejunal sacs incubated at 5 min in increasing concentrations of FCCP did not differ in the percentage of viable cells. Percentage viability of the cells at 0, 10, 20, 30, and 50 \(\mu M\) FCCP were 70.1, 68.5, 70.3, 71.0, 68.5, and 67.4%, respectively.

**DISCUSSION**

The characteristics of FA transport in the intestine of the laying hen were studied and the influence of naturally occurring folate derivatives (5-MTHF and 10-FTHF) and heme on FA absorption was further investigated. The absorption of FA in the intestine is believed to be one of the major factors affecting the transfer of dietary FA into the egg. It has long been determined that folate is absorbed in the intestine by a specific carrier-mediated transport system (Steinberg, 1984; Sirotnak and Tolher, 1999); however, the identity of the putative folate transporter had not been established. We have recently determined the presence and expression patterns of the reduced folate carrier and the proton-coupled folate transporter in the digestive tract of the laying hens (Jing et al., 2009, 2010); however, functional studies have yet to be conducted. In the present study, everted intestinal sacs were chosen as an in vitro model to characterize intestinal absorption of FA in laying hens because they offered the opportunity of investigating the initial stages of FA absorption under closely controlled conditions. Ball (2006) has noted that folate absorption studies involving whole animal models are often difficult to interpret because of problems associated with the enterohepatic circulation of folate via bile fluid and the complicating effect of changes in digesta transit time. The in vitro everted sac model system has been shown to be rapid and useful in predicting the trend of the absorptive response in intact animals (Hill et al., 1987).

As a function of FA concentrations, the rate of increase in mucosal-to-serosal uptake of FA in the jejunal section of the small intestine of the laying hen diminished at higher FA concentrations (>0.1 \(\mu M\)). This pattern is reminiscent of the response demonstrated by laying hens for egg folate concentration when dietary FA supplementation increased (House et al., 2002; Hebert et al., 2005). It was previously reported by Hebert et al. (2005) that increasing supplementation of FA at 2, 4, 8, 16, 32, 64, and 128 mg/kg of laying hen diet did not lead to a consistent increase in egg folate concentration. They noted that although the amount of folate in egg was enriched at lower supplementation rates (2 to 4 mg of FA/kg of the diet), higher FA supplementation did not lead to proportional folate enrichment; instead, it demonstrated features of saturation. Therefore, on the basis of these results, we surmise that the observed saturation in egg folate concentration after increased dietary supplementation of FA may be partly attributed to limitations in FA absorption in the intestine. At increased FA concentration in the mucosal medium, the maximum capacity of the intestinal folate transporter for FA uptake might have been achieved. This observation is in line with our earlier findings, which demonstrated that neither the metabolism of dietary-derived FA into its biologically active form of folate, the 5-MTHF (Tactacan et al., 2010), nor the capacity for transfer of circulating blood 5-MTHF into the egg (House et al., 2002; Hebert et al., 2005) are restricting the capacity of the laying hens for deposition of folate into the egg.

Folate transport activity with optimal function at acidic pH was first identified for intestinal folate absorption (Mason and Rosenberg, 1994). Optimal transport at an acidic condition is a distinct feature of intestinal folate transporter, which separates it from other cellular folate transporters (Said, 2004). In the present study, the mucosal-to-serosal uptake of FA as a function of pH appeared to involve a pH-dependent transport process, demonstrating maximum transport at the acidic condition of pH 6.0. The presence of FCCP in the mucosal medium, a dissipater of H\(^+\) gradients, confirmed this observation. With increasing concentration of FCCP in the intestinal mucosa, the mucosal-to-serosal uptake of FA was extensively reduced. Therefore, the transport of FA across the intestinal wall of the laying hen may involve a H\(^+\)-coupled transport process. These results are...
consistent with those observed in other species (Qiu et al., 2007; Inoue et al., 2008), and may provide evidence to suggest that proton-coupled folate transporter, which utilized a H⁺ gradient to transport folate, plays a role in the intestinal absorption of FA in the laying hen.

Proton-coupled folate transporter and reduced folate carrier are expressed along the entire intestinal tract of the laying hen (Jing et al., 2009, 2010) and rat (Inoue et al., 2008). In the present study, transport of FA was also observed in the duodenal, jejunal, ileal, and cecal section of the chicken intestine. The distribution profile for FA transport activity was highest in the jejunum and duodenum and decreased as it approached the ileum and cecum. Previous studies have also reported the existence of higher transport activity for FA in the upper half of the small intestine and lower transport activity for FA in the lower portion of the small intestine (Qiu et al., 2006; Inoue et al., 2008). Folate transport with optimal activity at the proximal portion of the small intestine is consistent with the acidic pH in the microenvironment of the absorptive surface of the upper small intestine (Said et al., 1987; McEwan et al., 1990; Ikuma et al., 1996), whereas the lower transport activity in the ileum and cecum is consistent with the alkaline condition in this region of the intestine (Engberg et al., 2002). The presence of transport activity of FA in the ceca, coupled with our previous demonstration of the presence of mRNA for both reduced folate carrier and proton-coupled folate transporter in the ceca (Jing et al., 2009, 2010) provide evidence that folate supplied by commensal microorganisms in the ceca may contribute to the folate status of the laying hen.

The role of endogenous folate supply in contributing to the folate status of the host has been proposed by others (Rong et al., 1991; Asrar and O’Connor, 2005). Gut microflora can synthesize considerable amounts of folate, and a significant portion of this folate exists in the lumen in the absorbable monoglutamate form (5-MTHF; Kim et al., 2004). The current demonstration of a measurable uptake of folate from cecal everted sacs has the potential to make significant contributions to our understanding of the extent to which nutrients derived from microbes contribute to daily requirements of the laying hen.

Beyond its low pH optimum, another distinguishing feature of intestinal folate transport is its demonstrated affinity not only for FA, but also for the natural folate derivatives (Zhao and Goldman, 2007). Because transport is nonspecific, the existence of different folate compounds in the lumen may result in potential interactions during cellular transport and absorption in the intestine. In the present study, the absorption of FA was impeded by the presence of 5-MTHF and 10-FTHF in the mucosal medium. The 5-MTHF was a more potent inhibitor of FA than the 10-FTHF. Another compound, heme, a dietary source of iron, was investigated because proton-coupled folate transporter was originally identified as a heme carrier protein (Shayeghi et al., 2005). However, the results of the uptake study provided evidence that folate transporters exhibited a very low affinity for heme, as compared with the 2 derivatives of folate. This result is physiologically relevant considering that these micronutrients may negatively influence the absorption of dietary FA and its eventual transfer to tissues, including the egg. Both 5-MTHF and 10-FTHF are the 2 most predominant folate derivatives found in plant-based feed ingredients (Bagley and Shane, 2005), whereas heme is found in significant amounts in diets containing animal-based ingredients, in particular meat and bone meal.

In summary, the mucosal-to-serosal uptake of FA in the jejunum everted sac of the laying hen has been characterized. The rate of increase in mucosal-to-serosal uptake of FA in the jejunum section of the small intestine of the laying hen diminished at higher FA concentrations. The transport of FA demonstrated maximum activity at acidic pH but was markedly reduced when H⁺ gradients were eliminated. This may indicate a H⁺-coupled transport process, a feature consistent with the functional characteristic of the proton-coupled folate transporter. Folic acid was absorbed in all regions of the intestine, showing greatest transport activity in the upper half and lowest transport activity in the lower half of the intestine. Although of lesser capacity, the absorption of FA in the ceca raises the possibility that bacterially derived folate may serve as an alternative source of exogenous folate for the chicken. The natural folate derivatives 5-MTHF and 10-FTHF, as well as the molecular compound heme, impeded FA absorption, thus potentially reducing its metabolic availability to tissues.

ACKNOWLEDGMENTS

This research was financially supported through a Collaborative Research and Development Grant from the Natural Sciences and Engineering Research Council of Canada, in collaboration with the Manitoba Egg Farmers (Winnipeg, Manitoba, Canada) and the Egg Farmers of Canada (Ottawa, Ontario, Canada). The help of the following members of the Department of Animal Science, University of Manitoba, is gratefully acknowledged: Gary Crow, Harry Muc, Jerry Levandski, Aurele Chartier, Jason Neufeld, and Naveen Gakhar; Bill Diehl-Jones from the Department of Nursing, University of Manitoba, is also acknowledged for his input in determining the cell viability of the everted sac preparations.

REFERENCES


dependent enzymes and indices of folate status
Zhao, R., and I. D. Goldman. 2007. The molecular identity and
carrier-mediated folate transporter—PCFT: Biological ramifications and impact on the activity of pemetrexed.
Cancer Metastasis Rev. 26:129–139.

Ball, G. F. M. 2006. Intestinal absorption and bioavailability of vita-