Under commercial industry practices and current standard feeding procedure, hatchlings are commonly held for 36 to 72 h from the time of actual hatch to placement (Decuypere et al., 2001). This is due to the wide “hatching window” in commercial hatcheries, in which hatchlings are not removed to hatchery treatments, such as sexing, vaccination, and transport, until the maximum number of eggs has hatched. Thus, most hatchlings are fasted for 48 h or more before they reach their first access to feed and water. Holding hatchlings without food and water for more than 24 h has long-lasting negative effects on both broilers and turkeys (Augustine, 1982; Tarvid, 1992; Noy and Sklan, 2001; Batal and Parsons, 2002; Juul-Madsen et al., 2004). When access to first feed is delayed, hatchlings become more susceptible to pathogens (Dibner et al., 2008), exhibit decreased BW (Pinchasov and Noy, 1993; Bigot et al., 2003), and have restricted development of critical tissues and organs, such as the intestine (Tarvid, 1992; Geyra et al., 2001b; Dibner et al., 2008), the immune system (Dibner et al., 2008), and the pectoral muscle (Halevy et al., 2000; Mozdziai et al., 2002a,b; Bigot et al., 2003; Halevy et al., 2003; Moore et al., 2005a,b).

Yet it is known that any disturbances occurring in the days immediately posthatch affect hatchling quality and subsequent performance (Christensen et al., 1999, 2003; Willemsen et al., 2010). This is explained by the physiological and metabolic processes and changes taking place in birds during the pre- and posthatch periods (Vieira and Moran, 1999a,b; Christensen et al., 2003;
The hatching chicks were monitored for time of hatch (overall hatchability was 92% in all groups). Each chick was weighed, sexed, and tagged. Male and female chicks (150 for each treatment group) were placed in cages (10 chicks/cage per treatment) that measured 150 × 48 × 48 cm (length, width, and height, respectively). All cages (15 cages per treatment group) were situated in hatching trays in the incubator according to the routine procedure.
in one computer-controlled environmental room that maintained a constant temperature with an accuracy of ±1.0°C, RH at ±2.5%, air velocity at ±0.25 m/s, and under continuous fluorescent illumination. For each treatment group, 11 cages were used for BW and ADG values (Tables 1 and 2). Birds (n = 8) were taken randomly from these cages on specific sampling days (day of hatch; 24 and 36 h posthatch; and d 3, 5, 14, and 35) for breast muscle weight and percentage, myoblast and myofiber analyses, and glycogen analysis (Figures 1, 2, 3, and 4). The 4 other cages (with an equal number of males and females in each cage) were not sampled and were used for FI and FCR data for each treatment group (Table 3). Water and feed were supplied ad libitum. The nutrient contents of the starter, grower, and finisher diets were calculated to meet the levels recommended by Cobb-Vantress (http://www.cobb-vantress.com/contactus/brochures/Cobb500_BPN_Supp_08_(EMEA).pdf). According to the different treatments, the EF groups (control-EF and IOF-EF) were provided first access to feed and water no later than 6 h posthatch, whereas the SP groups (control-SP and IOF-SP) had access to first feed only 36 h posthatch. Birds were fed a corn- and soybean meal-based diet (starter, d 0 to 10; grower, d 11 to 21; finisher, d 22 to 35, crumbles) from d 0 to 35 supplied for ad libitum consumption. Calculated nutrient content was based on ingredient composition data from the recommended broiler feed formulation for Cobb 500 (Brown Feed Mill, Hod HaSharon, Israel).

All birds were weighed at hatch, 24 and 36 h posthatch, and then on a weekly basis (7, 14, 21, 28, and 35 d), and their average BW and ADG were calculated. Feed consumption of chickens for each treatment group was determined weekly by cage (n = 4). Feed intake and FCR were determined from the data by period and cumulatively. The experimental procedures were carried out with the approval of the Animal Welfare Committee of the Faculty of Agriculture, Food and Environment of the Hebrew University of Jerusalem, and the Israeli Ethics Committee.

### Tissue Sampling

Samples from embryos and chicks, randomly selected, were taken following cervical dislocation.

#### Table 1. Body weight (g) of chickens from 4 treatments: standard first-feeding procedure (SP), that is, feeding the hatchlings 36 h after clearing the shell, without in ovo feeding (IOF; control-SP) and with IOF (IOF-SP), and early feeding procedure (EF), that is, feeding the hatchling not later than 6 h after clearing the shell, without IOF (control-EF) and with IOF (IOF-EF) from the day of hatch to d 35

<table>
<thead>
<tr>
<th>Age (d)</th>
<th>SP</th>
<th>EF</th>
<th>P-value</th>
<th>Feeding×IOF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>47.55 ± 0.39</td>
<td>48.21 ± 0.4</td>
<td>0.190</td>
<td>0.343</td>
</tr>
<tr>
<td>2</td>
<td>38.7 ± 0.58b</td>
<td>42 ± 0.58b</td>
<td>&lt;0.0002</td>
<td>0.0052</td>
</tr>
<tr>
<td>7</td>
<td>139.31 ± 2.2</td>
<td>138.16 ± 2.2</td>
<td>&lt;0.0002</td>
<td>0.362</td>
</tr>
<tr>
<td>14</td>
<td>438.03 ± 6.43</td>
<td>499.65 ± 6.36</td>
<td>&lt;0.0002</td>
<td>0.362</td>
</tr>
<tr>
<td>21</td>
<td>862.92 ± 13.9</td>
<td>926.27 ± 14.49</td>
<td>&lt;0.0002</td>
<td>0.145</td>
</tr>
<tr>
<td>28</td>
<td>1,380.2 ± 28.5</td>
<td>1,517.2 ± 27.7b</td>
<td>&lt;0.0002</td>
<td>0.145</td>
</tr>
<tr>
<td>35</td>
<td>1,995 ± 43.06c</td>
<td>2,209.93 ± 43.7b</td>
<td>&lt;0.0002</td>
<td>0.185</td>
</tr>
</tbody>
</table>

a–cWithin a row, values with different superscripts are significantly different (P < 0.05).

1BW values are mean ± SEM of 150 and 134 birds for d 0 and d 2, respectively; n = 104 birds for d 7 and 14, and n = 74 birds for d 21, 28, and 35 (equal number of males and females).

2Significant interaction at P < 0.05.

#### Table 2. Average daily gain (g) of chickens from the 4 treatments on the first 2 d posthatch (0 to 2 d), and the first (0 to 7 d), second (7 to 14 d), third (14 to 21 d), fourth (21 to 28 d), and fifth (28 to 35 d) weeks posthatch and for the entire growing period (0 to 35 d)

<table>
<thead>
<tr>
<th>Age (d)</th>
<th>SP</th>
<th>EF</th>
<th>P-value</th>
<th>Feeding×IOF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 2</td>
<td>−4.38 ± 0.2a</td>
<td>−3.11 ± 0.2b</td>
<td>&lt;0.0002</td>
<td>0.0012</td>
</tr>
<tr>
<td>0 to 7</td>
<td>12.95 ± 0.28</td>
<td>12.83 ± 0.28</td>
<td>&lt;0.0002</td>
<td>0.574</td>
</tr>
<tr>
<td>7 to 14</td>
<td>42.9 ± 0.72</td>
<td>41.58 ± 0.71</td>
<td>&lt;0.0002</td>
<td>0.096</td>
</tr>
<tr>
<td>14 to 21</td>
<td>60.24 ± 1.22b</td>
<td>67.49 ± 1.26a</td>
<td>&lt;0.0002</td>
<td>0.0012</td>
</tr>
<tr>
<td>21 to 28</td>
<td>77.46 ± 2.76c</td>
<td>85.13 ± 2.72ce</td>
<td>&lt;0.0002</td>
<td>0.0151</td>
</tr>
<tr>
<td>28 to 35</td>
<td>84.72 ± 2.52b</td>
<td>97.92 ± 2.56a</td>
<td>&lt;0.0002</td>
<td>0.0012</td>
</tr>
<tr>
<td>0 to 35</td>
<td>55.61 ± 1.22c</td>
<td>61.75 ± 1.24a</td>
<td>&lt;0.0002</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

a–cWithin an age, values followed by different superscripts differ significantly (P < 0.05).

1Standard first-feeding procedure (SP): feeding the hatchlings 36 h after clearing the shell, without in ovo feeding (IOF; control-SP) and with IOF (IOF-SP); early feeding procedure (EF): feeding the hatchling not later than 6 h after clearing the shell, without IOF (control-EF) and with IOF (IOF-EF).

2Significant interaction at P < 0.05.
from d 14 and 35 were taken from birds euthanized with CO2. For glycogen analysis in the liver and in the pectoral muscle, embryos were sampled randomly from the control groups (8 embryos from control-SP and 8 from control-EF) and the IOF groups (8 embryos from the IOF-SP group and 8 from the IOF-EF group) during the incubation (on E18, E19, E20, and E21) and posthatch periods (on the day of hatch and 24 and 36 h posthatch).

Embryos, hatchlings, and organs were weighed, and organs were immediately frozen and stored at −20°C for glycogen determination. On d 14 and 35, the pectoral (major and minor) muscle was removed from 30 randomly selected chickens (equal numbers of males and females) from the various treatment groups and weighed.

**Glycogen Determination**

Liver and pectoral muscle glycogen contents were determined by a colorimetric method based on iodine reduction as described by Dreiling et al. (1987). For each bird, 0.2 g of liver or pectoral muscle sample was placed in a 1.5-mL tube. To each tube was added 0.5 g of 1 mm³ zirconia/silica beads and 0.8 mL of 8% (vol/vol) HClO₄. Homogenization was performed by a Mini-BeadBeeper-96 cell disrupter (BioSpec Products, Bartlesville, OK). Homogenates were centrifuged at 12,000 × g at 4°C for 15 min. The supernatant was removed, and 1 mL of petroleum ether was added to each tube. After mixing, the petroleum ether fraction was removed, and samples from the bottom layer (10 and 100 µL for the liver and pectoral muscle, respectively) were transferred to a new tube containing 650 µL of color reagent (1.3 mL of solution containing 0.26 g of crystal iodine and 2.6 g of KI in 10 mL of double-distilled water added to 100 mL of CaCl₂). After 10 min of incubation at room temperature, the absorbance at 450 nm was determined by an ELISA reader (Sunrise, Tecan, Switzerland) and the amount of glycogen was calculated according to a standard curve. Values for total glycogen in the liver and pectoral muscle were calculated by multiplying the weight of the organ by the amount of glycogen per 1 g of wet tissue.

**Cell Cultures**

Skeletal muscle cells were cultured from the pectoralis (major and minor) muscle of experimental chicks at various ages, as described previously (Halevy et al., 2000; Piestun et al., 2009). On all days, cell cultures were prepared from either 1.5 or 6 g of muscle (for embryos and posthatch, respectively) sampled from a pool of chopped muscle from 10 embryos or chicks (Piestun et al., 2009). The following media were used: modified Eagle’s medium with 10% (vol/vol) horse serum and 3% (vol/vol) chicken embryo extract (Gibco, Paisley, UK) for cells derived from embryos, or 10% horse serum containing Dulbecco’s modified Eagle’s medium for cells derived from posthatch chicks. An enriched population of myogenic cells was recovered, with less than 5% of these cells being nonmyogenic, and the CV of the cell preparations was approximately 5% (Halevy et al., 2000). The prepared cells were immediately counted with a hemocytometer.

**Analysis of Myofiber Diameter**

Muscle samples were excised from the superficial regions of the proximal one-half of the left pectoralis major of 8 embryos on E19 and of 8 chickens on d 3 and 35 from each treatment group. The long axis of each sample was parallel to the direction of the muscle fibers. Myofiber diameter was determined by analyzing the lesser myofiber diameter values, as described previously (Halevy et al., 2004). Briefly, muscle samples were fixed in 4% (wt/vol) paraformaldehyde and em-
bedded in paraffin, and muscle sections (5 µm) were cut. Sections were stained with hematoxylin and eosin, and at least 10 arbitrary fields in 2 to 3 serial sections of each muscle sample were photographed. In each muscle sample, the lesser fiber diameter was measured for individual myofibers with Cell^B software (Olympus, Hamburg, Germany). Because no statistically significant differences were observed among chicks, all data for the same treatment were pooled for further analysis.

Statistical Analyses

Least squares means ± SE of all measurements are reported in all tables and figures. The data from each measurement were subjected to 2-way ANOVA, with time of first feeding, IOF, and their interaction as main effects according to the following model:

\[ y = \mu + \text{feeding} + \text{IOF} + \text{feeding} \times \text{IOF} + e, \]

where \( y \) is the dependent variable, \( \mu \) is the grand mean, and \( e \) is the random error term. The Tukey-Kramer significant difference test (Kramer, 1956) was used for multiple comparisons among least squares means of feeding and IOF combinations, and values were considered statistically different at \( P < 0.05 \). All statistical analyses were carried out with JMP software (SAS Institute, Cary, NC).

RESULTS

BW, ADG, FI, and FCR Values

On d 2, both SP groups (feed and water provided only 36 h posthatch) showed reduced BW from hatch.
until d 2 relative to the BW increase for hatchlings in the EF group (feed and water provided 6 h after clearing the shell). However, the IOF-SP group exhibited significantly higher average BW than the control-SP group (Table 1). Although no significant differences in BW were observed between the 4 treatments in the first and second weeks posthatch, from the third week until marketing (d 35), the control-SP group exhibited the lowest BW, whereas the EF groups exhibited the highest values and the IOF group showed an intermediate value (Table 1).

This is also reflected in the data presented in Table 2, which shows that on d 2, the EF groups had gained BW (7.74 g for the control-EF group and 7.95 g for the IOF-EF group), whereas the SP groups had lost BW. However, the IOF-SP group lost significantly less BW than the control-SP group (−3.11 vs. −4.38 g). Table 2 shows that in the third and fifth weeks posthatch, the average BW gain of chickens in the IOF-SP group was similar to the average BW gain of the EF groups, whereas the values for chickens in the control-SP group were significantly lower.

In the first week posthatch, FI values for both EF groups were significantly higher ($P < 0.05$) than those for the SP groups (Table 3). Significant effects of the timing of first feeding and IOF were also observed; however, at this age no interaction was observed between these factors. An effect of time of first feeding was observed for FI value only on the first, second, and the third weeks of growth. Calculating the FI values along the growing period (0 to 35 d) showed that the EF groups and the IOF-SP group had significantly higher FI values compared with that in the control-SP group. Feed conversion ratio values in the first week posthatch were higher in all IOF treatments compared with control values. However, in the last week of growing (28 to 35 d), an interaction between the time of first feeding and IOF was observed, together with the highest FCR value in the IOF-SP group. Calculating the FCR value for the entire growth period demonstrated that the IOF-EF group had the optimal FCR.

**Pectoral Muscle Weight and Percentage**

On d 14, pectoral muscle weight was significantly higher in both EF groups (control-EF and IOF-EF) compared with the control-SP group ($P$ (interaction) = 0.4964; Figure 1A). Moreover, the IOF groups (IOF-SP and IOF-EF) also demonstrated significantly higher muscle weight than the control-SP group. When pectoral muscle was calculated as a percentage of BW, a significantly higher percentage was observed for both IOF groups on d 14. The IOF-SP group exhibited a 16.9% increase over the control-SP group, and the IOF-EF group exhibited a 6.5% increase over the control-EF group ($P$ (interaction) = 0.113; Figure 1B). Birds in the IOF treatments showed higher pectoral muscle weights than their respective controls on d 35 ($P$ (interaction) = 0.227). At this age, the pectoral muscle-to-BW ratio was highest for chickens in the IOF-EF group (Figure 1B).

**Glycogen Levels in the Liver and Pectoral Muscle During the Preand Posthatch Periods**

Analysis of glycogen reserves in the liver before hatch (Figure 2A) showed a pattern of glycogen accumulation on E19 and E20 and a reduction in glycogen reserves on the last day of incubation (E21). Embryos from the IOF group had higher levels of glycogen in the livers, by 2.55-fold on E19 (24 h post-IOF procedure) and by 1.53-fold on E20, in comparison with embryos from the control group. On E21, no significant difference was observed in glycogen reserves between the IOF and control groups.

In this prehatch period, glycogen levels in the pectoral muscle (Figure 2C) remained almost unchanged in the control group. However, a significant increase was observed 24 h after the IOF procedure in the IOF...
IN OVO FEEDING AND INTERACTION WITH FIRST-FEED TIMING

Figure 4. (A) Myofiber diameter distribution in pectoral muscle from the 4 treatment groups on d 3 (upper panel) and d 35 (lower panel) of age (n = 8): standard feeding procedure (SP), that is, feeding the hatchlings 36 h after clearing the shell, without in ovo feeding (IOF; control-SP) and with IOF (IOF-SP), and early feeding procedure (EF), that is, feeding the hatchling not later than 6 h after clearing the shell, without IOF (control-EF) and with IOF (IOF-EF) on d 14 and 35. Myofibers are clustered in bin intervals of 5 µm, and myofiber diameter values are ranked in ascending order within each treatment group. Results are presented as percentage of total fibers. (B) Mean myofiber diameter calculated for each group. Within an age, bars with different letters (a–d) differ significantly (P < 0.05).
**Table 3.** Feed intake (FI) and feed conversion ratio (FCR) of chickens from the 4 treatments\(^1\) on the first (0 to 7 d), second (7 to 14 d), third (14 to 21 d), fourth (21 to 28 d), and fifth (28 to 35 d) weeks posthatch and for the entire growing period growing (0 to 35 d)

<table>
<thead>
<tr>
<th>Age (d)</th>
<th>Item</th>
<th>Control</th>
<th>IOF</th>
<th>Control</th>
<th>IOF</th>
<th>Feeding</th>
<th>IOF</th>
<th>Feeding × IOF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 7</td>
<td>FI</td>
<td>154.1 ± 3.79</td>
<td>175.9 ± 3.54</td>
<td>226.91 ± 4.05</td>
<td>236.46 ± 4.1</td>
<td>&lt;0.000</td>
<td>2</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>7 to 14</td>
<td>FCR</td>
<td>1.71 ± 0.05</td>
<td>1.99 ± 0.04</td>
<td>1.7 ± 0.05</td>
<td>1.87 ± 0.05</td>
<td>0.986</td>
<td>0.409</td>
<td>0.948</td>
</tr>
<tr>
<td>14 to 21</td>
<td>FI</td>
<td>424.4 ± 9.68</td>
<td>410.39 ± 9.04</td>
<td>523.36 ± 10.32</td>
<td>507.30 ± 10.46</td>
<td>&lt;0.000</td>
<td>2</td>
<td>0.133</td>
</tr>
<tr>
<td>21 to 28</td>
<td>FCR</td>
<td>1.40 ± 0.03</td>
<td>1.34 ± 0.3</td>
<td>1.52 ± 0.04</td>
<td>1.49 ± 0.04</td>
<td>&lt;0.000</td>
<td>2</td>
<td>0.249</td>
</tr>
<tr>
<td>28 to 35</td>
<td>FI</td>
<td>618.20 ± 22.28</td>
<td>695.82 ± 20.89</td>
<td>734.59 ± 24.86</td>
<td>767.70 ± 24.18</td>
<td>&lt;0.000</td>
<td>2</td>
<td>0.018</td>
</tr>
<tr>
<td>0 to 35</td>
<td>FCR</td>
<td>1.45 ± 0.05</td>
<td>1.49 ± 0.05</td>
<td>1.45 ± 0.06</td>
<td>1.5 ± 0.06</td>
<td>0.986</td>
<td>0.409</td>
<td>0.940</td>
</tr>
</tbody>
</table>

\(^{a,b}\)Within a row, values with different superscripts are significantly different (\(P < 0.05\)).

\(^1\)Standard first-feeding procedure (SP): feeding the hatchlings 36 h after clearing the shell, without in ovo feeding (IOF; control-SP) and with IOF (IOF-SP); early feeding procedure (EF): feeding the hatchling not later than 6 h after clearing the shell, without IOF (control-EF) and with IOF (IOF-EF). FI and FCR values are mean ± SEM of 4 cages (10 birds/cage) from each treatment group (equal number of males and females).

\(^2\)Significant interaction at \(P < 0.05\).

The IOF procedure had an immediate effect on promoting myoblast proliferation: on E19, cell numbers in the IOF group were significantly higher than those in the control group (Figure 3). On d 3 posthatch, the myoblast numbers in both EF groups were significantly higher than those in both SP groups, whereas the numbers in the EF-IOF group were higher than those in the control-EF group. As expected, all cell numbers declined on d 5 posthatch as the cells underwent terminal differentiation (Halevy et al., 2004, 2006); the myoblast number in the control-SP group was the lowest, whereas that in the IOF-SP group was the highest. Cell numbers in the IOF-EF group were still higher than those in the control-EF group.

**Myofiber Diameter in Posthatch Chicks**

An analysis of the myofiber diameter distribution was performed on d 3 to evaluate the immediate effect of delayed feeding and the IOF procedure on myofiber development. Myofiber diameter values of all groups displayed typical Gaussian curves: this curve was sharpest for the control-SP group with the lowest diameter values, whereas the group that underwent the IOF procedure with delayed feeding (IOF-SP) had a slightly flatter curve with a rightward shift to the higher diameter bins (Figure 4A). Both EF groups demonstrated the flattest distribution with the highest percentage of myofibers with higher diameter values. On d 35, the distribution of myofiber diameter values was in the range of 5 to 85 µm in all groups; however, the curve peaks of the EF groups were shifted slightly toward the higher diameter bins compared with the delayed-feed groups, with that of the IOF-SP group having a slight shift to the right (Figure 4A, lower panel). The curve distribution was reflected in the mean myofiber diameter values: they were lowest in the control-SP group and highest in the EF groups (Figure 4B). The IOF procedure contributed to enlargement of the myofiber diameters because these groups exhibited higher means than their respective controls.

**DISCUSSION**

Chick feeding is commonly delayed for the first 36 h posthatch because of the logistics of production. Although the effect of fasting for the first 48 to 72 h
posthatch has been examined in several studies, all of which documented attenuated increases in BW, intestinal development and functionality (Vieira and Moran, 1999a,b; Geyra et al., 2001b), and breast muscle development (Halevy et al., 2000; Mozdzial et al., 2002a,b), this study focused on milder fasting and its interaction with prehatch feeding by examining these variables during the pre- and posthatch periods until marketing.

The present study shows that even the standard hatchery procedure, which delays first feed by only 36 h from the moment of hatch, leads to irreversible growth depression, as expressed by reduced BW on d 35 and reduced muscle weight. This is clearly shown by the 2-way ANOVA results with respect to BW (Table1) and ADG (Table 2) in which the timing of the first feeding factor had a significant effect along the growth period. Taken together with previous reports, it is conceivable that any first-feed delay of at least 36 h will negatively affect BW and muscle weight.

It can be concluded that injecting a solution of carbohydrates and HMB in the amniotic sac of the embryo several days before hatch supports the growth of broilers subjected to delayed access to feed and water because their BW and muscle weight were only marginally affected at an early age as well as at later ages. Indeed, the two-way ANOVA implied a beneficial effect of IOF over SP, at least with respect to BW and ADG.

In the current study, although prehatch control embryos suffered from physiological limitations and depleted glycogen reserves in the liver, the IOF-treated birds showed benefits from the carbohydrate source included in the IOF solution on E19, E20, and at hatch. In addition, during the posthatch period, although the SP treatments showed the expected results of minimal glycogen stores (3 mg) in the liver, the EF birds exhibited 120 to 140 mg of total glycogen in the liver. It can be concluded that the administration of carbohydrate before hatch (by IOF) together with EF resulted in maximal carbohydrate availability during the pre- to posthatch period.

Among the 2 EF groups, the IOF-EF group showed higher glycogen stores. This difference was probably due to the enhanced capacity of birds in the IOF group to digest and absorb their first feed because of the enhanced development of the small intestine by the IOF procedure (Tako et al., 2004; Smirnov et al., 2006; Foye et al., 2007). Although, at 36 h posthatch, liver glycogen levels were similar in both the control-EF and IOF-EF groups, the advantage of greater glycogen reserves in the immediate posthatch period (24 h posthatch) probably supported the development of critical systems, among them the immune and skeletal systems (Dibner and Richards, 2004; Bar-Shira and Friedman, 2006; Dibner et al., 2007). In addition, these higher glycogen reserves probably reduced the need for glucose synthesis via gluconeogenesis from muscle proteins.

In contrast to the glycogen dynamics in the liver during the pre- to posthatch period, only minor changes in glycogen concentration could be observed in the muscle. However, the IOF treatment elevated glycogen levels in the pectoral muscle before hatch (on E19, E20, and E21) and the EF elevated glycogen reserves posthatch.

The results imply that both IOF and EF affected similar processes that occurred during the pre- to posthatch period and had long-term effects on performance. One such process was likely early stimulation of the gastrointestinal tract (GIT).

The GIT develops throughout incubation, but the functional abilities of the small intestine begin to develop only in the last quarter of incubation, as evidenced by extensive morphological, cellular, and molecular changes in this organ (Uni et al., 2003b; Dibner and Richards, 2004; Uni, 2006). During the last days of incubation (Uni et al., 2003b), there is a significant increase in the weight of the intestine relative to the weight of the embryo (1.4% on E17 to 3.4% on the day of hatch). The activity and RNA expression of brush-border enzymes, which digest disaccharides and short peptides, and of the major transporters begin to increase on E16 and have increased by 15- to 40-fold by E20 (Uni et al., 2003b). Research using real-time PCR and gene-array methods has shown that the peptide transporter, 10 different amino acid transporters, and 4 sugar transporters are expressed from E17 and exhibit elevated expression toward hatch (Gilbert et al., 2007; de Oliveira et al., 2009).

Studies have shown that immediate feeding posthatch accelerates the development of the small intestine (Noy and Sklan, 1999; Noy et al., 2001), whereas delayed access to external feeding for up to 48 h attenuates the morphological and functional development of the small intestine mucosal layer (Uni et al., 1998, 2003a; Geyra et al., 2001b). Furthermore, birds whose access to first feed is denied for 24 to 48 h posthatch show decreases in villus length (Yamauchi et al., 1996), crypt size and number of crypts per villus, and enterocyte migration rate (Geyra et al., 2001a). In addition, delayed access to feed for 48 h posthatch results in changes in mucin dynamics, which affects the absorptive and protective functions of the small intestine (Uni et al., 2003a). Experiments focusing on ways to advance development of the intestine have shown that the injection of 1 mL of IOF solution containing dextrin (as a carbohydrate source), Na+, Cl−, and HMB markedly enhances enteric development (Tako et al., 2005; Smirnov et al., 2006; Foye et al., 2007). It was concluded, based on several experiments, that the small intestine of IOF birds is at a functional stage similar to that of conventionally fed 2-d-old chicks.

The second process that underlies the later improved performance of chicks in the IOF and EF groups is muscle development during the pre- and immediate posthatch periods. Indeed, IOF on E18 had an immediate enhancing effect on muscle cell numbers, as observed on E19 and d 5. Likewise, early feeding at 6 h posthatch, a critical period for satellite cell proliferation (Haley et al., 2000, 2003), had a beneficial effect on cell numbers on d 3 and 5. It is interesting that the combination of
IOF and EF had the greatest effect on cell numbers on d 3, the peak day of satellite cell proliferation in broilers (Halevy et al., 2004, 2006), in agreement with earlier reports in turkey poultys (Moore et al., 2005a,b). The promoting effects of IOF, EF, and particularly their combination were manifested as early as d 3 in myofiber development, with more myofibers having larger diameters, corroborating previous results documenting delayed myofiber maturation attributable to late feeding (Halevy et al., 2000; Moore et al., 2005a,b). At the later posthatch period, the beneficial effect of EF and its combination with IOF was clearly observed in terms of hypertrophy and pectoral muscle weight. The beneficial effect of IOF on muscle growth in chickens grown under the standard 36-h delay in first feeding was still observed on d 35, but was less marked than the difference during the early growth phase, again implying the dramatic adverse long-term effects of delayed feeding even for 36 h, and their almost complete reversal by IOF.

The posthatch enhancing effects of IOF and EF, compared with the adverse effects of delayed first feeding, on muscle growth could be attributed to increased liver and muscle glycogen reserves (Uni et al., 2004), accelerated development of the small intestine (Tako et al., 2004; Smirnov et al., 2006; Foye et al., 2007), a higher reservoir of muscle progenitors (satellite cells; Halevy et al., 2000, 2003; Mozdziaik et al., 2002a; Moore et al., 2005a,b), or their combination. Conceivably, adequate glycogen reserves and sufficient protein synthesis are crucial for higher functionality of the GIT, and hence for improved body and muscle growth.

However, it should be noted that the experiment was conducted using Cobb 500 broilers, which are characterized by their rapid growth right after hatch, whereas other major broiler lines have different growth curves. This fact applies for all growing parameters measured in this study. Moreover, although the numbers of satellite cells and their activity have been found to be maximal early after hatch (Halevy et al., 2001, 2006; Piestun et al., 2009), some shift in the kinetics could occur in different broiler lines.

In summary, this study emphasizes the importance of feeding at critical stages of GIT and muscle development during the pre- and posthatch periods in broilers. Moreover, IOF has a long-term effect in supporting BW and posthatch muscle growth via enhancement of liver and muscle glycogen reserves and satellite cell proliferation, even when first feeding is delayed.

REFERENCES


