FROM THE DIAGNOSTIC LAB:
GETTING THE BEST RESULTS FROM VETERINARY HISTOPATHOLOGY*

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*Article is modified from “Getting the Best from Veterinary Histopathology.” By Mark Stidworthy and Simon Priestnall, In Practice June 2011 | Volume 33 | 252–260.

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Broiler Live Production Cost | Average Company
---|---
Feed Cost/ton w/o color ($) | 410.44
Feed cost /lb meat (c) | 35.62
Days to 4.6 lbs | 38
Chick cost / lb (c) | 5.48
Vac-Med cost/lb (c) | 0.00
WB & ½ parts condemn. Cost/lb | 0.22
% mortality | 3.15
Sq.Ft. @ placement | 0.84
Lbs/sq. ft. | 7.41
Downtime (days) | 19

Data for week ending October 27th, 2012
Samples taken for histopathology can provide diagnostic results that are timely and inexpensive for the clinicians when done properly. For histological examination, a sample removed from an animal needs to be converted from a three-dimensional tissue into a stained section, approximately 4mm thick, adhered to a glass slide. This is examined under a microscope, essentially in two dimensions. Each stage of this sequential process is important for the final section quality, including the collection of a representative tissue sample, fixation, laboratory trimming/sampling, tissue processing, embedding, cutting, staining, mounting and, where appropriate, the use of special stains or immunohistochemistry.

The aim of fixation is to maintain fresh tissue in a state that stabilizes its architecture and chemical components in a form that enables it to be processed for histological staining and long-term preservation. Formaldehyde based fixatives are routinely used in diagnostic setting on the grounds of cost, efficacy, versatility and relative safety. Buffered 4 to 10% formaldehyde solutions are best, as this limit the amount of artifact requiring interpretation by the pathologist. Good fixation is easy to achieve, providing a few simple principles are applied. Formaldehyde penetrates tissue at a rate of about 5 mm per 24 hours. Therefore, material that is too thick will not be fixed in the center. This results in poor processing and that the appearance of the final histological section may not accurately reflect the underlying process. An adequate volume of fixative is also essential. In general, the volume of fixative should be at least 10 times the volume of the piece of tissue. It is best to add the tissue to the fixative to avoid one surface of the sample adhering to the wall of the container. For larger samples or multiple tissues following postmortem examination, the volume of fixative required may be too great to send through the mail. Case material can be left to fix for an adequate period of time before being submitted. Changing the fixative may be necessary to have adequate preservation of numerous tissues, bloody tissues like spleen, liver or lungs, or large tissue samples. Some organs (spleen, testes) require cutting of the capsule to allow penetration of the fixative. The excess fixative should be poured off before submission and the tissues sent to the laboratory moist with a small amount of fixative in a sealed container. It is also helpful to remember that tissues stiffen after fixation. A large soft sample that fits through a narrow opening when fresh will not necessarily fit back through the same
opening once it has been fixed. This is a source of frustration and potential hazard for laboratory staff that have to remove material from the container in which it is trapped. It can also result in the specimen being damaged during removal. It is therefore important to use containers with wide lids. Some samples require a different fixative for optimum preservation. Eyeballs should be fixed in Davison solution instead of formaldehyde based fixative to decrease the amount of artifacts during fixation. Davison penetrates the tissue faster and prevents the retina from floating off the choroid and appearing like a detached retina when examined.

When a fixed sample arrives at PDRC, a technician will trim it for histological processing. Most material is processed using standard histological cassettes for presentation on standard glass slides. Standard cassettes are about 40 x 28 mm in size, which means that any submitted sample will be trimmed down into pieces smaller than this to enable examination. Bone samples may have to be decalcified before processing in order to be sectioned on the microtome which will add time to when the slides are ready for examination. Indicating what samples submitted on the form will help the technician make sure all pieces of tissue are present or if they are missing.

Routine histology processing involves dehydration of the sample using graded alcohols, permeation using a solvent (clearing agent) of equivalent density to wax and, finally, replacement of the solvent by the wax itself. The speed of this process will depend on the type of tissue, processor and reagents used. PDRC runs this process overnight, so that samples submitted on one day are ready for sectioning and staining the following morning. Some tissues (bone) may require more prolonged processing. The only exception for this situation is samples submitted for ILT diagnosis. Tracheas and eyelids can be short processed and slides made the same day if the samples are here at PDRC by 11am. Once the material is permeated with wax, it is orientated for sectioning in a mould, and surrounded by molten wax. As the wax hardens, it forms a supportive block that can be mounted on a microtome and sectioned to retain the tissue architecture. Once embedded in an appropriate orientation in a wax block, the tissue is serially sectioned using a microtome until the full surface of the embedded sample is exposed. Sections of about 4 μm thickness are then cut (as ribbons of wax
containing the tissue) and placed on to a warm water bath to flatten out before being laid out on glass slides. These are allowed to dry, after which the supporting matrix of wax is removed with solvents and the sections are rehydrated before staining. The routine stain used for histological examination is hematoxylin and eosin. PDRC pathologists will generally examine all tissues submitted using this stain before proceeding to any additional special stains. Once stained, sections are mounted under a glass cover slip and the mounting solution allowed to set before examination. PDRC archives copies of both the original slides produced and assessed by the pathologist and the paraffin block containing the embedded processed tissues, so that further sections or special stains can be produced at a later date. Tissues not trimmed in are kept for 30 days incase addition slides are needed.

Special stains that are available at PDRC include Giemsa, PAS, GMS, Acid Fast, Trichrome, Warthin Starry, Von Kossa, Brown and Hopps, and Perl’s Iron. We have immunohistochemistry for ILT. We can also cut sections for PCR from formalin fixed paraffin embedded samples in cases were fresh samples are not sent or cannot be shipped (international samples).

Additional information regarding the signalment is very helpful for interpretation of the changes seen as some are age related. Bursal sections are routinely submitted for scoring to the PDRC pathologists. However, samples from birds less than 17 days old have tremendous variation in follicle size and lymphoid content; it is difficult to determine if the changes are significant or normal variation. Information regarding any gross lesions seen during the necropsy is helpful when submitted fixed tissues. “Tissue for Histo” is not a reason for submission, it indicates the requested test. Summaries of necropsy findings (3 out of 4 birds with pneumonia, 3 out of 5 birds with bursal and thymic atrophy) will help the PDRC pathologist determine if the lesions seen are a flock problem or an individual bird problem that might be an outlier.
Below are examples of cases submitted to PDRC, both improper and proper submission of fixed tissues.

Small container has too many tissues for amount of formalin. Large container has proper ratio of formalin to tissue. Having the proper amount of formalin solution allows penetration of the tissues to fix them completely. Submitted samples that are bloody usually have the formalin changed to increase the likelihood of complete fixing of the tissues.

In some cases, international samples are submitted in wax blocks. Here, the wax melted during transportation, leaving tissues exposed (white cassettes). Blue cassette demonstrates the proper amount of wax for comparison. These samples had to be re-embedded before sectioning. Problems arise if the tissues come off the submitted blocks and more than one case is submitted. Laboratory personnel may not be able to determine which case the tissue belongs to.
Remember when submitting histology samples:

1. Proper formalin to tissue ratio (10:1) for fixation.

2. Include any important gross lesions.

3. When submitting neoplasia, include with the mass some normal tissue if possible.

4. When suspecting a viral neoplasia problem, submit a complete set of tissues, not just affected organs. Include brain and eyeballs with optic nerve still attached.

If you are interested in sending samples to the lab or have any additional questions please contact us at 706-542-5657 or pdrc@uga.edu. Additional documents and information are also available on our website at:

http://www.avian.uga.edu/diagnostic/dxlab.php
Chickens and Eggs
Released November 23, 2012, by NASS, Agricultural Statistics Board, USDA

October Egg Production Up 2 Percent

Please note that Missouri will now be published separately in the Broiler-Type Chicks Hatched table.

U.S. egg production totaled 7.90 billion during October 2012, up 2 percent from last year. Production included 6.88 billion table eggs, and 1.02 billion hatching eggs, of which 952 million were broiler-type and 69 million were egg-type. The total number of layers during October 2012 averaged 340 million, up 1 percent from last year. October egg production per 100 layers was 2,321 eggs, up slightly from October 2011.

All layers in the U.S. on November 1, 2012, totaled 342 million, up 2 percent from last year. The 342 million layers consisted of 291 million layers producing table or market type eggs, 49.1 million layers producing broiler-type hatching eggs, and 2.82 million layers producing egg-type hatching eggs. Rate of lay per day on November 1, 2012, averaged 75.4 eggs per 100 layers, up 1 percent from November 1, 2011.

Egg-Type Chicks Hatched Up 4 Percent

Egg-type chicks hatched during October 2012 totaled 39.1 million, up 4 percent from October 2011. Eggs in incubators totaled 35.0 million on November 1, 2012, down 7 percent from a year ago. Domestic placements of egg-type pullet chicks for future hatchery supply flocks by leading breeders totaled 196 thousand during October 2012, down 82 percent from October 2011.

Broiler-Type Chicks Hatched Up 2 Percent

Broiler-type chicks hatched during October 2012 totaled 727 million, up 2 percent from October 2011. Eggs in incubators totaled 569 million on November 1, 2012, down 1 percent from a year earlier. Leading breeders placed 6.22 million broiler-type pullet chicks for future domestic hatchery supply flocks during October 2012, down 1 percent from October 2011.

Broiler Hatchery
Released November 28, 2012, by NASS, Agricultural Statistics Board, USDA

Broiler-Type Eggs Set In 19 Selected States Down Slightly

Commercial hatcheries in the 19-State weekly program set 195 million eggs in incubators during the week ending November 24, 2012. This was down slightly from the eggs set the corresponding week a year earlier. Average hatchability for chicks hatched during the week was 85 percent. Average hatchability is calculated by dividing chicks hatched during the week by eggs set three weeks earlier.

Broiler-Type Chicks Placed Down 4 Percent

Broiler growers in the 19-State weekly program placed 151 million chicks for meat production during the week ending November 24, 2012. Placements were down 4 percent from the comparable week a year earlier. Cumulative placements from January 1, 2012 through November 24, 2012 were 7.60 billion, down 2 percent from the same period a year earlier.

Turkey Hatchery
Released November 15, 2012, by the NASS, Agricultural Statistics Board, USDA

Eggs in Incubators on November 1 Down 1 Percent from Last Year

Turkey eggs in incubators on November 1, 2012, in the United States totaled 27.3 million, down 1 percent from November 1, 2011. Eggs in incubators were up 1 percent from the October 1, 2012 total of 27.0 million eggs. Please note that regional estimates have been discontinued: NASS will no longer publish regional Turkey Hatchery estimates. Only estimates at the United States level will be published due to the limited number of hatcheries involved.

Poults Hatched During October Up 4 Percent from Last Year

Turkey poults hatched during October 2012, in the United States totaled 23.3 million, up 4 percent from October 2011. Poults hatched were up 9 percent from the September 2012 total of 21.4 million poults.

Net Poults Placed During October Up 6 Percent from Last Year

The 22.7 million net poults placed during October 2012 in the United States were up 6 percent from the number placed during the same month a year earlier. Net placements were up 9 percent from the September 2012 total of 20.8 million.
## Current Month Charts

### Broiler Performance Data

#### Live Production Cost

<table>
<thead>
<tr>
<th></th>
<th>SW</th>
<th>Midwest</th>
<th>Southeast</th>
<th>Mid-Atlantic</th>
<th>S-Central</th>
<th>Average Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Cost/ton w/o color ($)</td>
<td>401.38</td>
<td>392.88</td>
<td>419.92</td>
<td>412.77</td>
<td>416.35</td>
<td>410.44</td>
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<tr>
<td>Feed cost/lb meat (c)</td>
<td>34.45</td>
<td>33.62</td>
<td>35.99</td>
<td>37.04</td>
<td>36.66</td>
<td>35.62</td>
</tr>
<tr>
<td>Days to 4.6 lbs</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Chick cost/lb (c)</td>
<td>4.93</td>
<td>5.36</td>
<td>5.89</td>
<td>5.01</td>
<td>5.37</td>
<td>5.48</td>
</tr>
<tr>
<td>Vac &amp; Med cost/lb (c)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.04</td>
<td>0.05</td>
<td>0.04</td>
<td>0.00</td>
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<tr>
<td>WB &amp; ½ parts condemn. Cost/lb</td>
<td>0.18</td>
<td>0.23</td>
<td>0.17</td>
<td>0.20</td>
<td>0.23</td>
<td>0.22</td>
</tr>
<tr>
<td>% mortality</td>
<td>2.87</td>
<td>3.18</td>
<td>3.13</td>
<td>3.41</td>
<td>3.10</td>
<td>3.15</td>
</tr>
<tr>
<td>Sq.Ft. @ placement</td>
<td>0.82</td>
<td>0.82</td>
<td>0.84</td>
<td>0.88</td>
<td>0.86</td>
<td>0.84</td>
</tr>
<tr>
<td>Lbs/sq. ft.</td>
<td>7.74</td>
<td>7.35</td>
<td>7.09</td>
<td>7.90</td>
<td>7.68</td>
<td>7.41</td>
</tr>
<tr>
<td>Downtime (days)</td>
<td>21</td>
<td>17</td>
<td>19</td>
<td>20</td>
<td>18</td>
<td>19</td>
</tr>
</tbody>
</table>

### Broiler Whole Bird Condemnation

<table>
<thead>
<tr>
<th></th>
<th>SW</th>
<th>Midwest</th>
<th>Southeast</th>
<th>Mid-Atlantic</th>
<th>S-Central</th>
<th>Average Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Septox</td>
<td>0.122</td>
<td>0.209</td>
<td>0.088</td>
<td>0.124</td>
<td>0.104</td>
<td>0.127</td>
</tr>
<tr>
<td>% Airsac</td>
<td>0.031</td>
<td>0.045</td>
<td>0.023</td>
<td>0.053</td>
<td>0.027</td>
<td>0.038</td>
</tr>
<tr>
<td>% I.P.</td>
<td>0.007</td>
<td>0.020</td>
<td>0.005</td>
<td>0.036</td>
<td>0.017</td>
<td>0.017</td>
</tr>
<tr>
<td>% Leukosis</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.004</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>% Bruises</td>
<td>0.001</td>
<td>0.001</td>
<td>0.003</td>
<td>0.003</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>% Other</td>
<td>0.004</td>
<td>0.003</td>
<td>0.030</td>
<td>0.008</td>
<td>0.010</td>
<td>0.011</td>
</tr>
<tr>
<td>% Total</td>
<td>0.165</td>
<td>0.278</td>
<td>0.149</td>
<td>0.228</td>
<td>0.162</td>
<td>0.196</td>
</tr>
<tr>
<td>% ½ parts condemnns</td>
<td>0.205</td>
<td>0.229</td>
<td>0.187</td>
<td>0.180</td>
<td>0.339</td>
<td>0.254</td>
</tr>
</tbody>
</table>

Data for week ending October 27th, 2012

## Previous Month Charts

### Broiler Performance Data

#### Live Production Cost

<table>
<thead>
<tr>
<th></th>
<th>SW</th>
<th>Midwest</th>
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<th>Average Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Cost/ton w/o color ($)</td>
<td>387.82</td>
<td>385.89</td>
<td>415.07</td>
<td>405.88</td>
<td>407.69</td>
<td>402.79</td>
</tr>
<tr>
<td>Feed cost/lb meat (c)</td>
<td>33.16</td>
<td>32.75</td>
<td>35.75</td>
<td>36.25</td>
<td>35.73</td>
<td>34.88</td>
</tr>
<tr>
<td>Days to 4.6 lbs</td>
<td>39</td>
<td>37</td>
<td>39.00</td>
<td>38</td>
<td>38</td>
<td>39</td>
</tr>
<tr>
<td>Chick cost/lb (c)</td>
<td>5.03</td>
<td>5.07</td>
<td>5.61</td>
<td>4.85</td>
<td>5.38</td>
<td>5.37</td>
</tr>
<tr>
<td>Vac &amp; Med cost/lb (c)</td>
<td>0.05</td>
<td>0.02</td>
<td>0.09</td>
<td>0.05</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>WB &amp; ½ parts condemn. Cost/lb</td>
<td>0.18</td>
<td>0.18</td>
<td>0.16</td>
<td>0.16</td>
<td>0.23</td>
<td>0.20</td>
</tr>
<tr>
<td>% mortality</td>
<td>2.87</td>
<td>3.09</td>
<td>3.44</td>
<td>3.16</td>
<td>3.70</td>
<td>3.34</td>
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<tr>
<td>Sq.Ft. @ placement</td>
<td>0.82</td>
<td>0.82</td>
<td>0.85</td>
<td>0.87</td>
<td>0.86</td>
<td>0.84</td>
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<tr>
<td>Lbs/sq. ft.</td>
<td>7.80</td>
<td>7.56</td>
<td>7.09</td>
<td>7.77</td>
<td>7.48</td>
<td>7.38</td>
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<tr>
<td>Downtime (days)</td>
<td>19</td>
<td>15</td>
<td>17</td>
<td>18</td>
<td>16</td>
<td>17</td>
</tr>
</tbody>
</table>

### Broiler Whole Bird Condemnation

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</tr>
</thead>
<tbody>
<tr>
<td>% Septox</td>
<td>0.121</td>
<td>0.152</td>
<td>0.082</td>
<td>0.093</td>
<td>0.089</td>
<td>0.103</td>
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<tr>
<td>% Airsac</td>
<td>0.034</td>
<td>0.026</td>
<td>0.021</td>
<td>0.043</td>
<td>0.031</td>
<td>0.035</td>
</tr>
<tr>
<td>% I.P.</td>
<td>0.008</td>
<td>0.021</td>
<td>0.004</td>
<td>0.028</td>
<td>0.026</td>
<td>0.018</td>
</tr>
<tr>
<td>% Leukosis</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.004</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>% Bruises</td>
<td>0.001</td>
<td>0.001</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>% Other</td>
<td>0.005</td>
<td>0.002</td>
<td>0.032</td>
<td>0.006</td>
<td>0.008</td>
<td>0.009</td>
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<tr>
<td>% Total</td>
<td>0.169</td>
<td>0.202</td>
<td>0.141</td>
<td>0.177</td>
<td>0.156</td>
<td>0.169</td>
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<tr>
<td>% ½ parts condemnns</td>
<td>0.207</td>
<td>0.217</td>
<td>0.194</td>
<td>0.164</td>
<td>0.344</td>
<td>0.249</td>
</tr>
</tbody>
</table>

Data for week ending September 22nd, 2012
Meetings, Seminars and Conventions

2012
November


2013
January


2013
February

February 1-5, 2013. Georgia International Poultry Short Course. This is a 3.5-day course designed by the Department of Poultry Science at UGA that will cover a range of topics in modern poultry meat production. Space is limited and registration closes on January 18, 2013, so please register early to ensure acceptance. For more info, please visit http://www.poultry.uga.edu/extension/InternationalShortCourse.html#info

February 21-22, 2013. Agricultural Outlook Forum. This Forum is organized by the U.S. Department of Agriculture and features the USDA commodity supply and demand and food price and farm income outlooks. Please visit http://www.usda.gov/oce/forum/

2013
March


2013
April

April 15-18, 2013. NIAA Annual Conference. The National Institute for Animal Agriculture will be holding its annual conference at the Galt House in Louisville, KY. Please visit http://www.animalagriculture.org/ for more info.


2013
July

July 20-23, 2013. AVMA/AAAP Annual Convention. This year it will be held in Chicago, IL. Please visit https://www.avma.org/Events/Convention/Pages/default.aspx


2013
August


Reminder
All previous issues of the Poultry informed Professional are archived on our website www.avian.uga.edu under the Online Documents and The Poultry Informed Professional links.