Occurrence of Aflatoxin B₁ in Poultry Feed and Feed Ingredients in Pakistan

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Abstract
A world trade in agricultural commodities has contributed significantly to the discussion about potential hazards involved and has increased in particular the awareness of mycotoxins. Safety awareness in food and feed production has also risen due to the simple fact that methods for testing residues and undesirable substances have become noticeably more sophisticated and more reliable at all points of the supply chain.

A 3-year survey program was initiated as decided in the 66th meeting of the Animal Feeds Sectional Committee held at Standards Development Centre, Karachi, Pakistan in order to evaluate the incidence of aflatoxin B1 in poultry feed and feed raw materials in some of the major poultry production areas. A total of 1021 analyses were performed on 639, 92, 77, 86 and 127 samples sourced from North, South, West, East and Central areas, respectively. Overall 61 percent of all aflatoxin B1 tests conducted on samples received from all selected survey areas tested positive. The percentages of positive evidenced in North, South, West, East and Central areas were 63, 49, 56, 53 and 69, respectively. Mean levels of aflatoxin B1 in feed ingredients and finished feeds (except layer grower, broiler starter, broiler finisher in mash forms and layer feed in crumb forms) were noted to be higher than safe limit of 20 µg/kg. This is the first comprehensive report on the determination of aflatoxin B1 in poultry feed raw material and finished feed from Punjab, Pakistan.

Key words: Aflatoxin B1, aflatoxin B1 occurrence, poultry ingredients, poultry feed

Introduction
Mycotoxins are secondary metabolites produced by filamentous fungi that cause a toxic response (mycotoxicosis) when ingested by higher animals. There are a wide variety of toxins, produced by numerous fungi, depending on the type of crop, geographical location and climatic conditions. Cereal plants may be contaminated by mycotoxins in two ways: fungi growing as pathogens on plants or growing saprophytically on stored plants. Mycotoxins are a potential threat to human health. Joint FAO/WHO expert committees are providing estimates of relative health risks associated with specific proposed maximum limits for particular toxins. Feeding contaminated materials to animals, especially single-stomached animals, impairs feed intake, efficiency of feed utilization, diminished body weight gain, increased disease incidence (due to immune –suppression) and reduced reproductive capacities which leads to economic losses.
Aflatoxins were first identified in 1961 in animal feed responsible for the deaths of 100,000 turkeys in the United Kingdom \(^{21}\). The most severe spontaneous outbreak of aflatoxicosis in poultry was described by Hamilton in North Carolina, in which 50\% of a flock of laying hens died within 48 h of being fed highly toxic corn (containing 100 ppm aflatoxin) and at the same time a 95\% drop in egg production occurred. Aflatoxins are produced by the common fungi *Aspergillus flavus* and the closely related species *Aspergillus parasiticus*. These are well-defined species: A. flavus produces only B aflatoxins, while A. parasiticus produces both B and G aflatoxins \(^{11,16}\). The major hosts of *A. flavus* among food and feed commodities are peanuts, corn, cottonseed and protein sources such as rapeseed meal, cottonseed meal, groundnut cake, sunflower cake, copra meal and palm kernel meal \(^{17,18}\). Feed is the major financial input in poultry production, amounting to 60-70\% of the total cost \(^3\). Generally, poultry feed contains 40-60\% grains, mainly corn, rice and wheat. According to the FAO, approximately 25\% of the world’s grain supply is contaminated with mycotoxins \(^4\).

Twenty different aflatoxins have been identified, with the major ones being B1, B2, G1, and G2, (14). Aflatoxin B1 (AFB1) is the most prevalent toxin in cereals used in feeds and presents the greatest toxigenic threat \(^{12}\). The AFB1 is an active hepatocarcinogen, which may be due to their inhibition of nucleic acid synthesis by either direct interaction with enzymes involved or by a toxin-DNA template \(^{26}\). AflatoxinB1 is rapidly absorbed from the small intestine into the mesenteric venous blood in poultry \(^7\). Only a few percentages or less of the ingested dose was found to be excreted unchanged in several species \(^{13}\).

Natural occurrence of aflatoxin in feed ingredients varies with growing and storage conditions, usually between 0 and 1 mg/kg. The aflatoxin problem has been reported to be more serious in tropical and subtropical regions of the world where climatic conditions of temperature and relative humidity favour the growth of *A. flavus / parasiticus*. Pakistan is located in the northwestern part of the South Asian subcontinent. Conditions favorable for natural aflatoxin contamination of foods occur at latitudes between 40°N and 40°S of the equator \(^{25}\). Pakistan lies between 24° and 40° north latitudes and its climate is conducive for growth of fungus. Poultry feed in the country is almost entirely dependent upon agricultural by-products \(^1\). Usually, and particularly in the developing countries, the best quality grains and cereals are exported or reserved for human consumption, whereas the poorer quality harvests are consumed for the production of animal feeds \(^{10}\). Moreover, inadequate storage facilities, humid environment and elevated temperature, particularly from May to November, are conducive for the growth of fungi, such as, *Aspergillus* species, which produce mycotoxins in these conditions \(^{20}\). Pakistan’s environment has been reported to favour aflatoxicosis, which is quite common in commercial broilers, breeders and layers \(^2,22\).

The aim of this paper is to report of the occurrence of AFTB1 in major animal production areas in Punjab. This is the first comprehensive report on the determination of AFTB1 in poultry feed raw material and finished feed from Punjab, Pakistan.
Materials and Methods

Samples and sampling procedure:
Samples of raw materials and finished feed were taken directly at poultry farms or poultry feed production sites, and analyzed at the feed testing laboratory, Poultry Research Institute, Rawalpindi during the period from June 2006 to June 2009. Sampling procedure followed the principles of the Romer® guide on “Sampling and sample preparation for mycotoxin analysis” 19. First it was collected a lot sample (500 g) which was composed from several small samples (100 g each) taken randomly from the whole lot. After grinding the full lot sample, a sub-sample is taken for the actual analytical process. A questionnaire was also designed for collecting information’s including date of collection, type of feed/ingredients, place, name of feed mill/farm, date of manufacture/purchase of feed, batch number, brand name, number of feed bags out of which sample collected, sample weight, storage conditions, number of birds at poultry farm, breed, age, mortality of flock etc.

Reagents:
Aflatoxin B₁ standard was purchased from Biopure® Referenzsubstanzen GmbH, Austria. Other chemicals were purchased from Merck AG, Germany.

Sample preparation and clean-up procedures:
For AFB1 clean-up, a total of 25 g of the sample was milled (for grinding and sub-sampling was accomplished using a Lab mill-1 QC-114, Hungary). Milled samples were well-mixed and extracted with 100 ml of acetonitrile: water (84:16) using a blender (USA) at high speed for 3 minutes. The extract was filtered through a folded filter (Whatman®, No. 1001185, England) and 4 ml of the filtrate was slowly pressed through a Mycosep® column number 226 was used and the residue was evaporated.

Thin layer chromatography (TLC):
The residue after evaporation was dissolved in toluene:acetonitrile 97:3 and then sample was spotted against the standard solution (0.4 ug/ml AFB1) with the use of Autospotter on TLC plate at 60°C, along with a standard series corresponding to 25, 50, 100, and 200 ng AFB1. The plate was developed in chloroform: acetone (9:1) to about 1 cm from the top of the plate, dried and dipped into methanol/sulphuric acid 90:10. After heating at 150°C spots became visible under long wave UV light (365nm), with reference to the standard spots.
All data were determined for average and median by using the SPSS version 9.5 (SPSS, Cary, NC, USA) statistical analysis program.
Results

In order to get a better overview about AFTB1 occurrence in certain Districts of the Punjab, data were grouped as follows:

North Area: Islamabad, Attock, Chakwal, Dina, Gujrat, Rawalpindi, Sargodha, Murree, Gujar Khan, Gujranwala, Jhelum, Sialkot, Khoshab, Johar Abad.

Central Area: Jhang, Kamalia, Summandri, Gojra, Toba Tek Singh, Faisalabad.

South Area: Bahawalpur, Lodhran, Multan, Hasilpur, Vehari.

West Area: Bhakkar, Mianwali, Dera Ghazi Khan, Layyah, Mianwali, Muzafargarh.

East Area: Arifwala, Bahawalnagar, Lahore, Pakpatton, Sahiwal, Nankana Sahib, Sheikupura, Kasur, Okara.

Aflatoxin B1 occurrence in respect of the sourcing areas:

A total of 639 samples of feed ingredients and finished feed were received from North area and analyzed. Table 1 gives an overview of numbers of analytical tests performed on samples with regard to their sourcing areas. Maximum samples were registered from North area because Feed Testing Laboratory, Poultry Research Institute exists in this area, routine samples were also included in these analysis. Table 1 gives the respective arithmetic mean, median and maximum levels detected. The frequency of samples expressed as a percentage, positive for AFTB1 was 63 % (405 out of 639). The highest level detected was in both cotton seed meal and fish meal samples sourced Chakwal and Rawalpindi areas with 156 µg/kg. The average contamination level for AFTB1 as accounted by arithmetic mean was 30 µg/kg. The median level (calculated from all positives) was 26 µg/kg.

A total of 92 analyses were performed on samples sourced in South area, with 49 % (45 of 92) detected as positive. The highest AFTB1 level detected was 39 µg/kg in guar meal and layer feed samples from Lodhran and Bahawalpur, respectively. Mean and median levels were 24 and 26 µg/kg, respectively.

Despite the relatively low number of samples received from West area (77), there was high toxin occurrence (56 %). All samples from this area were finished poultry feed. Broiler starter feed sample from Bhakkar yielded the highest level of AFTB1 at 76 µg/kg. Mean and median levels were 34 and 26 µg/kg, respectively.

Only 4 poultry ingredients samples (corn, canola meal, sunflower meal and rape seed meal) out of 86 samples (82 finished poultry feed) were received from East area. The prevalence of AFTB1 was 53 % (46 of 86), with a median contamination positive tests was 19 µg/kg. The highest AFTB1 level detected was 78 µg/kg in broiler starter feed sample from Bahawalnagar. Out of 4 feed ingredients, AFTB1 was detected in only one sample (rape seed meal) at a very low level of
19µg/kg.
A total of 127 feed ingredients and finished feed samples were received from Central area. Maximum samples were registered from Central area after North area because this area is rich in poultry population. Most of poultry farmers are prepared feed by using home mixing plants. The incidence of AFTB1 was 69 % (87 of 127), which is relatively high than other areas. The highest AFTB1 level detected was 80 µg/kg in layer feed sample from Kamalia, Distt. Toba Tek Singh. Mean and median levels were 27 and 26 µg/kg, respectively.

**Aflatoxin B1 occurrence in respect of the commodities:**
Table 2 & 3 give overview of contamination of feed ingredients and finished feed tested, stating the total number of each commodity tested, the number of positives, arithmetic mean and median of positive samples as well as the maximum level identified per commodity.

**Aflatoxin B1 occurrence in feed ingredients:**
A total of 344 different feed ingredients samples were received and analyzed. Overall incidence of AFTB1 in feed ingredients samples was 58 %. The average contamination level, median and maximum level were 30, 27 and 156 µg/kg, respectively.
Corn was the most commonly used feed ingredient accounting incidence for 38 % (11 of 29) of all areas samples analyzed. The average contamination level, median and maximum level were 31, 20 and 80 µg/kg, respectively. The above-mentioned average was mainly due to a single highly contaminated sample from North area (Rawalpindi).
For all 22 rice broken samples tested, AFTB1 was found in 59 % (maximum of 26 µg/kg, median 19 µg/kg). However, occurrence and concentration of aflatoxin B1 was relatively high in rice polishing samples (64 %) compared to rice broken samples (maximum of 56 µg/kg, median 21 µg/kg).
There was no evidence of AFTB1 contamination in the 4 wheat samples tested and only low incidence (1.0%) was observed (data not mentioned in the table). Generally, in Pakistan, wheat as an ingredient is not included in the poultry feed due to direct competition with human diet. However, out of 19 wheat bran samples, 6 samples were found positive (maximum of 39 µg/kg, median 19 µg/kg).
Only 10 cotton seed meal samples were tested, with all contaminated by aflatoxin B1, at an average level of 50 µg/kg and median of 56 µg/kg.
Maximum number of canola meal samples (50) were received and analysed. About half of the samples were found positive for aflatoxin B1. The average contamination level, median and maximum level was 25, 20 and 78 µg/kg, respectively.
Maximum incidence of AFTB1 (84 %) was found in guar meal samples (with an average 24 µg/kg, median 20 µg/kg and maximum level 58 µg/kg). Maximum samples were received from north area (Islamabad). However, concentration of toxin was almost at par with other vegetable protein sources.
Soybean meal is the most commonly used feed ingredient as vegetable protein source due to ideal amino acid profile. For all 36 soybean meal samples tested, occurrence and concentration of toxin was relatively high (incidence 72 %, mean 28 and maximum level 76 µg/kg).
A total of 26 sunflower meal samples were provided indicating medium toxin occurrence. The average concentration, median and maximum level was 20, 24 and 78 µg/kg, respectively.

Aflatoxin B1 was a common contamination of corn gluten meal (30 & 60%) samples with a contamination rate of 55 %. The maximum level detected was 56 µg/kg.

Only 13 rape seed meal samples were tested, with eight contaminated by AFTB1, at an average level of 21 µg/kg and a median of 19 µg/kg. The maximum level was 26 µg/kg received from Central area (Gojra, Distt.Toba Tek Singh).

Fish meal is the most commonly used feed ingredient as animal protein source due to balance/standard amino acid profile. A total of 32 fish meal samples were received which had high contaminated levels and incidence (156 µg/kg & 75 %, respectively).

Only 4 bone meal samples were provided indicating one positive sample yielded high toxin value (78 µg/kg).

**Aflatoxin B1 occurrence in finished feed:**
A total of 677 different finished feed samples were received from different areas and tested for AFTB1 detection. The results gathered from finished feed samples, the incidence of aflatoxin B1 was 66 %. The average contamination level, median and maximum level were 22, 26 and 80 µg/kg, respectively. The results showed that incidence of AFTB1 were more in the finished feed samples than those of feed ingredients. However, mean concentration of AFTB1 was less in the finished feed samples compared to feed ingredients.

Despite the relatively low number of chick starter (4); layer breeder (11); grower layers (6) and broiler breeder layer (9) feeds samples in crumbs form, there was a clear indication of high AFTB1 incidence (100, 82, 83 and 89 %, respectively). The highest level found was 80 µg/kg in layer breeder (crumb) sample from North area (Chakwal).

The samples of layer chick starter; layer; broiler breeder starter and broiler breeder grower; broiler breeder layer feeds in mash forms and layer & layer chick starter in crumbs forms had almost same occurrence of contamination (73 %). The highest level of contamination found was 78 µg/kg in both layer and broiler breeder starter feeds in mash forms. Maximum samples (103) of layer feed in crumbs form were received for analysis, however, maximum level, mean and median was relatively low (40, 19 & 19 µg/kg, respectively).

A total of 72 samples of broiler starter (mash) feed were analysed, with 42 % detected as positive, which is the lowest numbers among all finished feeds samples. The maximum level was observed as 76 µg/kg in only one sample received from West area (Bhakkar). The mean and median was 13, 26 µg/kg, respectively.

The samples of layer grower; broiler finisher; layer breeder layer feeds (mash forms) and broiler starter; broiler finisher and broiler breeder male feeds (crumbs forms) were detected almost 50-60% as positive. Maximum level found was 80 µg/kg in both the samples of layer breeder layer (mash) and broiler starter (crumbs).

**Discussion**
In summary, overall 61% of all aflatoxin B1 tests conducted on samples received from all selected survey areas tested positive. The positive AFTB1 evidenced in North, South, West, East...
and Central areas were 63, 49, 56, 53 and 69 %, respectively. Mean levels of AFTB1 in feed ingredients and finished feeds (except layer grower, broiler starter, broiler finisher in mash forms and layer feed in crumb forms) were noted to be higher than safe limit of 20 µg/kg recommended by FDA. It was observed that variations in the levels of AFTB1 in poultry feeds and ingredients were due to marked fluctuations in the environmental temperature and humidity conditions during the course of the year. *Aspergillus flavus* is not normally present at harvest and prevention of the formation of aflatoxins therefore relies mainly on avoidance of contamination after harvest, using rapid drying and good storage practice. Presumably, the constituted feeds stored under appropriate conditions were subject to lesser direct influence of temperature and humidity. However, increased production of AFTB1 in feedstuffs may be expected if the storage was for a longer period under unsatisfactory ventilation and storage conditions. Thus it can be concluded that the incidence of AFTB1, relevant for poultry production, is quite high in poultry feed, although an assessment of the relevance of levels occurring is difficult to undertake.

During 2004 and 2005, a survey was conducted to study the incidence of aflatoxin in various feed ingredients and finished feeds collected from different states of the India. Out of 984 samples analyzed, 824 samples were found to be positive for the presence of aflatoxin, ochratoxin and T-2 toxin. Of these, 91, 94, 97 and 97% of cereals, cereal by-products, oilseed meals and finished feeds, respectively, tested positive for aflatoxins.

In another survey of Asian feed ingredient sources, 1200 raw ingredients and feed samples collected from Pakistan, Bengal, China, Korea, Malaysia, the Philippines, Singapore, Sri Lanka, Thailand and Vietnam between 1998 and 2001 were analyzed for aflatoxin B1. Twenty-nine samples of feed were received from Pakistan and analyzed. The average contamination and maximum level was found 109 and 585 µg/kg, respectively. This concentration of contamination was much more than the present survey, quantum of samples was too small and highly contaminated samples were used in above-mentioned report.

Zinedine *et al.* reported that corn and poultry feed samples for AFB1 were analyzed, it was noted that level of contamination ranged from 0.23 to 11.2 and 0.05 to 5.38 ng/g for corn and poultry feeds respectively, the results showed that the contamination of 10% of samples of corn was higher than the limit set by EU regulations for AFB1. In the present study, corn was accounting incidence of AFB1 for 38%, which was higher than the above report.

Mycotoxin surveys from around the world indicate that protein sources such as rapeseed meal, cottonseed meal, canola meal, sunflower meal, corn gluten meal and guar meal are more susceptible to aflatoxins contamination than conventional raw materials such as soybean meal. Owing to high prices of conventional raw materials during certain years, feed manufacturers have been forced to opt for alternatives to soybean meal and this has increased the potential for aflatoxicoses for poultry. Similarly, the cost of corn has forced a look at other energy sources, including byproducts such as rice bran, wheat bran and rice polishing. The rising cost of the traditional mainstay ingredients, corn and soybean meal, has forced producers to consider greater use of by-products. This is likely to further increase the prevalence of aflatoxicoses since these alternative feed ingredients are more likely to contain aflatoxins. Trade in raw ingredients around the region exacerbates the problem and makes prediction of aflatoxin incidence difficult.

Diagnosis of poultry mycotoxicosis is based on experimental studies with specific toxins and
specific age of poultry, very often under well-defined toxicological laboratory conditions, so that the results of such studies can be far from real-life or natural situation. Furthermore factors such as breed, sex, environment, nutritional status, as well as other toxic entities can affect the symptoms of intoxication and may contribute to the significance of aflatoxins damage on economic output and poultry health. Diagnosis is very much dependent on receiving a sample of feed that was ingested prior to intoxication, but also on data from another representative group of animals of the facility and results of a post-mortem examination.

The economic costs of mycotoxins are impossible to determine accurately but the US Food and Drug Administration (FDA) estimated based on a computer model, that in the US the mean economic annual cost of crop losses from the mycotoxins aflatoxins, fumonisins, and deoxynivalenol are $932 million USD.

Table 1 Aflatoxin B1 contamination levels (µg/ kg) detected in samples from different areas in Punjab, Pakistan

<table>
<thead>
<tr>
<th>Areas</th>
<th>Mean</th>
<th>Median</th>
<th>Maximum level</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>30</td>
<td>26</td>
<td>156</td>
</tr>
<tr>
<td>South</td>
<td>24</td>
<td>26</td>
<td>39</td>
</tr>
<tr>
<td>West</td>
<td>34</td>
<td>26</td>
<td>76</td>
</tr>
<tr>
<td>East</td>
<td>27</td>
<td>19</td>
<td>78</td>
</tr>
<tr>
<td>Central</td>
<td>27</td>
<td>26</td>
<td>80</td>
</tr>
</tbody>
</table>

While mycotoxin associated losses in industrial countries are typically market losses as a result of rejected crops, necessary redirection of their use or even disposal in cases of severe contamination, developing countries suffer additionally from health impacts. Chronic exposure to high levels of mycotoxins, often combined with malnourishment, causes different levels of toxicosis including death.

The findings of the present study highlight the risk to which poultry industry exposed to poultry feed and ingredients contaminated with aflatoxin. These data be used for risk assessment that can
serve as a basis for establishing particular regulatory limits to minimize the level of aflatoxin contamination in the poultry feed and thereby protect the human and animal health.

Table 2 Incidence of Aflatoxin B1 contamination in poultry feed ingredients sourced in Punjab, Pakistan

<table>
<thead>
<tr>
<th>Feed Ingredients</th>
<th>Total No of Samples analyzed</th>
<th>No of Positive Samples</th>
<th>Mean (µg/kg)</th>
<th>Median (µg/kg)</th>
<th>Max Level (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>29</td>
<td>11</td>
<td>31</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Rice broken</td>
<td>22</td>
<td>13</td>
<td>21</td>
<td>19</td>
<td>26</td>
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<tr>
<td>Rice polish</td>
<td>28</td>
<td>18</td>
<td>23</td>
<td>21</td>
<td>56</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>19</td>
<td>6</td>
<td>24</td>
<td>19</td>
<td>39</td>
</tr>
<tr>
<td>Cotton seed meal</td>
<td>10</td>
<td>10</td>
<td>50</td>
<td>56</td>
<td>156</td>
</tr>
<tr>
<td>Canola meal</td>
<td>50</td>
<td>26</td>
<td>25</td>
<td>20</td>
<td>78</td>
</tr>
<tr>
<td>Guar meal</td>
<td>25</td>
<td>21</td>
<td>24</td>
<td>20</td>
<td>58</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>36</td>
<td>26</td>
<td>28</td>
<td>19</td>
<td>76</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>26</td>
<td>14</td>
<td>20</td>
<td>24</td>
<td>78</td>
</tr>
<tr>
<td>Corn gluten meal (30%)</td>
<td>22</td>
<td>12</td>
<td>29</td>
<td>26</td>
<td>56</td>
</tr>
<tr>
<td>Corn gluten meal (60%)</td>
<td>28</td>
<td>14</td>
<td>26</td>
<td>23</td>
<td>40</td>
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<tr>
<td>Rape seed meal</td>
<td>13</td>
<td>4</td>
<td>21</td>
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<td>Fish meal</td>
<td>32</td>
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<td>Bone meal</td>
<td>04</td>
<td>1</td>
<td>78</td>
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</table>
Table 3 Incidence of Aflatoxin B₁ contamination in poultry feed sourced in Punjab, Pakistan

<table>
<thead>
<tr>
<th>Feed</th>
<th>Total No of Samples analyzed</th>
<th>No of Positive Samples</th>
<th>Mean (µg/ kg)</th>
<th>Median (µg/ kg)</th>
<th>Max Level (µg/ kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer Chick Starter (Mash)</td>
<td>62</td>
<td>44</td>
<td>20</td>
<td>26</td>
<td>76</td>
</tr>
<tr>
<td>Layer Grower (Mash)</td>
<td>14</td>
<td>7</td>
<td>14</td>
<td>19</td>
<td>56</td>
</tr>
<tr>
<td>Layer (Mash)</td>
<td>89</td>
<td>66</td>
<td>23</td>
<td>26</td>
<td>78</td>
</tr>
<tr>
<td>Broiler Starter (Mash)</td>
<td>72</td>
<td>30</td>
<td>13</td>
<td>26</td>
<td>76</td>
</tr>
<tr>
<td>Broiler Finisher (Mash)</td>
<td>14</td>
<td>9</td>
<td>19</td>
<td>26</td>
<td>39</td>
</tr>
<tr>
<td>Broiler Breeder Starter (Mash)</td>
<td>11</td>
<td>8</td>
<td>30</td>
<td>33</td>
<td>78</td>
</tr>
<tr>
<td>Broiler Breeder Grower (Mash)</td>
<td>9</td>
<td>7</td>
<td>24</td>
<td>19</td>
<td>76</td>
</tr>
<tr>
<td>Broiler Breeder Layer (Mash)</td>
<td>43</td>
<td>31</td>
<td>21</td>
<td>26</td>
<td>60</td>
</tr>
<tr>
<td>Layer Breeder Layer (Mash)</td>
<td>47</td>
<td>32</td>
<td>20</td>
<td>23</td>
<td>80</td>
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<tr>
<td>Chick Starter (Crumbs)</td>
<td>4</td>
<td>4</td>
<td>44</td>
<td>39</td>
<td>58</td>
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<td>Layer Chick Starter (Crumbs)</td>
<td>30</td>
<td>22</td>
<td>21</td>
<td>26</td>
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<tr>
<td>Grower Layer (Crumbs)</td>
<td>6</td>
<td>5</td>
<td>29</td>
<td>19</td>
<td>78</td>
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<tr>
<td>Layer (Crumbs)</td>
<td>103</td>
<td>75</td>
<td>19</td>
<td>19</td>
<td>40</td>
</tr>
<tr>
<td>Broiler Starter (Crumbs)</td>
<td>96</td>
<td>56</td>
<td>18</td>
<td>26</td>
<td>80</td>
</tr>
<tr>
<td>Broiler Finisher (Crumbs)</td>
<td>54</td>
<td>31</td>
<td>19</td>
<td>26</td>
<td>78</td>
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<tr>
<td>Broiler Breeder Male (Crumbs)</td>
<td>3</td>
<td>2</td>
<td>22</td>
<td>33</td>
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<tr>
<td>Broiler Breeder Layer (Crumbs)</td>
<td>9</td>
<td>8</td>
<td>23</td>
<td>23</td>
<td>39</td>
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<td>Layer Breeder (Crumbs)</td>
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<td>9</td>
<td>23</td>
<td>20</td>
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References


