**SUPPORTING INFORMATION**

**The Impact of Probiotics and Egg Yolk IgY on Behavior and Blood Parameters in a Broiler Immune Stress Model**

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**TABLE S1** The statistics of standing activity time (sec/ 30 min) at 3rd week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen stand** | **Stand** | **Stand eat** | **Peck body** | **Peck head** | **Peck litter** | **Drink** | **Walk** | **Jump** | **Run** | **Total** |
| **1** | **Control** | 21.4 | 198.4 | 305.3 | --- | --- | 81.2 | 101.3 | 115.6 | --- | --- | 823.2±31.7 |
| **2** | **Probiotic** | 23.4 | 199.5 | 272.3 | 9.4 | --- | 70.3 | 84.5 | 139.3 | --- | 7.0 | 805.7±30.4 |
| **3** | **IgY** | 22.5 | 169.0 | 240.6 | 15.0 | --- | 21.6 | 109.9 | 61.7 | --- | --- | 640.3±27.4 |
| **4** | **Probiotic + IgY** | 12.0 | 177.0 | 340.3 | 3.8 | --- | 50.0 | 102.4 | 101.4 | --- | 5.3 | 792.2±36.2 |
| The mean time of behavioural patterns such as standing activity (e.g., preen stand, stand, stand eat, peck body, peck head, peck litter, drink, walk, jump and run) were recorded. Standing activity and lying time were presented as mean ± SEM. IgY: immunoglobulin Y; (---): not detected. | | | | | | | | | | | | |

**TABLE S2** The statistics of lying time (sec/ 30 min) at 3rd week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen lie** | **Lie/Rest** | **Lie eat** | **Lie sleep** | **Leg stretch** | **Wing stretch** | **Leg/wing stretch** | **Wing flap** | **Total** |
| **1** | **Control** | 32.2 | 721.1 | 121.0 | --- | 7.5 | 6.7 | 7.0 | 2.7 | 898.2±93.6 |
| **2** | **Probiotic** | 25.2 | 696.7 | --- | 299.9 | 2.8 | 5.8 | 5.5 | 5.5 | 1041.4±93.6 |
| **3** | **IgY** | 18.8 | 724.7 | 125.3 | 315.1 | --- | 5.5 | 4.2 | 4.3 | 1197.9±95.2 |
| **4** | **Probiotic + IgY** | 15.8 | 666.1 | 151.0 | 300.7 | --- | 4.2 | 5.9 | 4.1 | 1147.8±87.6 |
| The mean time of behavioural patterns such as rest/lying (e.g., preen lie, lie, lie eat, lie sleep, leg stretch, wing stretch, leg and wing stretch, wing flap) were recorded and analyzed using one-way-ANOVA. Standing activity and lying time were presented as mean ± SEM. IgY: immunoglobulin Y; (---): not detected. | | | | | | | | | | |

**TABLE S3** The statistics of frequency of activity (bout/ 30 min) at 3rd week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen stand** | **Stand** | **Stand eat** | **Peck body** | **Peck head** | **Peck litter** | **Drink** | **Walk** | **Jump** | **Run** | **Total** |
| **1** | **Control** | 3.2 | 5.0 | 5.6 | --- | --- | 5.0 | 3.7 | 11.6 | --- | --- | 34.1±0.9 |
| **2** | **Probiotic** | 2.6 | 7.4 | 4.0 | 3.1 | --- | 4.6 | 3.9 | 13.7 | --- | 2.5 | 41.8±1.2 |
| **3** | **IgY** | 3.3 | 7.4 | 3.9 | 1.8 | --- | 3.3 | 3.8 | 10.1 | --- | --- | 33.6±0.9 |
| **4** | **Probiotic + IgY** | 2.9 | 8.7 | 6.3 | 1.3 | --- | 4.8 | 5.0 | 16.1 | --- | 1.8 | 46.9±1.5\* |
| The mean frequency of behavioural patterns such as standing activity (e.g., preen stand, stand, stand eat, peck body, peck head, peck litter, drink, walk, jump and run) were recorded and analyzed using one-way-ANOVA. Standing activity frequency was presented as mean ± SEM. IgY: immunoglobulin Y; (\*), *P*< 0.05. (---): not detected. | | | | | | | | | | | | |

**TABLE S4** The statistics of frequency of lying (bout/ 30 min) at 3rd week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen lie** | **Lie/Rest** | **Lie eat** | **Lie sleep** | **Leg stretch** | **Wing stretch** | **Leg/wing stretch** | **Wing flap** | **Total** |
| **1** | **Control** | 3.3 | 7.9 | 2.0 | --- | 1.3 | 2.3 | 2.0 | 1.7 | 20.5±0.8 |
| **2** | **Probiotic** | 3.4 | 6.9 | --- | 1.2 | 1.5 | 1.9 | 2.3 | 2.8 | 20±0.7 |
| **3** | **IgY** | 3.8 | 6.9 | 1.6 | 3.1 | --- | 2.6 | 1.6 | 2.1 | 21.7±0.6 |
| **4** | **Probiotic + IgY** | 2.8 | 7.3 | 3.4 | 2.3 | --- | 1.9 | 2.3 | 2.1 | 22.1±0.7 |

The mean frequency of behavioural patterns such as rest/lying (e.g., preen lie, lie, lie eat, lie sleep, leg stretch, wing stretch, leg and wing stretch, wing flap) were recorded and analyzed using one-way-ANOVA. lying frequency was presented as mean ± SEM. IgY: immunoglobulin Y; (---): not detected.

**TABLE S5** The statistics of standing activity time (sec/ 30 min) at 4th week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen stand** | **Stand** | **Stand eat** | **Peck body** | **Peck head** | **Peck litter** | **Drink** | **Walk** | **Jump** | **Run** | **Total** |
| **1** | **Control** | --- | 24.7 | 255.3 | 2.2 | --- | 3.1 | 189.3 | 41.1 | --- | --- | 515.7±34.3 | |
| **2** | **Probiotic** | --- | 3.5 | 86.0 | 1.0 | --- | 1.7 | 94.2 | 14.9 | --- | --- | 201.3±13.9 | |
| **3** | **IgY** | --- | 48.5 | 371.8 | --- | --- | --- | 81.8 | 41.9 | --- | --- | 544±50.01 | |
| **4** | **Probiotic + IgY** | --- | 61.5 | 606.8 | 125 | --- | 200 | 166.1 | 183.8 | --- | --- | 1343.2±61.4\* | |
| The meantime of behavioural patterns such as standing activity (e.g., preen stand, stand, stand eat, peck body, peck head, peck litter, drink, walk, jump and run) were recorded and analyzed using one-way-ANOVA. Standing activity was presented as mean ± SEM. IgY: immunoglobulin Y. (\*), *P*< 0.05; (---): not detected. | | | | | | | | | | | | | |

**TABLE S6** The statistics of lying time (sec/ 30 min) at 4th week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen lie** | **Lie/Rest** | **Lie eat** | **Lie sleep** | **Leg stretch** | **Wing stretch** | **Leg/wing stretch** | **Wing flap** | **Total** |
| **1** | **Control** | 147.3 | 684.1 | 378.2 | 198.9 | 4.2 | 1.4 | --- | --- | 1414.1±92.06 |
| **2** | **Probiotic** | 129.2 | 246.7 | 299.4 | 101.2 | 2.9 | 0.2 | --- | --- | 779.6±43.6 |
| **3** | **IgY** | 49.8 | 1147.6 | 44.0 | --- | --- | --- | 4.1 | --- | 1245.5±197.2 |
| **4** | **Probiotic + IgY** | 59 | 361.6 | 34.0 | --- | --- | --- | 2.1 | --- | 456.7±58.8\* |
| The meantime of behavioural patterns such as rest/lying (e.g., preen lie, lie, lie eat, lie sleep, leg stretch, wing stretch, leg and wing stretch, wing flap) were recorded and analyzed using one-way-ANOVA. Lying time was presented as mean ± SEM. IgY: immunoglobulin Y; (\*), P< 0.05; (---): not detected. | | | | | | | | | | |

**TABLE S7** The statistics of frequency of activity (bout/ 30 min) at 4th week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen stand** | **Stand** | **Stand eat** | **Peck body** | **Peck head** | **Peck litter** | **Drink** | **Walk** | **Jump** | **Run** | **Total** |
| **1** | **Control** | --- | 1.9 | 2.0 | 2.1 | --- | 1.6 | 3.8 | 5.0 | --- | --- | 16.4±0.4 |
| **2** | **Probiotic** | 1.5 | 5.7 | --- | --- | --- | --- | 3.9 | 9.8 | --- | --- | 20.9±1.1 |
| **3** | **IgY** | 4 | 6 | 3 | 4 | --- | --- | 5.45 | 5 | --- | --- | 27.45±0.3 |
| **4** | **Probiotic + IgY** | 3 | 5 | 4 | 3 | --- | 1 | 4.48 | 5 | --- | --- | 25.48±0.4 |
| The mean frequency of behavioural patterns such as standing activity (e.g., preen stand, stand, stand eat, peck body, peck head, peck litter, drink, walk, jump and run) were recorded and analyzed using one-way-ANOVA. Standing activity frequency was presented as mean ± SEM. IgY: immunoglobulin Y; (---): not detected. | | | | | | | | | | | | |

**TABLE S8** The statistics of frequency of lying (bout/ 30 min) at 4th week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen lie** | **Lie/Rest** | **Lie eat** | **Lie sleep** | **Leg stretch** | **Wing stretch** | **Leg/wing stretch** | **Wing flap** | **Total** |
| **1** | **Control** | 2.8 | 5.2 | 1.7 | --- | --- | 1.2 | --- | --- | 10.9±0.6 |
| **2** | **Probiotic** | 3.9 | 7.6 | --- | --- | --- | 1.3 | 2.5 | --- | 15.3±0.9 |
| **3** | **IgY** | 3 | 7 | 1.07 | --- | 1 | 1 | 2 | --- | 15.07±0.8 |
| **4** | **Probiotic + IgY** | 2 | 6 | 1 | --- | 2 | 1.6 | --- | --- | 12.6±0.7 |
| The mean frequency of behavioural patterns such as rest/lying (e.g., preen lie, lie, lie eat, lie sleep, leg stretch, wing stretch, leg and wing stretch, wing flap) were recorded and analyzed using one-way-ANOVA. Lying frequency was presented as mean ± SEM. IgY: immunoglobulin Y; (---): not detected. | | | | | | | | | | |

**TABLE S9** The statistics of standing activity time (sec/ 30 min) at 5th week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen stand** | **Stand** | **Stand eat** | **Peck body** | **Peck head** | **Peck litter** | **Drink** | **Walk** | **Jump** | **Run** | **Total** |
| **1** | **Control** | --- | 39.3 | 284.3 | 48.2 | --- | 43.2 | 145.1 | 35.2 | --- | --- | 595.3±31.5 |
| **2** | **Probiotic** | 39.9 | 77.0 | 404.4 | 23.2 | 13.0 | 15.5 | 138.3 | 32.8 | --- | 7.3 | 751.4±40.2 |
| **3** | **IgY** | 15.8 | 72.9 | 261.8 | --- | --- | 39.2 | 200.4 | 66.6 | --- | 18.4 | 675.1±30.4 |
| **4** | **Probiotic + IgY** | 19.2 | 67.4 | 291.1 | 7.3 | --- | 31.0 | 146.6 | 62.1 | --- | --- | 624.7±31.6\*\* |
| The meantime of behavioural patterns such as standing activity (e.g., preen stand, stand, stand eat, peck body, peck head, peck litter, drink, walk, jump and run) were recorded and analyzed using one-way-ANOVA. Standing activity was presented as mean ± SEM. IgY: immunoglobulin Y. (\*\*), P< 0.01. (---): not detected. | | | | | | | | | | | | |

**TABLE S10** The statistics of lying time (sec/ 30 min) at 5th week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen lie** | **Lie/Rest** | **Lie eat** | **Lie sleep** | **Leg stretch** | **Wing stretch** | **Leg/wing stretch** | **Wing flap** | **Total** |
| **1** | **Control** | 43.0 | 923.0 | 48.1 | 202.3 | --- | 4.5 | 6.8 | --- | 1227.7±127.07 |
| **2** | **Probiotic** | 30.2 | 631.4 | 159.5 | --- | --- | 3.6 | 3.7 | 2.4 | 830.8±88.03 |
| **3** | **IgY** | 17.0 | 802.7 | 123.4 | 180.7 | --- | 6.5 | 6.7 | --- | 1137±109.2 |
| **4** | **Probiotic + IgY** | 36.7 | 585.2 | 104.8 | --- | 6.2 | 3.8 | --- | 3.8 | 740.5±81.1 |
| The meantime of behavioural patterns such as rest/lying (e.g., preen lie, lie, lie eat, lie sleep, leg stretch, wing stretch, leg and wing stretch, wing flap) were recorded and analyzed using one-way-ANOVA. Lying time was presented as mean ± SEM. IgY: immunoglobulin Y. (---): not detected. | | | | | | | | | | |

**TABLE S11** The statistics of frequency of activity (bout/ 30min) at 5th week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen stand** | **Stand** | **Stand eat** | **Peck body** | **Peck head** | **Peck litter** | **Drink** | **Walk** | **Jump** | **Run** | **Total** |
| **1** | **Control** | --- | 3.3 | 3.1 | 3.1 | --- | 1.9 | 4.8 | 5.0 | --- | --- | 21.2±0.37 |
| **2** | **Probiotic** | 2.6 | 4.1 | 4.1 | 2.8 | --- | 2.1 | 4.5 | 6.6 | --- | 1.5 | 28.3±0.51 |
| **3** | **IgY** | 3.0 | 5.9 | 4.1 | --- | --- | 3.3 | 3.1 | 9.8 | --- | 2.4 | 31.6±0.81\*\* |
| **4** | **Probiotic + IgY** | 3.3 | 3.3 | 4.0 | 2.5 | --- | 4.3 | 3.1 | 6.3 | --- | --- | 26.8±0.39 |
| The mean frequency of behavioural patterns such as standing activity (e.g., preen stand, stand, stand eat, peck body, peck head, peck litter, drink, walk, jump and run) were presented as mean ± SEM and analyzed using one-way-ANOVA. IgY: immunoglobulin Y. (\*\*), *P*< 0.01; (---): not detected. | | | | | | | | | | | | |

**TABLE S12**The statistics of frequency of lying (bout/ 30min) at 5th week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen lie** | **Lie/Rest** | **Lie eat** | **Lie sleep** | **Leg stretch** | **Wing stretch** | **Leg/wing stretch** | **Wing flap** | **Total** |
| **1** | **Control** | 2.8 | 7.2 | 1.6 | 1.8 | --- | 1.8 | 2.0 | --- | 17.2±0.76 |
| **2** | **Probiotic** | 3.9 | 6.6 | 1.9 | --- | --- | 1.7 | 1.0 | --- | 15.1±0.80 |
| **3** | **IgY** | 2.5 | 6.6 | 3.3 | 1.8 | --- | 1.8 | 2.1 | --- | 18.1±0.65 |
| **4** | **Probiotic + IgY** | 4.1 | 6.9 | 1.9 | --- | 1.1 | 1.6 | --- | --- | 15.6±0.85 |
| The mean frequency of behavioural patterns such as rest/lying (e.g., preen lie, lie, lie eat, lie sleep, leg stretch, wing stretch, leg and wing stretch, wing flap) were recorded and analyzed using one-way-ANOVA. Lying frequency was presented as mean ± SEM. IgY: immunoglobulin Y; (---): not detected. | | | | | | | | | | |

**TABLE S13** The statistics of standing activity time (sec/ 30min) at 6th week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen stand** | **Stand** | **Stand eat** | **Peck body** | **Peck head** | **Peck litter** | **Drink** | **Walk** | **Jump** | **Run** | **Total** |
| **1** | **Control** | --- | 11.7 | 311.0 | 7.0 | 2.7 | 91.0 | 137.3 | 21.9 | --- | 3.1 | 585.7±108.004 |
| **2** | **Probiotic** | 14.7 | 96.2 | --- | 24.9 | --- | 78.4 | 136.3 | 26.3 | --- | --- | 376.8±48.6 |
| **3** | **IgY** | 9.6 | 63.0 | 276.4 | 8.4 | --- | 105.6 | 98.9 | 26.6 | --- | 5.6 | 594.1±91.08 |
| **4** | **Probiotic + IgY** | --- | 55.8 | 229.4 | 9.9 | --- | 184.2 | 216.0 | 18.5 | --- | --- | 713.8±101.83\*\* |
| The meantime of behavioural patterns such as standing activity (e.g., preen stand, stand, stand eat, peck body, peck head, peck litter, drink, walk, jump and run) were recorded and analyzed using one-way-ANOVA. Standing activity time was presented as mean ± SEM. IgY: immunoglobulin Y. (\*\*) P < 0.01; (---): not detected. | | | | | | | | | | | | |

**TABLE S14** The statistics of lying time (sec/ 30min) at 6th week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen lie** | **Lie/Rest** | **Lie eat** | **Lie sleep** | **Leg stretch** | **Wing stretch** | **Leg/wing stretch** | **Wing flap** | **Total** |
| **1** | **Control** | 32.6 | 1047.1 | 479.7 | --- | 97.0 | 4.1 | 12.8 | 5.0 | 1678.3±139.55 |
| **2** | **Probiotic** | 46.9 | 828.0 | 286.3 | --- | 47.7 | 6.0 | 10.1 | 1.9 | 1226.9±107.73 |
| **3** | **IgY** | 49.8 | 844.3 | 241.1 | --- | 58.4 | 4.3 | 10.1 | 3.6 | 1211.6±108.71 |
| **4** | **Probiotic + IgY** | 31.4 | 696.6 | 189.8 | --- | --- | --- | --- | 3.9 | 921.7±113.63\*\* |
| The meantime of behavioural patterns such as rest/lying (e.g., preen lie, lie, lie eat, lie sleep, leg stretch, wing stretch, leg and wing stretch, wing flap) were recorded and analyzed using one-way-ANOVA. Lying time was presented as mean ± SEM. IgY: immunoglobulin Y. (\*\*) *P*< 0.01; (---): not detected. | | | | | | | | | | |

**TABLE S15** The statistics of frequency of activity (bout/ 30min) at 6th week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen stand** | **Stand** | **Stand eat** | **Peck body** | **Peck head** | **Peck litter** | **Drink** | **Walk** | **Jump** | **Run** | **Total** |
| **1** | **Control** | --- | 1.6 | 2.0 | 1.5 | 1.0 | 3.8 | 6.5 | 6.0 | --- | 1.3 | 23.7±0.69 |
| **2** | **Probiotic** | 4.2 | 4.2 | --- | 2.1 | --- | 4.8 | 6.9 | 7.6 | --- | --- | 29.8±0.63 |
| **3** | **IgY** | 2.4 | 2.5 | 3.2 | 1.5 | --- | 5.4 | 9.5 | 8.5 | --- | 2.0 | 35±0.97 |
| **4** | **Probiotic + IgY** | --- | 4.1 | 1.9 | 1.8 | --- | 4.6 | 8.0 | 7.1 | --- | --- | 27.5±0.81 |
| The mean frequency of behavioural patterns such as standing activity (e.g., preen stand, stand, stand eat, peck body, peck head, peck litter, drink, walk, jump and run) were recorded and analyzed using one-way-ANOVA. Standing activity frequency was presented as mean ± SEM. IgY: immunoglobulin Y; (---): not detected. | | | | | | | | | | | | |

**TABLE S16** The statistics of frequency of lying (bout/ 30min) at 6th week of broilers supplemented with variant feed additives.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Group** | **Preen lie** | **Lie/Rest** | **Lie eat** | **Lie sleep** | **Leg stretch** | **Wing stretch** | **Leg/wing stretch** | **Wing flap** | **Total** |
| **1** | **Control** | 4.2 | 7.1 | 2.6 | --- | 1.3 | 1.9 | 2.0 | 1.8 | 20.9±0.72 |
| **2** | **Probiotic** | 4.5 | 7.9 | 2.7 | --- | 1.7 | 1.9 | 1.4 | 1.2 | 21.3±0.85 |
| **3** | **IgY** | 5.1 | 8.5 | 3.2 | --- | 2.7 | 1.9 | 1.4 | 1.9 | 24.7±0.88 |
| **4** | **Probiotic + IgY** | 4.8 | 6.1 | 2.9 | --- | --- | --- | --- | 1.8 | 15.6±0.67 |
| The mean frequency of behavioural patterns such as rest/lying (e.g., preen lie, lie, lie eat, lie sleep, leg stretch, wing stretch, leg and wing stretch, wing flap) were recorded and analyzed using one-way-ANOVA. Lying frequency was presented as mean ± SEM. IgY: immunoglobulin Y; (---): not detected. | | | | | | | | | | |

**TABLE S17** Calculation of mortality% of chicken broilers (n=15/each group) during the experiment.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **No.** | **Group** | **Day** | **Dead** | **Mortality%** |
| **1** | **Control-saline** | 7 | 1 | 13 |
| 34 | 1 |
| **2** | **Probiotic** | --- | --- | 0 |
| **3** | **IgY** | --- | --- | 0 |
| **4** | **Probiotic + IgY** | --- | --- | 0 |
| IgY: immunoglobulin. (---): not detected. | | | | |