**Supplementary information**

**A multi-residue analytical method for assessing the effects of stacking treatment on anti-microbial and coccidiostat degradation in broiler litter**

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**Supplementary information**

**S1 Table.** Antibiotics and coccidiosats approved for use in poultry by national regulatory authorities in the USA, Brazil, China, Poland, United Kingdom, Germany, France, Israel and Spain, based on national reports as described in Section 1.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Coccidiostates** | **Fluoroquinolones** | **beta-Lactams** | **Tetracyclines** | **Macrolides** | **Sulfonamides** |
| Lasalocid | Ciprofloxacin\*\* | Amoxicillin | Chlortertacycline | Tilmicosin | Sulfachloropyrazine |
| Maduramicin | Danofloxacin | Ampicillin | Doxycycline | Tylosin | Sulfachloropyridazine |
| Monensin | Enrofloxacin |  | Oxytetracycline | Erythromycin\* | Sulfadiazine |
| Narasin | Norfloxacin |  | Tetracycline |  | Sulfadimethoxine |
| Salinomycin |  |  |  |  | Sulfisoxazole\* |
| Semduramycin |  |  |  |  | Sulfadimidine |
| Diclazuril |  |  |  |  |  |
| Robenidine |  |  |  |  |  |
| Decoquinate |  |  |  |  |  |
| Clopidol |  |  |  |  |  |
| Nicarbazine |  |  |  |  |  |

Source, Israeli Drug Registry [27].

\* Not approved in Israel

\*\* Metabolite of enrofloxacin and used in humans

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Group** | **Analyte** | **0.1** mg•kg-1  | **0.5 ppm** mg•kg-1 | **1.5 ppm** mg•kg-1 | **RSD %** |
| **Coccidiostats** | Narasin | 76 | 86 | 92 | 4 |
|  | Diclazuril | 95 | 113 | 96 | 10 |
|  | Clopidol | 101 | 103 | 97 | 3 |
|  | Nicrbazine | 80 | 98 | 95 | 10 |
|  | Maduramicin  | 88 | 90 | 84 | 3 |
|  | Monensin  | 87 | 96 | 96 | 5 |
|  | Robenidine  | 116 | 111 | 105 | 6 |
|  | Salinomycin  | 70 | 90 | 95 | 13 |
|  | Semduramycin | 112 | 115 | 101 | 7 |
|  | Lasalocid | 113 | 113 | 101 | 7 |
|  | Decoquinate | 105 | 80 | 105 | 14 |
| **Sulfonamides** | Sulfachloropyrazine | 101 | 102 | 102 | 1 |
|  | Sulfachloropyridazine | 110 | 98 | 101 | 6 |
|  | Sulfadiazine | 112 | 117 | 109 | 4 |
|  | Sulfadimidine | 130 | 112 | 99 | 16 |
|  | Sulfaquinoxaline | 87 | 96 | 98 | 6 |
|  | Sulfadoxine | 124 | 117 | 107 | 9 |
| **Macrolides** | Tilmicosin | 103 | 111 | 104 | 4 |
|  | Tylosin | 84 | 94 | 94 | 6 |
|  | Erythromycin | 116 | 97 | 104 | 10 |
| **Fluoroquinolones** | Danofloxacin | 124 | 106 | 103 | 11 |
|  | Ciproflaxacin | 128 | 105 | 105 | 13 |
|  | Norfloxacin | 85 | 97 | 101 | 8 |
|  | Enrofloxacin | 114 | 119 | 103 | 8 |
| **Tetracyclines** | Doxycycline hyclate | 100 | 102 | 111 | 6 |
|  | Oxytetracycline  | 102 | 95 | 97 | 4 |
|  | Chlortetracycline  | 116 | 110 | 104 | 6 |
|  | Tetracycline | 84 | 118 | 102 | 17 |
| **b-Lactams** | Amoxicillin trihydrate | 102 | 94 | 112 | 9 |
|   | Ampicillin | 96 | 104 | 102 | 4 |

**S2 Table.** Intra-day analytical recovery (in %) of 30 anti-microbials and coccidiostats in poultry litter as described in Section 2.1.1.



**S3 Figure**. Antimicrobial and coccidiostat residues in Israeli broiler litter (2019-2021) as described in Section 2.2.

**S4 Table.** Average poultry litter pH (n=4) as described in Section 2.3.

|  |  |  |  |
| --- | --- | --- | --- |
| Before treatment |  | After treatment |  |
| Upper layer | Middle layer | Upper layer | Middle layer |
| 7.94 | 7.94 | 8.90 | 8.43 |
| 6.86 | 6.37 | 8.66 | 8.98 |
| 7.28 | 7.76 | 9.13 | 9.03 |
| 6.37 | 7.36 | 8.73 | 8.50 |

**S5 Table.** Reference to some drug residue degradation related to temperature and pH in turkey and broiler litter composting methods as described in Section 3.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Drugs** | **Treatment (duration)** | **Treatment** | **Reduction** | **Reference** |
| Chlortetracycline | Windrow composting (35) | turkey | spiked composting  | >99% reduction | Dolliver et al., 2008.  |
| Sulfamethazine | Windrow composting (35) | turkey | spiked composting  | no change |   |
| Monensin | windrow comosting(35) | turkey | spiked composting  | 54% reduction |   |
| Tylosin | windrow composting (35) | turkey | spiked composting  | 76% reduction |   |
| Tilmicosin | in-vessel (40) | broiler | spiked composting  | below MQL | Ho et al., 2013. |
| Sulfadiazine | in-vessel (40) | broiler | spiked composting  | below MQL‡ |   |
| Doxycycline | in-vessel (40) | broiler | spiked composting  | >99% |   |
| Enrofloxacin | in-vessel (40) | broiler | spiked composting  | >99% |   |
| Norfloxacin | in-vessel (40) | broiler | spiked composting  | below MQL (>99%) |   |
| Tylosin | in-vessel (40) | broiler | spiked composting  | below MQL (99%) |   |
| Salinomycin | composting (38) | poultry | excreted composting | 99% reduction | Ramaswamy et al., 2010 |
|   |   |   |   |   |   |
| Narasin | turned (150 days) | poultry | excreted composting | 40% |  |
|   | Piled |   |   | 55% |   |
| Salinomycin | turned (150 days) | poultry | excreted composting | 70% | Munaretto et al., 2016 |
|   | Piled |   |   | 60% |   |
| Monensin | turned (150 days) | poultry | excreted composting | 15% |   |
|   | Piled |   |   | 20% |   |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment** | **Moisture** | **Temperature** | **Duration** | **pH** | **Reference** |
| PL turned | 33-40% | 53°C | 3 days |  | Munaretto et al., 2016 |
| anaerobic-composting | 67°C | 5 days | 7.1-8.1 |  |
|  |  |  |  |  |  |
| PL stock piles | 29-34% | 55°C | 3 days |  | Gupta et al., 2021 |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

**S6 Table.** Reference and case report on intoxication of drug residues in ruminants as described in section 3.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Group**  | **Compound** | **Prevalence**  | **Residue conc.** | **Degradation%** | **Cattle Intoxication**  | **reference** |
|  |  |  | **Median (mg/kg)** | **after stacking treatment** |  **conc. (mg/kg)**  |  |
| Macrolides | Erythromycin | N.D | N.D | In high conc (20 ppm)  |   |   |
|   | Tilmicosin | N.D | N.D | 44-60 |   |   |
|   | Narasin | 20/42 | 2.88 | 43-58 | 100-200 | Alexander et al., 2007 |
|   | Diclazuril | 1/42 | 1.92 | 17-53 |   |   |
|   | Clopidol | N.D | N.D | 58-67 |   |   |
| Coccidiostats | Nicarbazin | 25/42 | 6.06 | 21-49 |   |   |
|   | Maduramicin | 2/42 | 0.28 | 0-10 | <4.8 | Shlosberg et al., 1992 |
|   | Monensin  | 24/42 | 6.97 | 5-27 | <200 | Gonzalez et al., 2005 |
|   | Robenidine  | 6/42 | 1.35 | 52-66 |   |   |
|   | Decoquinate | 3/42 | 0.19 | 0 |   |   |

**S7 Table.** Comparison of antimicrobial and coccidiostat degradation in poultry litter upon drying by oven or lyophilzation described in Section 4.2.

| **Compound** | **Oven to Lyophilisation ratio** | **Control to Lyophilisation ratio** | **Compound** | **Oven to Lyophilisation ratio** | **Control to Lyophilisation ratio** |
| --- | --- | --- | --- | --- | --- |
| Sulfachloropyrazine | 0.28 | 0.95 | Monensin | 0.54 | 0.76 |
| Sulfadimidine | 0.58 | 0.96 | Salinomycin | 0.43 | 0.9 |
| Sulfadiazine | 0.11 | 0.93 | Semduramycin | 0.42 | 0.9 |
| Sulfadoxine | 0.57 | 0.78 | Lasalocid | 0.49 | 0.95 |
| Sulfadimethoxine | 0.53 | 1.1 | Narasin | 0.4 | 0.82 |
| Sulfaquinoxaline | 0.85 | 0.9 | Decoquinate | 0.34 | 0.57 |
| Sulfachloropyridazine | 0.43 | 1 | Robenidine | 0.5 | 0.74 |
| Doxycycilin | 0.07 | 0.95 | Maduramycin | 0.51 | 0.7 |
| Oxytetracycline | 0.53 | 0.62 | Nicarbazine | 0.75 | 0.9 |
| Chlortetracycline | 0.47 | 1.03 | Enrofloxacin | 0.71 | 0.89 |

**S8 Figure.** Calibration curves for some anti-microbials and coccidiostats in fortified PL (n=5) as described in Section 4.4.1.