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Allometric analysis of tylosin tartrate pharmacokinetics in growing male turkeys

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ABSTRACT

Background: Despite common use of tylosin in turkeys, the pharmacokinetic (PK) data for this drug in turkeys is limited. Within a few months of growth, PK of drugs in turkeys undergoes changes that may decrease their efficacy due to variable internal exposure.
Objectives: The objective of this study was to investigate the influence of age on the PK of a single intravenous (i.v.) and oral administration of tylosin to turkeys at a dose of 10 and 50 mg/kg, respectively.
Methods: Plasma drug concentrations were measured using high-performance liquid chromatography with UV detection. The PK parameters were assessed by means of non-compartmental approach and were subjected to allometric analysis.
Results: During a 2.5-month-long period of growth from 1.4 to 14.7 kg, the median value for area under the concentration-time curve after i.v. administration increased from 2.61 to 7.15 mg × h/L and the body clearance decreased from a median of 3.81 to 1.42 L/h/kg. Over the same time, the median elimination half-life increased from 1.03 to 2.96 h. For the oral administration a similar trend was noted but the differences were less pronounced. Bioavailability was variable (5.76%–21.59%) and age-independent. For both routes, the plasma concentration of the major tylosin metabolite, tylosin D, was minimal. Protein binding was age-independent and did not exceed 50%. Allometric analysis indicated a relatively poor predictivity of clearance, volume of distribution and elimination half-life for tylosin in turkeys.
Conclusions: Age has a significant impact on tylosin PK in turkeys and dosage adjustment may be needed, particularly in young individuals.

Keywords: Tylosin; Turkey; allometry; pharmacokinetics; growth

INTRODUCTION

Tylosin is a broad spectrum time-dependent macrolide antibiotic commonly used in companion and farm animals. Its mode of action is based on the inhibition of bacterial protein synthesis that leads to bacteriostatic effect [1]. Typically, the drug is used as a mixture of closely related compounds: tylosin A, B, C and D. Tylosin A is the predominant form (> 80%)

and tylosin D is also the major metabolite being formed *in vivo* [2]. Typical indications include infections caused by *Lawsonia intracellularis* in pigs as well as *Bordetella sp., Pasteurella multocida* and *Mycoplasma spp.* in a wide range of animal species [3-5]. In poultry, primary indications include chronic respiratory disease in chickens and infectious sinusitis in turkeys caused by *Mycoplasma gallisepticum* as well as joint and respiratory disease caused by *Mycoplasma synoviae* [6]. In spite of the fact that tylosin is still considered an important anti-mycoplasmal drug, its use is associated with an increasing problem of acquired resistance in poultry pathogens [7].

There is evidence that tylosin is consistently underdosed on farms [8] which may lead to the development of antimicrobial resistance as a result of selection pressure for more resistant strains [9]. Underdosing on farms may result from human error or variability in drug uptake by the animals. However, it may also be a result of significant physiological changes in treated animals. Several studies indicate that within the short time of massive weight gain in turkeys and broiler chickens, the disposition of drugs is subject to major changes [10-12]. It was reported for metronidazole that the internal exposure (as measured by the area under the concentration-time curve, AUC) after the same dose was 3-fold lower in 5-week-old turkeys as compared to 15-week-old turkeys [11]. Numerous studies exist that describe the pharmacokinetics (PKs) of tylosin in chickens [13-15]. But in the case of turkeys, only a single report on drug residues is available in the peer-reviewed literature [2] and it is not known whether growth has an effect on tylosin PK in this species.

This study aimed at investigating the influence of age and body weight gain on tylosin PK after a single intravenous (i.v.) and oral (p.o.) administration. These interrelations were assessed using allometric scaling and the resulting models were checked for predictivity and possible application for dose adjustments.

MATERIAL AND METHODS

Animals
Three-week-old male broiler turkeys (n = 20) were purchased from a commercial breeding facility in Słupia Kapitulna, Poland. Only male individuals were selected as the model to maximize the effect of weight gain on the PK. The birds were identified individually and housed in an aviary with temperatures ranging between 20°C and 23°C. Commercial diets (without antimicrobial or antiparasitic agents) and water were provided *ad libitum*. Turkeys were clinically examined by a poultry veterinarian upon arrival and during the following two-week acclimatization period and experiments. No abnormalities were found. Turkeys were randomly picked from a homogenous flock and divided into two groups with 10 birds each: one received the drug i.v. and the other was administered orally. The experiment was approved by the Local Animal Experimentation Committee in Wrocław (approval no. 33/2016). All procedures involving animals were performed in accordance with national and international laws and policies including the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (USA). All efforts were made to minimize animals' suffering and to reduce the number of animals used.

PK study
Turkeys were subjected to a single-dose PK studies four times, that is when they reached the age of 5; 9; 12 and 16 weeks (which corresponded to the body weight of: 1.4 ± 0.1; 4.6 ± 0.3; 9.0 ± 0.6 and 14.7 ± 0.8 kg, respectively). All animals were fasted for 10 h before

drug administration and the experiments commenced at 7:00 A.M. Tylosin tartrate (pharmaceutical grade, VETOS-FARMA, Poland) was administered i.v. over 1 min into *vena brachialis* or orally via a soft tube into the crop at a single dose of 10 or 50 mg/kg, respectively (dose calculated per tylosin base). For i.v. administration, blood samples (1 mL) were collected into heparinized syringes by jugular venipuncture before the experiment (blank control) and at 2; 15; 30 min and 1; 2; 4; 6; 8 and 12 h after drug administration. In the p.o. studies, the first blood sampling took place at 7 min and since 15 min all sampling points were the same as in the i.v. study. Plasma was rapidly separated by centrifugation (10 min, 3,000 ×g) and samples were stored at −70°C until assayed.

Determination of tylosin A and D in plasma

Plasma concentrations of tylosin A and D were measured by an in-house developed high-performance liquid chromatography (HPLC) method. Waters Alliance HPLC system (Waters, USA) equipped with a 2996 PDA detector and a Hypersil GOLD aQ (5 μm) 150 × 4.6 mm column (Thermo Fisher Scientific, USA) were used to separate and quantify both compounds. The mobile phase comprised 70% 0.02 M KH_2PO_4 (Sigma-Aldrich, Japan) at pH 2.4 and 30% acetonitrile (J.T. Baker, USA) and was set at a flow rate of 1 mL/min. Tylosin A and D were detected by UV absorption at 286 nm. Retention time for tylosin A was 10.5 min and for tylosin D it was 8.2 min. Plasma samples (0.5 mL) were extracted with 1.5 mL ethyl acetate for 15 min. To facilitate separation, samples were centrifuged at 37,000 ×g for 15 min and frozen in −70°C for 3 min. After that, supernatant was collected and dried in vacuum at 45°C. Dry residues were dissolved in 250 μL of water and transferred to autosampler vials. Plasma concentrations of tylosin A and D were calculated based on calibration curves prepared using blank plasma spiked with analytical standards (Sigma-Aldrich, Germany). Linearity, specificity, recovery, inter-assay and intra-assay coefficient of variation (CV) were assessed. The limit of quantification (LOQ) was determined as 10:1 signal-to-noise ratio, while limit of detection (LOD) was determined at a ratio of 3:1.

Non-compartmental PK analysis

Non-compartmental analysis was used to estimate the PK parameters of tylosin A (TP4.1 software, ThothPro, Poland). Since tylosin D was found at very low concentrations for a very short time, no PK calculations for the metabolite were possible. The area under the concentration–time curve from time 0 to infinity (AUC_{inf}), the area under first moment curve from time 0 to infinity ($AUMC_{inf}$), mean residence time (MRT), body clearance (Cl_B), apparent volume of distribution at steady state (Vd_{ss}), elimination half-life ($T_{1/2el}$) and biological half-life ($T_{1/2}$) were calculated. For the determination of $T_{1/2el}$, at least three last datapoints from the linear portion of the terminal slope were used. Biological half-life ($T_{1/2}$) was calculated as follows: $T_{1/2}$ = MRT × 0.693. For the p.o. study, peak plasma concentration (C_{max}) and the time when it was observed (T_{max}) were assessed. Mean absorption time (MAT) after oral administration was calculated as follows: MAT = median MRTp.o. − median MRTi.v. The bioavailability (F) of orally administered drug was calculated as follows: F (%) = 100 × [(median AUC_{inf}p.o.×Dose i.v.)/(median AUC_{inf}i.v.×Dose p.o.)]. For more information on non-compartmental equations please refer to [16] or standard handbooks for PKs.

Allometric modelling and model validation

PK data for i.v. administration was analysed for correlations to body weight by means of allometric scaling. The absolute values for Cl_B, V_{ss} and $T_{1/2el}$ were plotted against the body weight and a trend line was fitted according to the equation:

$$Y = aBW^b$$

where: Y is the PK parameter; a is the allometric coefficient; BW is the body weight, and b is the allometric exponent [17,18]. The parameters a and b were calculated using linear regression (Excel 2019, Microsoft Corporation, USA) based on the log-transformed allometric equation [19,20]. The resulting model was further subject to the leave-one-out (LOO) cross-validation [21]. The coefficient of determination (R^2) of the observed versus predicted data and squared cross-validated correlation coefficient (Q^2) were determined. Q^2 was calculated according to the formula [21]:

$$Q^2 = 1 - [\frac{\Sigma(Y_{obs} - Y)^2}{\Sigma(Y_{obs} - Y_m)^2}]$$

where: Y_{obs}, observed value for the i-th object; Y, value of the i-th object estimated by the model; Y_m, average value of the validation set. The differences between Q^2 and R^2 were calculated as measure of the internal performance and model predictive ability. Difference of ability between fitting and predictive ability was analysed using the difference between asymptotic Q^2 (Q^2_{asym}) and Q^2. Q^2_{asym} was calculated according to the formula:

$$Q^2_{asym} = 1 - (1 - R^2) \times [\frac{n}{n - n_p}]^2$$

where: R^2, coefficient determination; n, number of the objects (internal validation set); n_p, number of parameters in the final model [21]. Validation acceptance criteria were: $Q^2 \geq 0.65$, $R^2 \geq 0.85$, $Q^2 - R^2 < 0.3$, $Q^2_{asym} - Q^2 > 0$ and $p < 0.05$ in the F test [21,22]. Only a model that simultaneously met all the criteria was considered validated.

Drug-plasma protein binding

Drug-plasma protein binding was assessed using ultrafiltration technique. The centrifugal tubes (Nanosep 10 kDa, Pall, USA) were pre-treated with Tween 20 to prevent nonspecific binding [23]. After the wash, 500 μL of fresh plasma spiked with tylosin A was placed in the tubes and incubated for 1 h at 37°C. Then, samples were centrifuged first at 1,000 g for 10 min, next at 2,000 g for 20 min. The total plasma and ultrafiltrate tylosin A concentration was determined by standard analytical method. Protein binding level was calculated using equation:

$$Protein\ binding\ (\%) = \frac{C_{TP} - C_{FP}}{C_{TP}} \times 100$$

where: C_{TP} is total plasma concentration and C_{FP} is filtrated plasma concentration [24]. Each experiment was made in triplicate.

Statistical analysis

Due to the lack of normal distribution in a significant proportion of the parameters (assessed by Shapiro-Wilk test), all PK parameters are presented as median and range. To determine the statistical significance of the differences, Kruskal–Wallis analysis of variance followed by the median test was used (Statistica 13.3, TIBCO, USA). The differences with $p < 0.05$ were considered significant.

RESULTS

The HPLC method for tylosin A and D was found to be specific and linear ($R^2 = 0.999$ for both drugs). For tylosin A, LOD was 0.003 μg/mL and LOQ was 0.011 μg/mL. For tylosin D, LOD was 0.034 μg/mL and LOQ was 0.104 μg/mL. Assay validation for tylosin A indicated

an intra-assay CV of 2.81; 2.65 and 9.50% at the concentration of 25, 3.13 and 0.39 µg/mL, respectively. For tylosin D intra-assay CV was 5.44; 5.28 and 17.38% at the concentration of 5, 0.63 and 0.15 µg/mL, respectively. For tylosin A inter-assay CV was 2.79; 6.39 and 15.84% at the concentration of 25, 3.13 and 0.39 µg/mL, respectively. For tylosin D inter-assay CV was 7.90; 9.35 and 19.28% at the concentration of 5, 0.63 and 0.15 µg/mL, respectively. The recovery rate for tylosin A and D was 102% and 158%, respectively (at 6.25 µg/mL).

The age-dependent change in the concentration-time profile of tylosin A in turkeys is shown in **Fig. 1**. After i.v. administration (panel A), a distinct distribution phase was followed by a relatively short elimination phase. The duration of both phases increased in an age-dependent manner. A similar trend in the prolongation of the elimination phase was seen after p.o. administration (panel B), although the differences in absorption phase seem not to be related to age. **Table 1** summarises the PK parameters of tylosin A after i.v. administration. The value of AUC significantly increased over time, however, this change seems to be more pronounced at the earlier age. The CV for the pooled AUC values from all age groups was 45.3%. A significant drop in Cl_B developed over time but Vd_{ss} was not affected by age. In contrast, the increase in $T_{1/2el}$ was seen throughout the whole study. Similarly, the median maximal concentration of tylosin D increased steadily. After oral administration, the age-dependent trend in the PK parameters is less clear (**Table 2**). Although the median AUC in the heaviest birds is significantly higher as compared to the youngest ones, the values for the 9- and 12-week-old birds do not follow any visible trend. This seems to be related to very high interindividual variability (CV for the pooled AUC in all age groups was 68.5%). No age-dependent pattern was observed in parameters describing either absorption (MAT, C_{max}, T_{max}, F) or elimination ($T_{1/2el}$, $T_{1/2}$). Plasma protein binding of tylosin A is below 50% and shows no clear age-dependence (**Table 3**).

Fig. 2 shows the allometric models fitted to the empirical data on Cl_B, Vd_{ss} and $T_{1/2el}$. **Table 4** summarizes the performance of these models and the LOO cross-validation outcome. Although all models were statistically significant, neither of them passed stringent validation criteria. The coefficient of determination (R^2) was highest for Cl_B, intermediate for Vd_{ss} and lowest for $T_{1/2el}$.
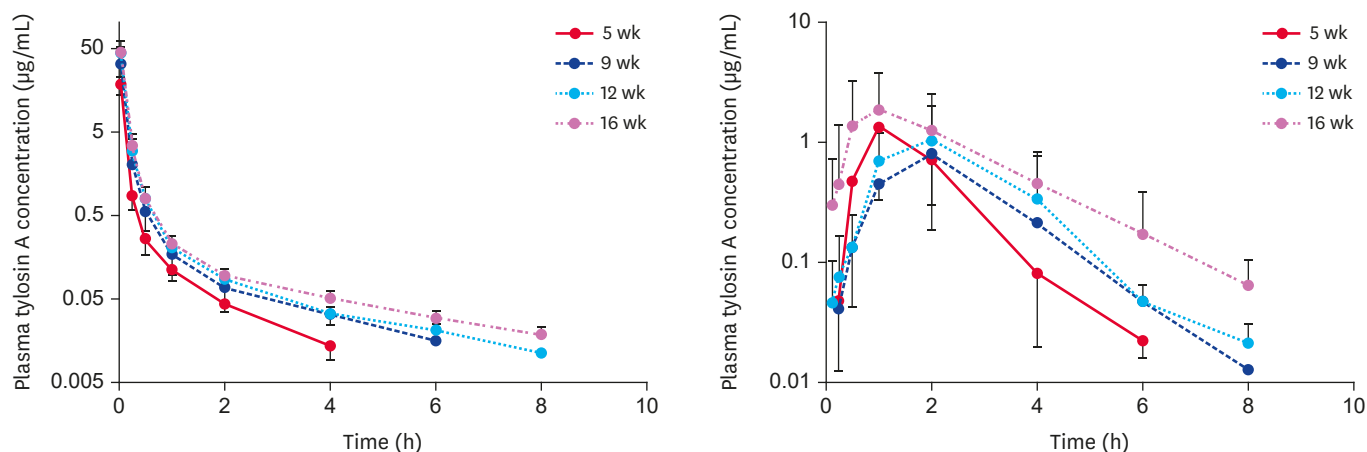
Fig. 1. Plasma tylosin A concentrations (mean ± SD) after single intravenous administration of tylosin tartrate at a dose of 10 mg/kg (panel A) and single oral administration of tylosin tartrate at a dose of 50 mg/kg (panel B) to 5-, 9-, 12- and 16-week-old turkeys.

Table 1. Pharmacokinetic parameters (median and range) of tylosin A in plasma after single i.v. administration of tylosin tartrate at a dose of 10 mg/kg to turkeys of four different ages

Parameters	5 wk (n = 10)	9 wk (n = 10)	12 wk (n = 9)	16 wk (n = 9)
Body weight (kg)	1.4 ± 0.1	4.6 ± 0.4	9.0 ± 0.7	15.0 ± 0.6
AUC_{inf} (mg × h/L)	2.61 (2.07–3.82)[a]	4.88 (3.06–6.96)[ab]	7.41 (4.65–8.41)[b]	7.15 (4.11–11.07)[b]
$AUMC_{inf}$ (mg × h²/L)	0.55 (0.30–0.66)[a]	1.39 (0.48–1.76)[ab]	1.93 (1.22–2.49)[bc]	2.79 (2.16–5.75)[c]
MRT_{inf} (h)	0.19 (0.13–0.30)[a]	0.27 (0.10–0.43)[ab]	0.25 (0.23–0.42)[ab]	0.42 (0.21–1.09)[b]
$Cl_{B(rel)}$ (L/h/kg)	3.81 (2.65–4.93)[a]	1.93 (1.45–3.31)[ab]	1.36 (1.20–2.19)[b]	1.42 (0.79–2.50)[b]
$Vd_{ss(rel)}$ (L/kg)	0.54 (0.26–0.89)	0.30 (0.14–1.02)	0.26 (0.21–0.63)	0.44 (0.13–0.90)
$T_{1/2el}$ (h)	1.03 (0.54–1.59)[a]	1.46 (0.53–3.57)[ab]	2.10 (1.85–2.89)[b]	2.96 (1.73–7.04)[b]
C_{max} (µg/mL)	17.04 (13.25–26.30)[a]	33.45 (16.18–46.15)[ab]	47.5 (29.33–52.40)[b]	42.49 (24.58–69.70)[b]
$C_{max,met}$ (µg/mL)	0.88 (0.65–1.22)[a]	1.45 (0.10–2.04)[ab]	2.28 (1.45–2.45)[bc]	2.53 (0.13–3.60)[c]

Body weight is presented as mean ± SD whereas other parameters are presented as median with range in brackets.
AUC_{inf}, area under the curve; $AUMC_{inf}$, area under the first moment curve; MRT, mean residence time; $Cl_{B(rel)}$, relative body clearance; $Vd_{ss(rel)}$, relative volume of distribution at steady state; $T_{1/2el}$, elimination half-life; C_{max}, maximal concentration of tylosin A; $C_{max,met}$, maximal concentration of tylosin D.
Values in a row not sharing a common superscript letter are statistically different, $p < 0.05$. Lack of superscript indicates lack of statistical difference.

Table 2. Pharmacokinetic parameters (median and range) of tylosin A in plasma after oral administration of tylosin tartrate at a dose of 50 mg/kg to turkeys at four different ages

| Parameters	5 wk (n = 10)	9 wk (n = 10)	12 wk (n = 10)	16 wk (n = 9)
Body weight (kg)	1.4 ± 0.1	4.6 ± 0.3	9.0 ± 0.6	14.4 ± 0.9
AUC_{inf} (mg × h/L)	2.82 (0.77–4.17)[ab]	2.16 (0.77–3.58)[a]	2.14 (1.60–6.28)[ab]	4.70 (2.34–10.75)[b]
$AUMC_{inf}$ (mg × h²/L)	4.23 (1.67–7.08)[a]	5.18 (2.54–8.23)[ab]	5.17 (3.75–14.84)[ab]	13.25 (3.57–22.97)[b]
MRT_{inf} (h)	1.79 (1.38–2.5)[a]	2.65 (1.88–3.79)[b]	2.36 (1.90–3.66)[ab]	2.21 (1.23–8.39)[ab]
MAT^* (h)	1.60	2.38	2.11	1.79
$T_{1/2el}$ (h)	0.82 (0.68–2.92)	1.1 (0.76–1.48)	1.35 (0.71–2.43)	1.18 (0.75–6.01)
$T_{1/2}$ (h)	1.24 (0.96–1.73)[a]	1.84 (1.30–2.63)[b]	1.64 (1.32–2.54)[ab]	1.53 (0.85–5.81)[ab]
C_{max} (µg/mL)	1.42 (0.29–2.42)[ab]	0.84 (0.23–1.87)[a]	1.09 (0.69–2.94)[ab]	2.17 (0.98–4.98)[b]
T_{max} (h)	1.00 (1.00–2.00)	2.00 (1.00–4.00)	1.50 (1.00–4.00)	1.00 (0.12–4.00)
F^* (%)	21.59	8.84	5.76	13.15

Body weight is presented as mean ± SD whereas other parameters are presented as median with range in brackets.
AUC_{inf}, area under the curve; $AUMC_{inf}$, area under the first moment curve; MRT, mean residence time; MAT, mean absorption time; $T_{1/2el}$, elimination half-life; $T_{1/2}$, biological half-life; C_{max}, maximal concentration; T_{max}, time when the C_{max} is reached; F, bioavailability.
*Value calculated for the median value from oral administration and the median value from the i.v. administration. Values in a row not sharing a common superscript letter are statistically different, $p < 0.05$. Lack of superscript indicates lack of statistical difference (not valid for F and MAT as statistically incomparable).

Table 3. *In vitro* protein binding analysis for tylosin A (percent of the bound fraction, results expressed as mean ± SD) in fresh plasma samples collected from turkeys at four different ages

Tylosin A concentration (µg/mL)	5 wk	9 wk	12 wk	16 wk
20	33.32 ± 3.60	31.42 ± 4.80	40.39 ± 0.32	31.54 ± 1.21
4	30.58 ± 2.71	31.00 ± 7.30	42.43 ± 2.13	24.53 ± 7.29
0.8	37.95 ± 3.38	24.34 ± 9.56	44.49 ± 2.16	25.36 ± 7.65
0.16	45.13 ± 21.80	33.11 ± 18.38	47.22 ± 10.17	28.68 ± 9.11
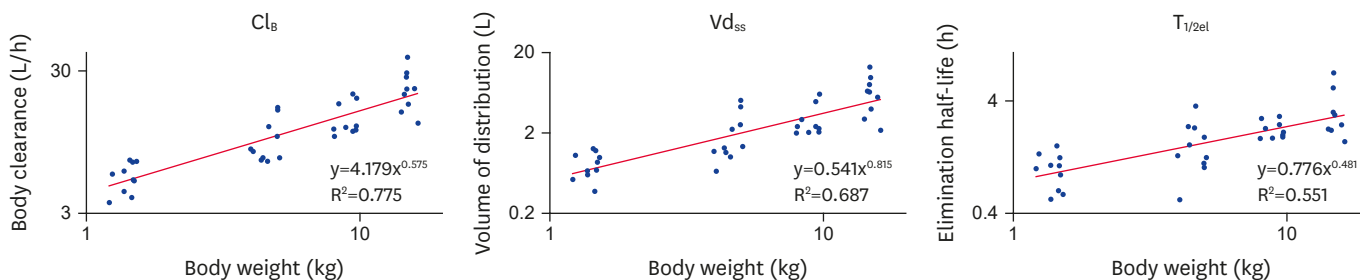
Fig. 2. Allometric models fitted to empirical values of total body clearance (Cl_B, upper panel), volume of distribution at steady state (Vd_{ss}, middle panel) and elimination half-life ($T_{1/2el}$, lower panel) vs. body weight.

Table 4. Coefficients and LOO cross-validation parameters of allometric models describing selected pharmacokinetic parameters of tylosin A in turkeys of four different body weights (pooled)

Parameters	Cl_B	Vd_{ss}	$T_{1/2el}$
Allometric models for PK parameters			
Unit	l/h	l	h
N	38	38	38
Coefficient	4.179	0.541	0.776
Exponent	0.575	0.815	0.481
R^2	0.775	0.687	0.551
F	71.37	39.77	27.35
P	< 0.01	< 0.01	< 0.01
LOO cross-validation parameters			
R^2	0.665	0.525	0.432
Q^2	0.614	0.466	0.338
$Q^2 - R^2$	0.051	0.032	0.094
Q^2_{asym}	0.646	0.499	0.338
$Q^2_{asym} - Q^2$	0.032	0.032	0.062
SS	2,218.71	312.58	60.33
PRESS	856.40	166.79	39.93
Validation outcome	-	-	-

F and *P* determined by linear regression analysis of observed vs. predicted values. For validation outcome, "-" denotes that model does not meet all validation criteria.
LOO, leave-one-out; Cl_B, body clearance; Vd_{ss}, volume of distribution at steady state; $T_{1/2el}$, elimination half-life; N, number of individual parameters used to develop the model; SS, sum of squares; PRESS, predicted residual error sum of squares; Q^2_{asym}, Q^2 asymptotic.

DISCUSSION

Although tylosin is commonly used in poultry to treat respiratory infections, the available scientific literature describing the drug's PK in turkeys is almost non-existing. For this species, the authors have found only a tissue depletion study [2]. The intention of the current study is to fill this gap in knowledge.

As can be appreciated from **Fig. 1**, in turkeys tylosin A is rapidly removed from the body, regardless of the route of administration. Over the course of the experiment, the primary parameter of elimination, Cl_B, decreased from 3.81 to 1.42 L/h/kg with the latter value (for the heaviest turkeys) being similar to Cl_B found in chickens by Ji et al. [14] 1.61 L/h/kg and Abu Basha et al. [15] 1.70 L/h/kg. Since in both these studies, chickens were weighing less than 2 kg, it is apparent that turkeys of similar body weight (1.4 kg) remove tylosin A more than twice as fast. This indicates a significant interspecies difference between turkeys and chickens in regard to tylosin A PK. However, the intra-species change in tylosin A PK in turkeys is also substantial. This age-dependent change seems to be related mainly to changes in hemodynamics [11]. Value of Cl_B reported by Kowalski et al. [13] is even lower (0.32 L/h/kg). Duthu [25] investigated the Cl_B values from 3 mammalian species using allometry and found a very high correlation coefficient for the fitted model ($R^2 = 0.997$). Surprisingly, in the current study the intra-species allometric scaling for Cl_B resulted in a poorer fit ($R^2 = 0.775$). On one hand, this difference may be accidental and caused by the small number of species in the study by Duthu [25]. On the other, the predominant route of elimination may be also responsible. Tylosin is eliminated with faeces and urine, however, the relative importance of these routes may vary among species [26]. Since drugs eliminated with bile are usually poor candidates for allometric scaling [27], the predictability of the model may be highly affected by the selection of species. Unfortunately, the excretion ratio for tylosin in turkeys is not known.

In contrast to Cl_B, Vd_{ss} did not show significant age-related differences. The present findings (0.26–0.54 L/kg) suggest rather limited distribution to tissues and are similar to the findings of Kowalski et al. [13] 0.69 L/kg and lower than values reported by Ji et al. [14] 1.09 L/kg (both in chickens). It is known, though, that tylosin penetrates well to avian tissues [28]. The exponent of 0.815 is relatively close to 1 which is typical for models describing this parameter [27]. The relatively poor performance of the model for Vd_{ss} cannot be explained by plasma protein binding which was low and not affected by age [27]. In the study by Duthu [25] on tylosin PK in mammals an exponent of 0.75 was found.

The $T_{1/2el}$ (i.v. administration) changed significantly with age from 1.03 to 2.96 h. This is close to the values for chickens found by Abu Basha et al. [15] and Ji et al. [14]: 2.06 and 1.16 h, respectively. For intramuscular administration of tylosin, Locke et al. [28] found $T_{1/2el}$ of 1.2 h in quails, pigeons and cranes but 4.7 h in emus. In the current study, the allometric model for $T_{1/2el}$ had the poorest predictivity. This is a common finding as this is a hybrid parameter depending on both Cl_B and Vd [27]. However, for a limited set of 3 mammalian species, Duthu [25] described a very high R^2 of her fit.

Oral administration resulted in a less clear age-dependent trend which is probably associated with very low and variable bioavailability of 5.76 to 21.59%. For tylosin tartrate in chickens, Ji et al. [14] found the bioavailability of 27% (fasted chickens) whereas Kowalski et al. [13] provides the value of 31% (feeding status unknown). On the other hand, Abu Basha et al. [15], who administered an oral dose of 25 mg/kg of tylosin tartrate to fasted chickens, estimated the bioavailability to be 35%–41%. It is difficult to say whether lower tylosin tartrate bioavailability in turkeys found in this study is a result of some physiological differences as compared to chickens or of the higher dose applied. Other parameters of absorption (T_{max}, MAT) were similar to the values reported in chickens by Ji et al. [14] and Kowalski et al. [13]. For the oral route, $T_{1/2el}$ did not reflect the age-dependent prolongation observed for i.v. administration. Current values of $T_{1/2el}$ are similar to those reported by Ji et al. [14] but shorter than those reported by Kowalski et al. [13] and Abu Basha et al. [15]. After oral administration, the concentration of 0.05 µg/mL—the minimum inhibitory concentration for *Mycoplasma gallisepticum* [29]—was maintained for 4.75, 5.7, 5.8, and 8 h in turkeys of respective age groups. This suggests the need for dosage adjustments in turkeys of different age, e.g. more frequent dosing and/or higher dose in younger birds. However, it should be kept in mind that concomitant feed access under field conditions may add further variability to bioavailability and the age-dependent trend may be somehow obscured. Despite this, the importance of tylosin dose modification in turkeys of different age is supported by the higher incidence of mycoplasmosis in young birds as compared to adult ones [30].

Concentrations of tylosin D in plasma were low and quantifiable only in the first two sampling times after i.v. administration. Interestingly, in an earlier tissue depletion study on turkeys, in liver only tylosin D was detected but no parent compound was found [2]. This report together with our current findings (very low levels of tylosin D in plasma) suggest that tylosin D may be formed in the liver and then eliminated efficiently with bile.

In veterinary medicine, allometric scaling of drug dose is usually applied for species for which no dosage protocol is available. However, broad physiological and metabolic differences between species often limit the applicability of this tool [27]. In our recent studies, we have suggested a different application of allometry – to scale drug dosage in rapidly growing poultry

species in which drug elimination (measured by Cl_B) changes rapidly over a short period of time. In this method, allometric exponent for Cl_B can be used to modify the dose in order to obtain a more uniform AUC in different age groups [12]. The new dosage protocol is calculated based on the non-compartmental equation for clearance: $Cl_B = D/AUC$ [16]. If we aim at achieving constant AUC in different age groups, then the dose should change proportionally to Cl_B according to the standard allometric equation: $D = aBW^{0.58}$. If we assume now that the AUC in the largest turkeys was satisfactory (e.g. because it translated into the longest T > MIC), then we can use this equation to calculate the coefficient: $a = D/BW^{0.58}$, where D is the average dose given to the largest turkeys (150 mg) and BW is their average body weight (15 kg). This allows us to create the new non-linear dosage protocol: $D = 31.6 \times BW^{0.58}$. If applied to turkeys weighing 1.5, 4.5, 9, and 15 kg, we obtain the dosage of 26.6, 16.7, 12.4, and 10 mg/kg, respectively. Such dosage should neutralize the age-dependent effect of Cl_B on the variability of AUC. This approach is expected to optimize the efficacy of concentration-dependent antimicrobials (as their efficacy is strongly affected by the AUC). The applicability of this dosage to optimize the efficacy of time-dependent antimicrobials (e.g. tylosin) is yet unknown. Although previous interspecies allometric analyses may suggest promising results [25], empirical studies are needed to verify whether intra-species allometric scaling of tylosin dosage in turkeys provides more predictable internal exposure as compared to traditional linear calculation of dose.

It is concluded that tylosin is eliminated faster in turkeys as compared to broilers of similar body weight. Moreover, significant age-related change in the drug's PK suggests a need for dosage adjustments in turkeys of different age. The applicability of allometry for this purpose requires further studies.

ACKNOWLEDGMENTS

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