IVERMECTIN RESIDUE DEPLETION IN NELLORE BEEF CATTLE

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Abstract – Anatomical and/or physiological differences in residues depletion must be taken into account when implementing control strategies to ensure safety intake of products of animal origin. This work aimed to evaluate the effects of dynamic distribution and depletion Ivermectin (IVM) in 3.5% concentration with respect to the application types(subcutaneous, muscular), different tissues evaluated (N=14), as well as different periods after applying of the IVM in beef cattle (7, 21, 36, 63, 91, 105 and 122 days). A Completely Randomized Design (CRD), with cross-classification 2x14x7 was used in analysis. The estimative of mean and standard deviation for IVM concentration in original scale (µg.kg^{-1}) were 1,011.9±5,073.7, ranging from 0.0 to 58,300.0 µg.kg^{-1}. The results of this work suggest that the dynamic of 3.5% IVM distribution is dependent on application type, of tissues collected and period after application

I. INTRODUCTION

The macrocyclic lactones (ML) are widely used for the control and treatment of parasitic diseases in domestic animals for food production. Among the ML, ivermectin (IVM) is the best known of these drugs and has been used to treat animals for over 20 years [1], due to its wide spectrum of activity and high efficacy. Like any therapeutic agent administered to domestic animals for food production, ivermectin residues in animal tissues are important matter of concern involving safety consumers of products of animal origin [2], as well as the Industries, up to the limits established in different international markets. Anatomical and/or physiological differences in residues depletion, must be taken into account when implementing control strategies. The highly lipophilic nature of IVM, and the different proportions of fat in the muscles may be the reason for the unequal distribution of residuals. This work aimed to evaluate the effects of dynamic distribution and depletion of Ivermectin in 3.5% concentration with respect to the application types, tissues evaluated, as well as different periods after applying of the IVM in beef cattle.

II. MATERIALS AND METHODS

A total of 168 Nellore cattle with average weight of 418 ± 15 kg and average age of 20 months were used in this experiment. The animals were divided in two groups representing subcutaneous and muscular application of Ivermectin in 3.5% concentration. The withdrawal time recommended in the label of the product was 120 days. After application, the animals were slaughtered in seven different days (7, 21, 36, 63, 91, 105 and 122 days after initial application). In each slaughter, 14 tissues were collected for residues research. The tissues collected were: application site, bleeding, fat brisket, left chuck roll, left shoulder, liver, loins, right chuck roll, right shoulder, rump, subcutaneous tissue close to the application site, tendons, tongue and topside. The concentration of IVM in the tissues was determined by Ultra Performance Liquid Chromatography (UPLC) directly coupled to a Mass Spectrometer (MS) (Xevo ® TQD, Watters Corporation). For analysis IVM concentrations in the samples, we used the scale transformation "natural logarithm of the concentration of ivermectin+1" (LN_IVM), proceeding to the analysis of variance and Tukey Test with the transformed data. To assess the concentrations of IVM in transformed scale, according to the comparison groups described above, we adopted a Completely Randomized Design (CRD), with cross-classification 2x14x7, according to the model specified below.

$$Y_{ijkl} = \mu + S_i + T_j + D_k + ST_{ij} + SD_{ik} + TD_{jk} + STD_{ijk} + e_{ijkl}$$

where,

Y_{ijkl} = is the observed value for the concentration of ivermectin in transformed scale (LN_IVERM) related to the animal l, for k day after application, in the type of tissue j and the application site i; μ = constant; S_i = is the effect of the i^{th} application type, being i=1 (SC=subcutaneous) or 2 (IM= muscular); T_j = is the effect of the j^{th} tissues collected, being j=1(Application site), 2(Bleeding), 3(Fat Brisket), 4(Left Chuck Roll), 5(Left Shoulder), 6(Liver), 7(Loins), 8(Right Chuck Roll), 9(Right Shoulder), 10(Rump), 11(Subcutaneous Tissue), 12(Tendon), 13(Tongue) ou 14(Topside); D_k = is the effect of the k^{th} day after application of IVM 3.5%, being j=1(7 days), 2(21 days), 3(42 days), 4(70 days), 5(91 days), 6(105 days) or 7(122 days); ST_{ij} = is the effect of double interaction between the application site i with tissue j; SD_{ik} = is the effect of double interaction between the application site i with day after application k; TD_{jk} = is the effect of double interaction between the tissue j with day after application k; STD_{ijk} = is the effect of double interaction between the application site i with tissue j with day after application k; e_{ijkl} = is the experimental error.

The analysis was performed using PROC MIXED procedure of Statistical Analysis System (SAS), version 9.1.3 [3].

III. RESULTS AND DISCUSSION

The estimative of means, standard deviation, minimum and maximum for IVM concentration in original (IVM) and transformed scale (LN_IVM) are presented in Table 1.

Table 1. Estimative of Means, Standard Deviation (SD), minimum value (Min.) and maximum value (Max.) for IVM concentration in original (IVM) and transformed scale (LN_IVM)

Trait	Mean	SD	Min	Max
IVM, ($\mu g \cdot kg^{-1}$)	1,011.9	5,073.7	0.0	58,300.0
LN_IVM	2.4	2.6	0.0	10.9

The triple interaction (STD_{ijk}) showed significant effect ($P<0.01$) in the ANOVA, implying that three main factors evaluated are dependent. Thus, we performed the study of the factor days after application within each combination of application types and tissue evaluated (Table 2).

In the subcutaneous application, the values of the concentrations of IVM 3.5% in transformed scale (LN_IVM) remain high at 122 days of application after application in the tissue Application site (LN_IVM=2.2200), Liver (LN_IVM=1.2050), Subcutaneous Tissue (LN_IVM=2.6057) and Tendon (LN_IVM=0.3765), with lower concentrations but different from zero. These values observed at 122 days after application in the original scale (IVM) amount to 8.2173$\mu g.kg^{-1}$ (for Application site), 2.3368$\mu g.kg^{-1}$ (for Liver), 12.5407$\mu g.kg^{-1}$ (for Subcutaneous Tissue) and 0.4572$\mu g.kg^{-1}$ (for Tendon). For all other tissues evaluated, the values of the C_SC site, tend to zero, suggesting the absence of residues of IVM.

For muscular application site, higher concentrations of IVM transformed scale (LN_IVM) at 122 days were observed in Application tissue site (LN_IVM=1.036), Fat Neck (LN_IVM= 0.2132), Chuck Roll Left (LN_IVM= 0.1781), Liver (LN_IVM=0.8715), Chuck Roll Right (LN_IVM=0.5904) and Subcutaneous Tissue (LN_IVM=1.9579). These values observed at 122 days when using the intramuscular application site (D_IM) in the original scale (IVM) amount to 1.8179 $\mu g.kg^{-1}$ (for Application site), 0.2376$\mu g.kg^{-1}$ (for Fat Neck), 0.1949$\mu g.kg^{-1}$ (Chuck for Roll Left), 1.3905$\mu g.kg^{-1}$ (for Liver), 0.8047$\mu g.kg^{-1}$ (Right to Chuck Roll) and 6.0844$\mu g.kg^{-1}$ (for Subcutaneous Tissue). The estimative verified for Fat Neck, Left Chuck Roll and Right Chuck Roll, when using the site D_IM application with 3.5% IVM can be assumed to be near zero in the original scale.

IV. CONCLUSION

The results suggest that the dynamic of 3.5% IVM distribution is dependent on application type, tissues collected and period after application. The residuals concentrations in tissues evaluated were within the internationally recommended, but they are not zero after withdrawal periods.

REFERENCES

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Table 2. Effect of days after application within each combination of application types and tissue evaluated on ivermectin concentrations in transformed scale

Tissue Collected	Days after Applicattion[1]	Subcutaneous Application		Muscular Application	
		Mean[2]		Mean	
Application Site	7	8.9698	A	8.4609	B
Application Site	21	8.5967	A,B	9.2634	A
Application Site	42	9.0995	A	7.6526	C
Application Site	70	8.0916	B	7.5099	C
Application Site	91	4.3466	C	2.7972	D
Application Site	122	2.2200	D	1.0360	E
Bleeding	7	4.6061	A	4.2261	A
Bleeding	21	4.3274	A	3.3557	B
Bleeding	42	0.8739	B	1.2682	C
Bleeding	70	1.3287	B	0.0000	D
Bleeding	91	0.0000	C	0.1583	D
Bleeding	122	0.0000	C	0.0000	D
Fat Neck	7	5.0246	A	5.1226	A
Fat Neck	21	4.1730	B	4.3391	B
Fat Neck	42	3.3337	C	2.3307	C
Fat Neck	70	2.9712	C	2.4001	C
Fat Neck	91	1.1745	D	0.1852	D
Fat Neck	122	0.0000	E	0.2132	D
Left Chuck Roll	7	4.1427	A	3.9769	A
Left Chuck Roll	21	3.5515	A	2.4636	B
Left Chuck Roll	42	1.6618	B	0.0000	C
Left Chuck Roll	91	0.0754	C	0.0000	C
Left Chuck Roll	122	0.0000	C	0.1781	C
Left Shoulder	7	3.6733	A	4.2143	A
Left Shoulder	21	3.6420	A	3.9297	A
Left Shoulder	42	0.1527	B	0.1608	B
Left Shoulder	91	0.0000	B	0.0000	B
Left Shoulder	122	0.0000	B	0.0000	B
Liver	7	6.3299	A	6.0829	A
Liver	21	5.1130	B	4.9834	B
Liver	42	5.0960	B	4.3659	B
Liver	91	2.4026	C	1.6149	C
Liver	122	1.2050	D	0.8715	D

[1] Means followed by the same letter in the same column and within each combination of application types and tissue evaluated, do not differ at 1% probability by Tukey test;
[2] Means in transformed scale [natural logarithm of the (µg.kg^{-1} of ivermectin+1)].

Table 2. Effect of days after application within each combination of application types and tissue evaluated on ivermectin concentrations in transformed scale (continuation)

Tissue Collected	Days after Applicattion[1]	Subcutaneous Application	Muscular Application
		Mean[2]	Mean
Loins	7	3.2615 A	2.8781 A
Loins	21	2.5955 A	2.7486 A
Loins	42	0.0000 C	0.0000 B
Loins	70	0.7043 B C	0.5500 B
Loins	91	1.1780 B	0.0000 B
Loins	122	0.0000 C	0.0000 B
Right Chuck Roll	7	4.2756 A	3.9211 A
Right Chuck Roll	21	3.7896 A	2.9789 B
Right Chuck Roll	42	0.8238 B	0.2619 C
Right Chuck Roll	91	0.0000 C	0.0000 C
Right Chuck Roll	122	0.0000 C	0.5904 C
Right Shoulder	7	3.5774 A	3.9701 A
Right Shoulder	21	3.5904 A	3.1701 B
Right Shoulder	91	0.0000 B	0.0000 C
Right Shoulder	122	0.0000 B	0.0000 C
Rump	7	3.0512 A	3.1659 A
Rump	21	1.6078 B	2.3179 B
Rump	42	0.6236 B,C	0.0000 C
Rump	70	0.6836 C	0.4588 C
Rump	91	0.0000 C	0.0000 C
Rump	122	0.0000 C	0.0000 C
Subcutaneous Tissue	7	5.0489 A	5.8066 A
Subcutaneous Tissue	21	2.6781 C	2.5628 B, C
Subcutaneous Tissue	42	3.3149 B	2.6072 B
Subcutaneous Tissue	91	1.3101 D	0.7985 D
Subcutaneous Tissue	122	2.6057 B, C	1.9579 C
Tendon	21	1.4744 A	2.1013 A
Tendon	42	0.2474 B	0.0000 B
Tendon	91	0.0780 B	0.1838 B
Tendon	122	0.3765 B	0.0000 B
Tongue	21	3.2602 A	2.9139 A
Tongue	42	1.1878 B	1.1541 B
Tongue	70	1.3622 B	1.0843 B,C
Tongue	91	0.8026 B	0.0000 C
Tongue	122	0.0000 C	0.0000 C
Topside	7	2.2689 A	3.0374 A
Topside	21	1.6190 A	1.8549 B
Topside	42	0.3041 B	0.0000 C
Topside	70	0.0000 B	0.0000 C
Topside	91	0.0000 B	0.0000 C
Topside	122	0.0000 B	0.0000 C

[1] Means followed by the same letter in the same column and within each combination of application types and tissue evaluated, do not differ at 1% probability by Tukey test;
[2] Means in transformed scale [natural logarithm of the ($\mu g.kg^{-1}$ of ivermectin+1)].