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Development, Efficacy and Toxicity Study of Novel Moxidectin Loaded Freeze Dried Seedmix for the Management of Internal Parasite In Caged and Aviary Birds

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Abstract:

Psittacine aviculture is constantly looking for the effective internal parasite control that is safe, effective and simple. In this study, an effort was made to develop moxidectin loaded freeze dried seedmix for caged and aviary birds. The seed mix consists of Canary, White proso millet and Dehaulled Oat. Different solvents combinations were assessed to check the loading efficiency of moxidectin in seedmix. Loading efficiency of seedmix was tested at various concentration of moxidectin. A comprehensive efficacy and toxicity study was conducted on wide ranges of birds in private and standard condition. Stability study was also conducted both at ambient and accelerated condition for 12 months. Histopathology study was conducted to assess the any toxic effect of moxidectin loaded seed mix at the recommended dose. Water/ethanol combination at 1:3 ratios was found to be effective in loading moxidectin during 48-hour infusion. Moxidectin concentration was 1/3 of total concentration in kernel. Moxidectin concentration in seedmix increased with the increase of moxidectin concentration in infusion solvent and it was almost linear. Efficacy and toxicity study demonstrated excellent efficacy including capillaries. Histopathology study results suggested that there is no toxic effect on various organs of birds at recommended dose. Stability studies at ambient and accelerated condition showed that seedmix is stable at both conditions.

Keywords: Freeze-drying, Safety & Efficacy, Internal parasites, Histopathology, Caged and aviary birds, Medicated seedmix

INTRODUCTION:

Moxidectin is a macrocyclic lactone that has been known as an antiparasitic agent for almost 30 years [1, 2]. It is a veterinary medicine with a broad spectrum of action, showing very high effectiveness against gastrointestinal and pulmonary nematodes, as well as for gadflies and mites. Because of its potency, moxidectin is considered to be an effective antiparasitic agent for cattle, pigs, sheep and avian birds [3-5]. Psittacine aviculture is constantly looking for effective internal parasite control that is safe, effective and simple. Worming of parrots has traditionally been achieved through in water medication or direct application to the beak. Both treatments have their advantages and disadvantages but neither offers a uniform, effective and simple method of worming parrots. Extensive literature study revealed that there is no study on moxidectin loaded seedmix for avian industries. Medicated seedmix concept is of great value to aviculture as it eliminates the vagaries of in water medication and the inherent dangers of direct administration (to the beak or crop needling). Development of medicated seedmix is always challenging as various variables may affect loading efficiency of moxidectin in seedmix. Treatment efficacy could be also affected if birds do not like the seedmix or inadequate intake during the treatment period. The current formulation (Smart Seed Parrot Wormer) relies on a unique technique of infusing standard parrot seeds with moxidectin to create an easy to use and effective wormer for use in parrots. Moxidectin has proven efficacy as a wormer in parrots and its safety margins are well recognised. By use of seed infusion and freeze drying technique, moxidectin can be transferred to the kernel of the seed (the part eaten by a parrot) and given the relatively constant seed intake (8 – 10)% of body weight; a therapeutic dose of moxidectin can be administered to the parrot simply by offering the medicated seed as a food source.

Considering these factors, an effort was made to characterize the formulation variables which may affect the loading efficiency and treatment efficacy. A wide range of parrots in aviculture were trialled under controlled conditions to ascertain the efficacy of the formulation. Comprehensive toxicity study including histopathology study of various organs following treatment on recommended dose was also conducted to confirm the safety margin of newly developed seedmix.

MATERIALS AND METHODS

Materials

Moxidectin EP grade was purchased from B.J Blue, Australia. Ethanol (Industry grade) was purchased from Chem supply, Australia. HPLC grade acetonitrile and methanol were purchased from Merck Australia. All other chemicals were of reagent grade or above and were used without further purification.

Methods

Seed infusion process

Required amount of moxidectin was dissolved in ethanol water mixture at the ratio of 3:1. The amount of solvents was 400 mL for 1 kg seed mix. Then required amount of seeds were mix to the above solution and mixed well. Mixing was repeated 2x a day for 48 hours. Then infused seed were placed in the freeze dryer for drying.

Analytical method

Moxidectin was analysed using the previously reported method used by Sigma Aldrich with slight modifications. An HPLC system (Shimadzu Scientific Instruments) consisting of a UV detector, Gemini C18 column (4.6 × 250 mm, 5 µm), a pump (LC 20AD), and an automatic

injector (SIL 20A) were used. The wavelength of the UV detector was 245 nm, the column temperature was maintained at 40^{o}C, the flow rate was 1.2 mL/min, with injection volume of 10 µL. The mobile phase consisted of methanol/water (97/3).

2.3 Study design for efficacy study

Efficacy and toxicity study was conducted according to VICH GL7 guidelines[6].To determine the efficacy of developed medicated seed over various species of parrots commonly held in aviculture, private aviaries were used. The selection of aviaries was based on the number of species held and the practicality of feeding and sample collection under avicultural conditions. In Australia, most parrots are held in outdoor enclosures and commonly use sand or gravel as a substrate. The collection of faecal samples, from the aviary, inevitably contains substrate material. By far the most common parasite found in parrots in Australian aviaries is the Roundworm (*Ascarid*)[7-9]**.** In avian veterinary practise elimination of this parasite from an enclosure has been extremely difficult. The other parasite which was found as much less degree compared to Ascarid are the Hookworm (*Acuraria*) and Wireworm (*Capillaria*). Differentiation of parasite eggs from the above is relatively easy under the microscope as each species produces a distinct egg shape. Both methods of detection (Faecalyser and McMaster) are capable of differentiating the egg type. Aviaries were selected from private breeders of parrots where there are a range of birds held and the conditions are deemed common to Australian Aviculture. Long established aviaries were preferred. Each flight was given an identifying number and the species and number of birds in each flight was recorded. Where a particular flight contains birds that are not suitable for the trial (especially where disturbance may cause stress or injury) the flight was not included during the data collection. Based on the species held, an estimation of body weight was performed. Individual catching and weighing of birds is very stressful and was not performed in this study. Selected aviaries were cleaned well prior to beginning the trial to reduce the risk of residual worm eggs contaminating the post treatment faecal samples. Such cleaning was involved removal of the upper 1 – 3 cm of substrate and sweeping and removal of all visible seed hull. Seed dishes were emptied and cleaned of residual seed matter prior to commencement of the trial. Aviaries were scaped clean between each faecal collection to reduce the chance of "carryover" parasite eggs between collections. After the initial cleaning of aviaries, a collection of faeces was performed to establish the presence, and level, of worm eggs within the flight. It was not possible to collect faeces from individual birds; rather the collection was a "pooled" sample from a particular flight. Collection was performed using a new wooden tongue depressor for each aviary and the faeces collected into zip lock bags numbered with the appropriate flight number. Collected samples were returned to the veterinary clinic for faecal parasite egg presence and egg species identification.

The medicated seed, containing a known amount of moxidectin, was given to the parrots. The amount given was such that the birds had ad lib access to the medicated seed and estimated such that the birds would not consume all the seed before the end of the trial period. The medicated seed was supplied for 3 days (approximately 72 hours). At the completion of the trial period (72 hours) the medicated seed was removed and the seed containers refilled with the bird's usual seed mix.

Faecal collections were repeated on Days 3, 10 and 20 post supply of medicated seed (Day 0). Determination of the efficacy of the Smartseed Parrot Wormer was performed on the Day 20 results obtained from the modified McMaster method.

2.4 Experimental design for Toxicity study

Two separate trials were conducted in order to assess the toxicity of Smart seed on birds. Trial I was carried out to assess the potential toxicity of a Moxidectin infused seed in Budgerigars under controlled conditions. The Australian budgerigar was selected for this trial as it is a very common avicultural bird and is an obligate grainvore. The budgerigar of 40 – 50 grams body weight is known to consume approximately 4 grams of seed per day (+/- 10 % of body weight) giving an estimate of seed consumed. 12 Budgerigars (*Melopsitticus undulatus*) ranging in weight from 40 – 50 grams to be ad hoc selected from a private collection. All birds were adults but not sex selected. Birds were held in modular cages of 1.2m cubed within an air-conditioned facility. Cage was fitted with feed and water containers and adequate perching for the species. Birds had free access to clean potable water. The birds were acclimatised for 7 days on a standard commercial "budgie mix" supplied in stainless steel seed containers common in the industry. At the start of the medication trial the feed container and cage were cleaned of any seed hull and uneaten seed from the acclimatisation period. At the beginning of the trial period the feed dish in the shelter had a known amount of SmartSeed added. Birds had free access to the seed at all times through the trial period. Birds were individually leg rung and weight of each bird recorded at the beginning and end of the trial. Birds were monitored daily by a qualified avian veterinarian to assess toxicity of the Smart Seed and results recorded. Trial II was carried out to assess the potential toxicity of a Moxidectin infused seed in a selection of parrot species. The Australian Budgerigar, the Cockatiel and the Indian Ringneck parrot were selected as the test species due to their common occurrence in aviculture, are mostly granivorous and range in size across the scope of common avicultural birds.

6 Grass Parrots (*Psephotus haematonotus*) ranging in weight from 50 – 70 grams were ad hoc selected from a private collection. All birds were adults but not sex selected. 6 Cockatiels (*Nymphicus hollandicus*) of body weight from 80 – 100 grams were selected ad hoc from a private collection. All birds were adults but not sex selected. 6 Ring Neck Parrots (Psittacula) of body weight from 120 – 180 grams were selected ad hoc from a private collection. All birds were adults but not sex selected. Birds are were held in modular cages of 1.2m cubed within an air-conditioned facility. Cages were fitted with feed and water containers and adequate perching for the species.

Birds had free access to clean potable water. The birds had acclimatised for 7 days on a standard commercial "budgie mix" supplied in stainless steel seed containers common in the industry. At the start of the trial the feed container and cage were cleaned of any seed hull and uneaten seed from the acclimatisation period. At the beginning of the trial period the feed dish in the shelter had a known amount of SmartSeed added. Birds had free access to the seed at all times through the trial period. Birds were monitored daily by a qualified avian veterinarian to assess toxicity of the Smart Seed.

2.5 Faecal Egg Count Procedures

Faecal samples were analysed by Modified McMaster method. The total number of eggs present in faeces was determined and the number of eggs present expressed in terms of eggs per gram (epg) of faeces following the WAAVP guidelines[10]. Larval species were identified by the School of Veterinary Science, Charles Sturt University, Wagga Wagga, Australia.

The anthelmintic efficacy of Smart seed was assessed by applying the Faecal Egg Count Reduction test (FECRT) as described by the World Association for the Advancement of Veterinary Parasitology[10]; the percent efficacy was calculated based on following Abbot's equation[11]:

% Efficacy=

(Mean number of egg in control animal- Mean number of egg in treated animal)

Mean number of egg in control animal) x 100

Anthelmintic treatment is considered to be effective if the percentage reduction in arithmetic mean faecal egg count is above 90%.

2.6 Stability Study

Stability study of 3 batches of freeze dried moxidectin loaded seed mix (Smart Seed) was conducted at room temperature (30 ^{0}C) and accelerated (40 ^{0}C) conditions in order to assess the commercial viability of the newly formulated product.

Histopathology

Histopathology of the liver, kidney, lungs were done following recommended dose using the Freeze dried seed mix at Veterinary Diagnostic laboratories, CSU, Wagga Wagga, NSW, Australia. 12 birds have been examined to allow statistically meaningful inter-group comparisons and interpretation of the histological parameters examined.

RESULT AND DISCUSSION

Effect/ Selection of infusion solvent:

Selection of appropriate infusion solvent is critical as uptake of moxidectin could be greatly influenced by the solvents. Various solvents system was tried to assess their effect on moxidectin uptake and seed integrity after 48 hours infusion. The results obtained are given in table 1.

Moxidectin uptake was almost similar for all seeds for 3 different types of solvent system. It was observed that dehaulled oat gets soft in presence of high percentage of water and lost the physical integrity in some extent. Considering the moxidectin upload and physical properties of seed water/ethanol (1:3) was selected for further evaluation.

Table 1: Optimization of solvent system

Solvent system	Seed	Moxidectin uptake	Physical integrity of seed
Water/PG (390:10)	Cannary	27.12 µg/g seed	Seeds looked good during infusion and after freeze drying
	Proso millet	28.5 µg/g seed	Seeds looked good during infusion and after freeze drying
	Dehaulled oat	27.9 µg/g seed	Seeds get soft during infusion and lost the physical integrity in some extent.
Water/ ethanol(1:1)	Cannary	28.45 µg/g seed	Seeds looked good during infusion and after freeze drying
	Proso millet	29.25 µg/g seed	Seeds looked good during infusion and after freeze drying
	Dehaulled oat	27.87 µg/g seed	Seeds get soft during infusion and lost the physical integrity in some extent.
Water/ethanol (1:3)	Cannary	28.40 µg/g seed	Seeds looked good during infusion and after freeze drying
	Proso millet	26.07 µg/g seed	Seeds looked good during infusion and after freeze drying.
	Dehaulled oat	28.10 µg/g seed	Seeds looked good during infusion and after freeze drying.

Selection of seed

Selection of appropriate seed is crucial in case of medicated seed mix. Birds are very particular in their food habit and emphasis was given to select a suitable seed mix. Extensive market study revealed that four types of seed (individual or as mix) are available for avian industry [12-14]. These are Cannary, proso millet, dehaulled oat and sun flower seed (Figure 1). These seeds were screened on the basis of moxidectin uptake following 48 hour infusion. Results are summarised in table 2.

A. Dehulled oat, B. Cannary, C. White proso millet, D. Sun flower

Fig.1 Seedmix

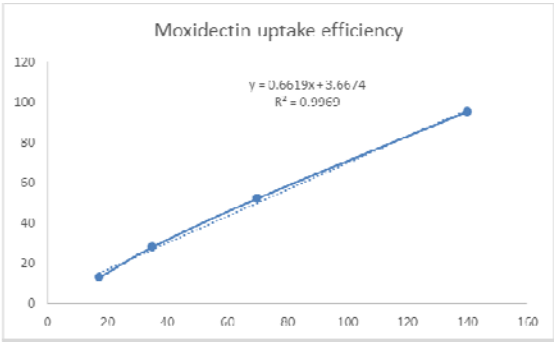
Table 2: Selection of seed for medicated seedmix

Solvent system	Seeds	Moxidectin uptake
Water/ethanol (1:3)	Cannary	28.0 µg/g seed
Water/ethanol (1:3)	Proso millet	27.40µg/g seed
Water/ethanol (1:3)	Dehulled oat	27.20µg/g seed
Water/ethanol (1:3)	Sun flower seed	15.10 µg/g seed

As we can see from the table that sun flower seed has poor moxidectin loading capacity in compare to other three seeds. Poor loading efficiency could be attributed to very hard outsell of sun flower seed. Based on the loading capacity and market research report, Cannary, proso millet and dehulled oat was selected for further evaluation.

Uptake efficiency of seed

Loading efficiency of seed/seed mix was evaluated at various drug concentrations. Concentration of moxidectin in seedmix increased with the increased concentration of moxidectin in infusion solution (Figure 2). The correlation coefficient between loading amount of moxidectin in infusion solvent and moxidectin uptake by seedmix was R^2=0.996. Uptake efficiency was around 74%. However, considering our target species and dosage regiment we have selected loading concentration of 35 µg/g seed mix optimum for further evaluation.



Uptake (µg/g seed)
Loading concentartion ox moxidectin (µg/g seed)

Figure 2: Correlation between loading amount and actual amount uptake by seedmix

Dose calculation based on actual concentration

It is well known that parrots/ budgerigars eat only kernel of proso millet[15-17] and canary and whole seed for dehulled oat which warrant the determination of precise calculation of moxidectin concentration in seed mix in order to determine the dosage of seedmix for the management of internal parasites in various species of parrot. In an attempt to determine the moxidectin concentration in kernel of Cannary and proso millet, moxidectin was extracted from kernel of proso millet and cannery seed to methanol and analysed by HPLC. Results are summarised in table 3.

Table 3: Drug concentration whole seed vs kernel

Seed name	Total concentration	Concentration in Kernel
Cannary seed	27.558 µg/g	10. 20 µg/g
Proso millet	28.526 µg/g	9.98 µg/g

As we can see from the results, moxidectin concentration in the Kernel for cannary and proso millet is around 1/3 of total concentration of respective seed. So for a seedmix of moxidectin concentration with for example 30 µg/g, ideally birds would consume 16-17µg moxidectin/g seed assuming equal consumption of each seed (subjective observation).

Stability study

The stability of Smart Seed was evaluated over 12 months at ambient (30°C, 65% relative humidity) and accelerated conditions (40°C, 75% relative humidity) (Table 4). There was no physical change in the seed mix; moreover, Moxidectin degradation in these three batches was not significant over a six-month period. The results suggest that the drug is stable in the seed mix for a sufficiently long period of time and suitable for commercialization.

Table 4: Stability study data

Stability Condition	Moxidectin (µg/g)		
	Batch A	Batch B	Batch C
Initial	29.386	28.774	29.872
3 months at 30°C	29.445	27.532	29.471
3 months at 40°C	28.173	28.086	29.217
6 months at 30°C	27.157	29.295	28.908
6 months at 40°C	26.957	28.777	27.652
9 months at 30°C	28.254	29.010	26.954
9 months at 40°C	27.381	26.984	27.694
12 months at 30°C	26.770	27.877	29.005
12 months at 40°C	26.805	26.891	28.882

Efficacy study result

A range of different parrot species with differing weights were treated with a Moxidectin infused seed mix (25 – 30ug Moxidectin/g seed mix) for the treatment of common internal nematodes. Moxidectin is well recognised as an efficient anthelmintic in many species and has been used by avian veterinarians “off label” to treat intestinal parasites for some years. Parrots from three separate aviaries were included in the trial. Aviary 3 was selected in particular as it had a long standing Capillaria infection. All birds were housed in “average “avicultural conditions with wire and metal flights and gravel or sand floors. Within the aviaries, flights were selected for the trial based on the presence of parasite eggs. Species and numbers were determined by the birds housed in the flights returning a positive coprological examination. Data are presented in tables 5, 6, 7.

The determination of amount of seed consumed can only be an estimate under avicultural conditions. Seed separation, scattering and vermin all created difficulties in achieving accurate consumption per aviary. Collection of faeces from the ground under perching sites proved the most effective and stress free method. Scattered faeces were collected (from under other perches and around feed stations) where possible to add to the volume for testing. It was inevitable that some sand, seed hull and other detritus would be collected with the faeces. The purpose of scraping the aviaries clean prior to the trials was to reduce the “carryover” contamination – however this cannot be guaranteed under avicultural conditions. The examination methods are relatively easy to perform in the veterinary clinic and appear to give consistent trends in reduction of parasite eggs. Variance between Faecalyser and McMaster Chamber results was expected. Because of the dilution factors of the McMaster chamber the presence of a single egg returns a value of “100 EPG”. Only McMaster data has been presented in tables 8, 9,10.

Table 5: Species, estimated weight, parasite identification and seed consumed (Aviary 1).

Cage No	Number of birds	Parrot Species	Estimated Weight of birds per cage(g)	Nematode ID	Amount of Smart Seed Supplied (g)	Uneaten Seed remaining (g) (approximate).
25	2	Princess	230	RW	350	321
26	2	Princess	230	RW	350	311
27	2	King Parrot	430	RW	400	351
22	2	Princess	230	RW	350	316
23	2	Red cap	200	RW	150	108
24	2	King Parrot	430	ST	400	290
20	2	Princess	230	RW	150	132
21	2	Galah	900	RW/ST	300	219
15	4	Malabar	450	ST	400	268
14	2	Cockatiel	200	RW	150	103
13	2	Conure	250	ST	150	71
10	3	Conure	250	ST	150	83
6	2	Quaker	250	RW	350	313
5	2	Princess	230	RW	150	124
4	1	Princess	120	RW	150	137
29	2	Conure	250	ST	150	72
30	4	Conure	450	ST	200	93
31	1	Red cap	90	RW	150	122

*RW – Ascarid. ST – Strongylid Type

Table 6: Species, estimated weight, parasite identification and seed consumed (Aviary 2)

Cage No	Number of Birds	Parrot Species	Estimated weight of birds per cage (g)	Nemtode ID	Amount of Smartseed supplied (g)	Uneaten Seed remaining (g) (Approximate)
2	2	Princess Superb	230	RW	200	178
3	2	Ringneck	300	ST	200	160
4	2	Princess	240	RW	200	155
5	2	Ringneck	320	ST	200	151
6	2	Princess	240	RW	200	158
7	2	Conure	160	RW	200	150

*RW – Ascarid *ST – Strongylid Type

Table 7: Species, estimated weight, parasite identification and seed consumed (Aviary 3)

Cage No	Number of Birds	Parrot Species	Estimated weight of birds per cage (g)	Nematode ID	Amount of Smart seed supplied (g)	Uneaten Seed remaining (g) (Approximate)
1	1	Alexandrine	230	C*	200	178
2	1	Alexandrine	300	C	200	160
3	1	Alexandrine	240	C	200	155
4	1	Alexandrine	320	C	200	151
5	1	Alexandrine	240	C	200	158
6	1	Alexandrine	260	C	200	150

*C – Capillaria type

Table 8: Smart Seed Clinical Trial: Aviary 1 Faecal Egg Count Results

Cage No	Species	No of Birds	Weight of birds (Gram)	FEC Day 0	Nematode ID	FEC Day 3	FEC Day 10	FEC Day 20
25	Princess	2	230	800	A	200	0	0
26	Princess	2	230	600	A	200	200	100
27	King Parrot	2	430	800	A	0	0	0
22	Princess	2	230	700	A	200	100	0
23	Red Cap	2	200	1800	A	300	200	100
24	King Parrot	2	430	600	S	0	0	0
20	Princess	2	230	200	A	200	0	0
21	Galah	3	900	1400	A/S	200	0	100
15	Malabar	4	450	600	S	100	100	0
14	Cockatiel	2	200	400	A	0	0	0
13	Conure	2	250	400	S	100	100	0
10	Conure	2	270	800	S	0	0	0
06	Quaker	2	250	1200	A	400	100	200
05	Princess	2	230	1000	A	200	100	0
04	Princess	1	120	900	A	100	0	0
29	Conure	2	25	500	S	0	0	0
30	Conure	4	450	900	A/S	0	0	0
31	Red cap	1	90	600	A	100	100	0

FEC reduction by day 20 of 96.5%.

Table 9: Smart Seed Clinical Trial: Aviary 2 Faecal Egg Count Results

Cage No	Species	No of Birds	Weight of Birds (Gram)	FEC Day 0	Nematode ID	FEC Day 3	FEC Day 10	FEC Day 20
2	PrincessSuperb	2	230	800	S	0	0	0
3	Ringneck	2	300	700	S	100	0	0
4	Princess	2	240	1200	A	0	100	100
5	Ringneck	2	320	1000	S	100	0	0
6	Princess	2	240	600	A	200	0	100
7	Conure	2	160	800	A	0	100	0
FEC reduction by day 20 of 94.1%								

Table 10: Smart Seed Clinical Trial: Aviary 3 Faecal Egg Count Results

Cage No	Number of birds	Parrot species	Estimated weight of birds per cage (g)	FEC Day 0	Nematode ID	FEC Day3	FEC Day10	FEC Day20
1	1	Alexandrine	230	800	C	600	0	0
2	1	Alexandrine	300	1200	C	800	100	0
3	1	Alexandrine	240	1300	C	300	100	0
4	1	Alexandrine	320	1000	C	400	300	0
5	1	Alexandrine	240	800	C	100	100	0
6	1	Alexandrine	260	900	C	200	200	100
FEC reduction by Day 20 of 98.3%								

Table 11: Aviary 1: Allometrically Scaled requirement vs Actual Consumption of Moxidectin

Cage No	Number of birds	Parrot Species	Estimated Weight of birds per cage(g)	Amount of Seed Eaten Over 3 days (g)	Allometrically Scaled Requirement (µg/day)	Actual Consumption Of Moxidectin (µg/day).
25	2	Princess	230	29	229	270
26	2	Princess	230	39	229	364
27	2	King Parrot	430	49	430	457
22	2	Princess	230	44	229	410
23	2	Red cap	200	42	200	392
24	2	King Parrot	430	110	430	1026
20	2	Princess	230	18	229	168
21	2	Galah	900	81	891	756
15	4	Malabar	450	132	449	1232
14	2	Cockatiel	200	47	200	438
13	2	Conure	250	79	249	737
10	3	Conure	250	67	249	625
6	2	Quaker	250	37	249	345
5	2	Princess	230	26	229	242
4	1	Princess	120	13	120	121
29	2	Conure	250	78	249	728
30	4	Conure	450	107	449	998
31	1	Red cap	90	28	89	261

Table 12: Aviary 2: Allometrically Scaled requirement vs Actual Consumption of Moxidectin.

Cage No	Number of Birds	Parrot Species	Estimated weight of birds per cage (g)	Amount Of seed Eaten over 3 days (g)	Allometrically Scaled Requirement (µg/day)	Actual Consumption Of Moxidectin (µg/day).
2	2	Princess Superb	230	22	229	205
3	2	Ringneck	300	40	300	373
4	2	Princess	240	45	239	420
5	2	Ringneck	320	49	320	457
6	2	Princess	240	42	239	392
7	2	Conure	160	50	159	466

Table 13: Aviary 3 Allometrically Scaled requirement vs Actual Consumption of Moxidectin

Cage No	Number of Birds	Parrot Species	Estimated weight of birds per cage (g)	Amount of seed eaten over 3 days (g)	Allometrically Scaled Requirement (µg/day)	Actual Consumption Of Moxidectin (µg/day).
1	1	Alexandrine	230	22	229	205
2	1	Alexandrine	300	40	300	373
3	1	Alexandrine	240	45	239	420
4	1	Alexandrine	320	49	320	457
5	1	Alexandrine	240	42	239	392
6	1	Alexandrine	260	50	260	466

It is not possible to draw a direct comparison between the two methods other than to look at overall trends in parasite oocyst presence over time following treatment. Where FEC reduction at Day 20 can be estimated, as a percentage of oocysts on Day 0, it ranged from 94.1 to 98.3. This reduction in FEC given the avicultural facilities, range of species and faecal collection techniques was considered very satisfactory.

Estimations of moxidectin consumed were tabulated and comparisons made between theoretical allometrically scaled requirement and that consumed by the birds under avicultural conditions. Allometric scaling for parrots gives a Moxidectin requirement in the vicinity of 1000 µg/Kg. During the trial consumption of Moxidectin by the parrots was consistently greater than the theoretical requirement on a daily basis. Data are presented in table 11, 12, 13.

Given the variance in amount of seed eaten (accepting the difficulty of measuring actual seed consumption) the intake over three days was effective in reducing parasite burden yet was below any observable toxic effect. The concentration of Moxidectin in the Smart Seed formula of 25 – 30 ug/g was considered necessary to ensure all birds within the aviary would consume adequate medication. The ad-lib feeding of a moxidectin treated seed mix, under avicultural conditions, to a range of parrot species, was successful in reducing the commonly encountered parasites. The simplicity of the delivery system, the consistent medication intake and the lack of toxicity make Smart seed Parrot Wormer a useful tool in aviculture.

TOXICITY STUDY RESULTS

Trial 1

Twelve adult male budgerigars within the protocol weight range were ad hoc selected from a private collection. Birds were placed in metal bird cage measuring 1.2 m cubed with food and water containers and adequate perches. The cage was placed in an air conditioned room at approximately 25 ^{0}C. The birds were acclimatised in the room for 7 days on potable water and commercial budgie mix (Avigrain). At the beginning of the test period the cage was cleaned of seed hulls and uneaten seed and 500 grams of the SmartSeed placed in a deep sided feed container. No other food of any description was given during the trial period. The birds selected had a body weight of between 40 and 50 grams (ring numbers and weights recorded in table below). Each bird had a unique aluminium budgerigar leg band applied (Chapman Rings, Mortdale, Sydney)and individual birds were placed in a small cardboard box which had been tared on a set of AND, model HF-300G scales (Max310g, d-0.001g) and weight recorded (rounded to the nearest whole gram). The birds were maintained on fresh potable water and SmartSeed Wormer for Parrots for 7 days. Birds in the aviary were observed each morning between 8 am and 10 am for any abnormal behaviour or demeanour by a qualified avian veterinarian. The toxicity study outcomes are summarised in table 00. The SmartSeed remaining in the feed dish was winnowed and weighed and found to contain 138 grams of whole seed. Consumption of Smartseed during the trial was 362 grams. During the period of testing the birds remained bright alert and responsive. There were no observable changes to behaviour or droppings. The clinical observations and bodyweight during toxicity trial have been presented in table 14.

Table 14: Body weight and clinical trial observations during toxicity trial 1

Leg Ring Number	Pre Trial Wt Grams	Post Trial Wt Grams	Observations over trial period.
VF 101	48	49	Nothing Remarkable
VF 102	42	45	NR
VF 103	44	43	NR
VF 104	49	49	NR
VF 105	45	42	NR
VF 106	42	43	Day 3 lameness*
VF 107	47	48	NR
VF 108	45	48	NR
VF 109	43	42	NR
VF 110	47	46	NR
VF 111	41	43	NR
VF 112	45	46	NR
Total weight	538 grams	544 grams	NA

*VF 106 Day 3 lameness: bird was observed clinically lame on the right leg. Bird was caught up and examined. The aluminium ring was overly tight on the leg causing some swelling. The ring was opened to relieve pressure and bird recovered uneventfully.

Budgerigars fed on Smart Seed for 7 days in an enclosed cage showed no discernible signs of toxicity. Behaviour and demeanour remained normal over the trial period indicating no acute toxicity had occurred. Weight variations within the group were observed but these are consistent with variation in crop fill at the time of weighing. Smart seed contains 30 micrograms moxidectin per gram of total seed mix. There are 3 seeds in the mix (oats, millet and canary seed). Millet and canary seed contain approximately 10 micrograms Moxidectin per gram of kernel while dehulled oats cannot be dehulled and contains 30 micrograms of Moxidectin per gram of seed. Testing confirms kernel alone contains 10 µg Moxidectin per gram. A gram of millet or canary is approximately 80% by weight kernel and 20% hull. Therefore approximately 8ug Moxidectin is consumed with each gram of millet or canary consumed. Assuming equal consumption of each seed (subjective observation) intake would equate to 46 micrograms of Moxidectin per 3 grams of consumed mix. So grams of seed eaten 362/3 x 46 = 5550 µg moxidectin consumed over 7 days by 12 birds of weight 538 grams (10,317 µg/Kg) or approximately 1473 µg/Kg/day. Adult budgerigars show no apparent toxicity to Smart Seed when given sole, ad lib access to the seed over a 7 day period.

Trial 2

6 budgerigars, 6 grass Parrots and 6 Ring Neck Parrots, within the protocol weight ranges, were ad hoc selected from private collections. The 6 birds of each species were placed, as a group, in metal bird cages measuring 1.2 m cubed with food and water containers and adequate perching. The cages were placed in an air conditioned room at approximately 25 C. All birds were acclimatised in the room for 7 days on potable water and commercial budgie mix (Avigrain). At the beginning of the test period the cages were cleaned of seed hulls and uneaten seed and

measured amounts of Smartseed placed in the feed containers. No other food of any description was given during the trial period. The birds selected were individually leg rung and weighed (ring numbers and weights recorded in table below). Each bird had a unique aluminium leg band applied (Chapman Rings, Mortdale, Sydney) and individual birds were placed in a cardboard box which had been tared on a set of AND, model HF-300G scales (Max310g, d-0.001g) and weight recorded (rounded to the nearest whole gram). The birds were maintained on fresh potable water and the Smart Seed Wormer for Parrots for 7 days. Birds in the aviary were observed each morning between 8 am and 10 am for any abnormal behaviour or demeanour by a qualified avian veterinarian.

During the period of testing the birds remained bright alert and responsive. There were no observable changes to behaviour or droppings. On day 8 the birds were reweighed and were returned to their original aviaries. The clinical observations have been presented in table 15, 16 and 17.

Table 15: Psephotus (Bodyweight and toxicity trial observations)

Leg Ring Number	Pre Trial Wt Grams	Post Trial Wt Grams	Observations over trial period.
VF 201	54	56	Nothing Remarkable
VF 202	61	61	NR
VF 203	64	65	NR
VF 204	58	55	NR
VF 205	52	55	NR
VF 206	62	66	NR
Total Bird Weight	351 grams	358	NA

Table 16: Nymphicus (Bodyweight and toxicity trial observations)

Leg Ring Number	Pre Trial Wt Grams	Post Trial Wt Grams	Observations over trial period.
VF 301	88	91	Nothing Remarkable
VF 302	92	96	NR
VF 303	96	93	NR
VF 304	84	89	NR
VF 305	95	90	NR
VF 306	81	83	*Discharge from eye
Total Bird weight	536 grams	542 grams	NA

*Discharge from eye of bird VF 306 was due to traumatic injury to eye. Recovered with treatment.

Table 17: Psittacula (Bodyweight and toxicity trial observations)

Leg Ring Number	Pre Trial Wt Grams	Post Trial Wt Grams	Observations over trial period.
VF 401	148	155	Nothing Remarkable
VF 402	157	160	Blood on feathers
VF 403	176	176	NR
VF 404	133	140	NR
VF 405	167	165	NR
VF 406	172	178	NR
Total Bird weight	953 grams	974 grams	NA

Blood on feathers of bird VF402 was found to be from a damaged blood quill.

Table 18: Seed consumption during toxicity trial 2. (Initial seed supplied based on estimated consumption of +/- 10% bodyweight per day x 7 days).

Group	Starting weight of Smartseed. (Grams)	Smartseed consumed. (Grams)	Total amount of Moxidectin consumed. µg	Moxidectin Intake µg/kg/day
Psephotus 351 g Bwt	300	220	3373	1372
Nymphicus 536 g Bwt	400	318	4876	1299
Psittacula 974 g Bwt	700	627	9614	1410

Following the trial, remaining seed was winnowed and weighed to calculate Smartseed consumed. The treatment groups of parrots fed on SmartSeed for 7 days in an enclosed cage showed no discernible signs of toxicity. Behaviour and demeanour remained normal over the period which would indicate no acute toxicity had occurred. Weight variations within the group were observed but these are consistent with variation in crop fill at the time of weighing and birds being confined to a cage. Smartseed contains 30 micrograms per gram of total seed mix. There are 3 seeds in the mix (oats, millet and canary seed). Millet and canary seed contain approximately 10 micrograms per gram of kernel while dehulled oats cannot be dehulled and contains 30 micrograms per gram of seed. Manual dehulling of individual millet and canary seeds and subsequent retesting of the kernel shows the kernels of both grains to contain approximately 10 micrograms of moxidectin per gram of kernel. A gram of millet or canary is approximately 80% by weight kernel and 20% hull. Therefore approximately 8 µg Moxidectin is consumed with each gram of millet or canary consumed. Assuming equal consumption of each seed (subjective observation) would equate to 46 micrograms of Moxidectin per 3 grams of consumed mix. Calculations of amount of Moxidectin consumed during the trial are in Table 18. Changes in weight of birds are to be expected due to relative crop fill and birds being in a confined space for the duration of the trial. Adult parrots of differing species and weight showed no apparent toxicity when given ad lib access to Smartseed for 7 days under controlled conditions.

Histopathological studies

The images of the tissue samples for the histopathological study of the lung, liver, and kidney following treatment with freeze dried seed for three days at usual dose are shown in Figure 2. The changes observed in the tissues examined were similar in both groups (control and treatment) as assessed in a qualitative way. No histological findings were noted that could potentially attribute pathologic changes to the administration (or not) of the drugs used. Minimal changes were seen in the liver, kidneys, and lungs in both groups, possibly representing background pathology as opposed to the changes caused by the effects of moxidectin administration. Moreover, these changes were not dissimilar to what is usually observed in

budgerigars and are not likely to be significant or represent the effects of moxidectin administration. The similar pattern of changes (Table 19) in various organs proves that the mild changes are independent of moxidectin administration, and the formulation is rather safe for budgerigars.

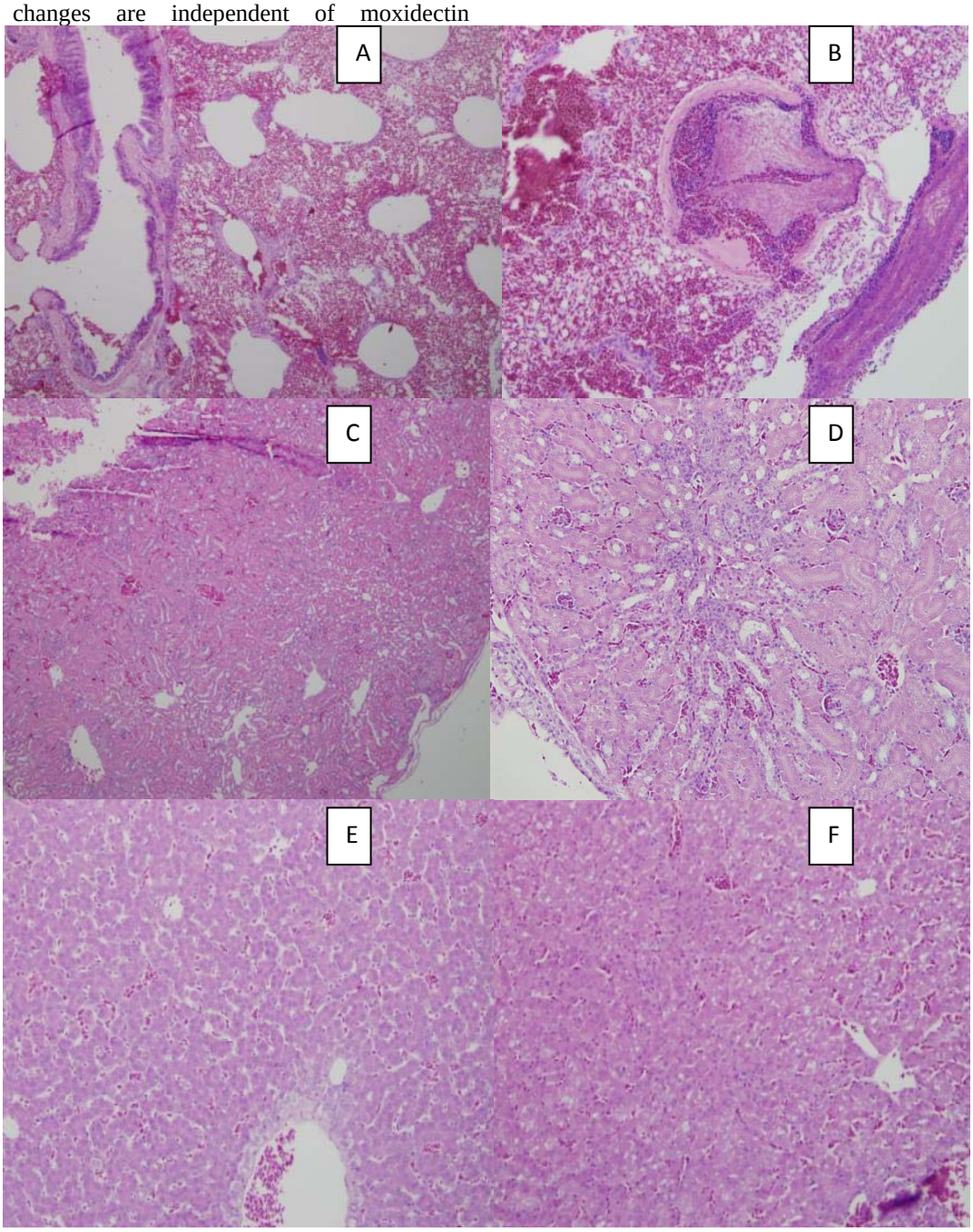


Figure 2. Histopathology study report (Selected data). Control - A. Lung, C. Kidney, E. Liver. Treated - B. Lung, D. Kidney, F. Liver.

Table 19: Histopathology study data at recommended dose

Group	Kidney	Liver	Lung
Control	A moderate number of renal tubular epithelial cells contained small amounts of haemosiderin.	Multifocal, mild hepatocellular vacuolation; no noteworthy changes.	Marked congestion.
Treatment	No noteworthy changes.	Moderate to marked congestion, multifocal, mild hepatocellular vacuolation; minimal to mild individual cell necrosis; minimal to mild evidence of hepatocellular regeneration	Marked congestion; several large and medium calliber vessels were partially occluded by thrombi

CONCLUSION

A safe, effective, stable novel medicated seedmix has been developed for the management of internal parasites in caged and aviary birds. Although, it has a good potential to become a convenient alternative approach with improved compliance, it still remains as a field to be explored to the fullest. In the future, we may see drugs formulated into seedmix in preference to other delivery systems to deliver drugs to large varieties of caged and aviary birds as a means of simple food intake. Drug delivery through medicated seedmix is fairly a new concept and will revolutionise the drug delivery concept in avian industry in near future.

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