

RIFAMPICIN, A NEW ANTIBIOTIC IN INSEMINATION OF QUEEN BEES

Bożena Chuda-Mickiewicz, Jarosław Prabucki

Department of Apiculture, Agricultural University, ul. Doktora Judyma 20, 71-466 Szczecin, Poland

S u m m a r y

The aim of the study was to verify the value of rifampicin in artificial insemination of queen bees. The obtained results permit to ascertain, that the number of spermatozoa reaching the spermatheca of queens inseminated with antibacterial protection of rifampicin and streptomycin was very similar, the difference being non-significant. Slightly better spermathecal spermatozoa count was observed with enlarged bee stocking. Queens inseminated with both antibiotics used for protection started egg laying in the same time. Microflora accompanying spermatozoa is fairly abundant, the majority of bacteria being classified among the genus *Bacillus*. *Bacillus* sp. undergoes more stronger reduction under the effect of rifampicin than under that of streptomycin. Rifampicin is an antibiotic that is inoffensive to spermatozoa, providing better antibacterial protection for drone semen when compared to streptomycin.

Keywords: queen bee, insemination, antibiotic, semen microflora.

INTRODUCTION

Efficiency of queen bees insemination depends on many factors, among other on the quality of semen, age of queens, or the conditions in which they are kept before and after insemination (Woyke 1979; Woyke and Jasiński 1978, 1979, 1980, 1982; Prabucki et al. 1987). An equally important factor is asepsis of the insemination treatment itself. Insemination apparatus, in particular these parts of it which are in direct contact with semen and queen, is subjected to disinfection with 72% alcohol or with disinfectants used for surgical instruments. For antibacterial protection of semen streptomycin is used (Vavilov 1978; Vavilov and Klepikova 1978; Poole and Taber, after Skowronek and Konopacka 1983). It is added to physiological fluid or to boiled water that draws semen in to capillary tube. This antibiotic, according to Kowszyk-Gindifer and Sobieczewski (1990), is characterised by narrow spectrum of activity. It is active against bacilli, effective against many Gram negative anaerobic bacilli and few staphylococci. According to the above cited authors, a water-soluble antibiotic with much broader activity spectrum is rifampicin. It is characterised by high activity towards Gram positive bacteria, most of all to staphylococci and streptococci, also being effective against Gram negative

coccidia. It is also distinguished by much lower toxicity than streptomycin, and by considerably higher stability. The presented qualities of rifampicin were a reason for taking up studies on the possibility to use it in instrumental insemination of queen bees. The lack of information in available literature on semen microflora of drones prompted us to make bacteriological assessment of semen collected from them.

MATERIAL AND METHODS

Examinations were carried out at Agricultural University of Szczecin in bee-keeping seasons of 1998 and 2000. They were made on queens reared from one-day larvae with Jenter's method. In the first year these were queens of the Caucasian, Carniolan and the Central European breeds, while in the second one those of the Central European breed. In the first year of the study sealed queen cells were introduced to boxes with approximately 250 bees before queens emerged, while in the second one to trapezoid mating hives settled with 400 bees. Queens were inseminated twice at the age of the 7th and the 9th day with a dose of 4 µl of semen collected from drones between day 14 and 18 of their life. When collecting the semen, two antibiotics had been used: rifampicin or streptomycin. They were added to the water drawing the semen in to syringe capillary tube in the amount of 100 mg rifampicin or 50 mg streptomycin per 100 ml boiled and cooled down water. In the inseminated queens was determined the number of spermatozoa reaching the spermatheca according to the method of Woyke (1979). In the first year spermatozoa were counted after forty-eight hours after the second insemination treatment, while in the second one on entering the egg laying by queens. The onset of oviposition had been established by daily inspection of mating hives beginning with the 4th day from second insemination.

Microbiological assays of semen were carried out in the second year of the study at the Department of Immunology and Microbiology of our University. In order to do this, 9 semen samples were collected from drones, 8 µl each. Semen was drawn in with physiological fluid to capillary tubes, thus being safe-guarded against drying. The collected semen was divided into three parts (groups): I - without antibiotics, II - with 0.2 mg streptomycin, and III - with 0.2 mg rifampicin. Semen without antibiotic, in the amount of 5 µl, was inoculated on 0.45 ml enriched broth bouillon. Decimal dilutions of semen were made in broth bouillon as well, from which inoculates, 0.1 ml each, were cultured on differential-selective mediums and Columbia agar. Thereafter, bacterial cultures were incubated for 24 hours at the temperature of 37°C. Semen of the second and third group was treated with alike procedure. It was inoculated in the amount of 5 µl on 0.5 ml enriched broth bouillon, followed by decimal dilutions, and inoculates, 0.1 ml each, were cultured on Columbia agar only. The bacterial colonies grown were stained with the method of

Gram. The obtained data were analyzed statistically with the use of Student's t-test

RESULTS

Queens that were kept with bees in boxes (first year of studies) after insemination with rifampicin or streptomycin protection had 4.05 million spermatozoa on the average in their spermathecas (Tab. 1). Slightly better spermatheca filling with spermatozoa was found in queens of the Middle European breed, in which mean number of spermatozoa was higher than that in Caucasian and Carniolan queens, by 0.97 and 0.56 million, respectively. Protection antibiotic used at semen collecting did not have greater effect on spermatozoa migration to the spermatheca. After insemination of queens with rifampicin as antibiotic protection, 3.99 million spermatozoa on the average was found in the spermatheca, the number of spermatozoa being higher by 0.14 million when streptomycin was used. Somewhat more clear differences were observed between breeds, the differences being however statistically non-significant in that case either.

Table 1

Number of spermatozoa in spermathecas of queens inseminated with rifampicin and streptomycin protection when kept in boxes - Liczba plemników w zbiorniczkach nasiennych matek inseminowanych z użyciem rifampicyny i streptomycyny przy przetrzymywaniu ich w skrzynkach

Breed - Rasa	Spermatozoa number (in millions) - Liczba plemników w mln							
	rifampicin - rifampicyna			streptomycin - streptomycyna			total - razem	
	n	range od- do	mean Średnio	n	range od- do	mean Średnio	n	mean Średnio
<i>A. m. caucasica</i>	3	3,04-3,74	3,38	4	3,04-3,50	3,36	7	3,37
<i>A. m. carnica</i>	5	3,10 -3,86	3,72	4	3,33-4,11	3,84	9	3,78
<i>A. m. mellifera</i>	11	2,08-6,72	4,27	14	2,48-6,55	4,39	25	4,34
Average - Średnio			3,99			4,13		4,05

In the second year of studies queens were kept in mating hives. In that case, queens' spermathecas were filled with 4.39 million spermatozoa on the average (Tab. 2). Spermathecal spermatozoa count had been connected with the series of queens rearing. The highest number of spermatozoa (4.49 million on the average) had been found in queens from series III, thus departing significantly from queens of rearing series I in which it was smaller by 1.2 million on the average. Intermediate spermatozoa number (4.23 million), not different significantly from those mentioned above, was found in queens from

series II. When rifampicin was added, 4.47 million spermatozoa on the average was the spermatheca. Slightly worse spermathecal spermatozoa count, by 0.16 million, was found in queens inseminated when streptomycin was used as antibiotic protection. On the other hand, significant differences in spermathecal spermatozoa count were found between series of inseminated queens, in particular when rifampicin was used as antibiotic screen. The differences stated, however, proved to be significant only between queens of series I and III.

Table 2

Number of spermatozoa in spermathecas of the Middle European queens inseminated with rifampicin and streptomycin protection when kept in mating hives - Liczba plemników w zbiorniczkach nasiennych matek rasy środkowoeuropejskiej inseminowanych z użyciem rifampicyny i streptonicyny przy przetrzymywaniu ich w ulikach weselnych

Rearing series Seria wychowu	Spermathecal spermatozoa count (in millions) Wypełnienie zbiorniczka nasiennego plemnikami (mln)							
	rifampicin - rifampicyna			streptomycin - streptomycyna			total - razem	
	n	range od - do	mean średnio	n	range od - do	mean średnio	n	mean średnio
I	8	2,93 - 4,44	3,88a*	7	2,02 - 5,90	3,80	15	3,85a
II	5	3,29 - 5,45	4,18ab	6	3,29 - 6,21	4,27	11	4,23ab
III	9	3,92 - 6,73	5,12b	8	3,75 - 5,98	4,79	17	4,97b
Average - Średnio			4,47			4,31		4,39

* values in columns followed by different letters differ significantly at $p = 0,05$

In the second year of the study, out of the total number of 46 queens inseminated 93.48% had entered egg laying (Tab. 3). Antibiotic used for protection did not have greater effect on the oviposition. With rifampicin used as antibiotic screen, 95.6% queen bees started egg laying, whereas the efficiency of insemination was smaller only by 4.3% when streptomycin was used for protection. Also the time of waiting for queens starting the oviposition was very similar for both antibiotics used, i.e. 9.3 (for rifampicin) and 8.9 days (for streptomycin) on the average, the difference being statistically non-significant. In the studies performed, the prevailing majority of queens, i.e. 91.3% (for rifampicin) and 82.6% (for streptomycin) started egg laying before the 14th day since the first insemination. Queens inseminated with semen with rifampicin antibacterial protection started laying eggs after 8.9 days, while those inseminated with streptomycin as antibiotic screen initiated oviposition after 8.9 days from the first insemination treatment, the difference in both groups being approximately 1.7 day.

Table 3

Onset of oviposition in inseminated queens with rifampicin and streptomycin used for protection - Podejmowanie czerwienia przez inseminowane matki przy wykorzystaniu rifampicyny i streptomycyny

Rearing series Seria wychowu	Antibiotic Antybiotyki	Number of inseminated queens Liczba matek inseminowanych	Onset of oviposition - Podejmowanie czerwienia							
			to day 14 after 1 treatment do 14 dnia po 1 zabiegu			to day 14 after 1 treatment do 14 dnia po 1 zabiegu			total razem	
			n	on the average after day średnio po dniach	%	n	on the average after day średnio po dniach	%	on the average after day średnio po dniach	%
I	rifampicin rifampicyna	8	7	9,6	87,5	1	16	12,5	10,4	100,0
	streptomycin streptomycyna	7	7	7,8	87,5	0	0	0	7,8	87,5
II	rifampicin rifampicyna	6	5	8,0	83,3	0	0	0	8,0	83,3
	streptomycin streptomycyna	7	6	6,8	85,7	0	0	0	6,8	85,7
III	rifampicin rifampicyna	9	9	9,0	100,0	0	0	0	9,0	100,0
	streptomycin streptomycyna	8	6	9,5	75,0	2	17	25,0	11,4	100,0
Total łącznie	rifampicin rifampicyna	23	21	8,9	91,3	1	16	4,3	9,3	95,6
	streptomycin streptomycyna	23	19	8,1	82,6	2	17	8,7	8,9	91,3

Table 4

Efficacy of antibiotics in antibacterial protection of drone semen (CFU/ μ l)
Skuteczność antybiotyków w ochronie antybakteryjnej nasienia trutni
(j.t.k./ μ l.)

Semen - Nasienie	Number of <i>Bacillus</i> sp. cells Liczba komórek <i>Bacillus</i> sp.
without antibiotic - bez antybiotyków	$1000,0 \times 10^{10}$
with rifampicin - z rifampicyną	$2,0 \times 10^2$
with streptomycin - ze streptomycyną	$7,0 \times 10^4$

CFU - Colony Forming Unit - j.t.k - jednostka tworząca kolonię

The performed microbiological assay of drone semen showed single yeast cells of *Candida* genus, sporocysts of *Ascospheara apis*, coccidia, and numerous Gram positive and Gram negative bacilli classified among *Bacillus*

genus. Antibacterial efficacy in drone semen protection is presented in Table 4. It turns out from the table that the number of *Bacillus* sp. cells undergoes rapid reduction to 7.0×10^4 when streptomycin is used, and to 2.0×10^2 for rifampicin when compared to semen without any contact with both antibiotics. Consequently, it may be stated that rifampicin is a much more effective antibiotic in respect of antibacterial activity than streptomycin.

CONCLUSIONS

1. The number of spermatozoa reaching the spermatheca of queens inseminated with antibacterial protection of rifampicin and streptomycin was very similar, the difference being non-significant. Slightly better spermathecal spermatozoa count was observed with enlarged bee stocking.
2. Queens inseminated with both antibiotics used for protection started egg laying in the same time,
3. Microflora accompanying spermatozoa is fairly abundant, the majority of bacteria being classified among *Bacillus* genus. *Bacillus* sp. undergoes stronger reduction under the effect of rifampicin than under that of streptomycin.
4. Rifampicin is an antibiotic that is inoffensive to spermatozoa, providing better antibacterial protection for drone semen when compared to streptomycin.

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RIFAMPICYNĄ NOWY ANTYBIOTYK W INSEMINACJI MATEK PSZCZELICH

Chuda-Mickiewicz B., Prabucki J.

S t r e s z c z e n i e

Badania wykonano w sezonie 1998 i 2000 roku. Celem ich było określenie przydatności rifampicyny w procesie sztucznego unasienniania matek pszczelich oraz zbadanie mikroflory w pobieranym od trutni nasieniu. W pierwszym roku obserwacje prowadzono na matkach przetrzymywanych w skrzynkach z ok. 250 pszczołami w drugim zaś, w ulikach weselnych nasiedlonych ok. 400 robotnicami. Unasieniając matki (2 x 4 μ l nasienia) korzystano z dwóch antybiotyków rifampicyny i streptomycyny. Dodawano je do wody zasysającej nasienie w kapilarze strzykawki, w ilości 100 mg rifampicyny lub 50 mg streptomycyny na 100 ml wody. U unasiennionych matek określono liczbę plemników w zbiorniczkach. W pierwszym roku wypreparowywano zbiorniczki i liczono plemniki dwie doby po drugim zabiegu inseminacyjnym w drugim zaś po podjęciu czerwienia przez matki.

Nasienie przeznaczone do badań mikrobiologicznych pobierano do kapilar używając płynu fizjologicznego. Ocenie mikrobiologicznej poddano nasienie do którego po pobraniu dodawano 0,2 mg rifampicyny lub streptomycyny na 5 μ l nasienia oraz nasienie bez dodatku antybiotyków.

Antybiotyk stosowany przy pobieraniu nasienia nie miał większego wpływu na przechodzenie plemników do zbiorniczków nasiennych. U matek przetrzymywanych w skrzynkach z pszczołami, po inseminacji z użyciem rifampicyny w zbiorniczkach znajdowało się przeciętnie po 3,99 mln plemników zaś z użyciem streptomycyny tylko o 0,14 mln plemników więcej. W przypadku przetrzymywania matek w ulikach weselnych stosując dodatek rifampicyny do zbiorniczków przechodziło przeciętnie po 4,47 mln plemników. Nieznacznie gorsze wypełnienie zbiorniczków o 0,16 mln miały matki unasiennione z wykorzystaniem streptomycyny.

Z ogólnej liczby 46 matek przetrzymywanych w ulikach weselnych 93,48% podjęło czerwienie. Zastosowany antybiotyk nie miał większego wpływu na ich czerwienie. Używając rifampicyny 95,6% matek rozpoczęło składać jaja. Stosując streptomycynę efektywność unasiennień była niższa jedynie o 4,3%. Również czas oczekiwania na

rozpoczęcie czerwienia matek przy stosowanych antybiotykach był bardzo zbliżony i wynosił średnio 9,3 dnia (dla rifampicyny) i 8,9 dnia (dla streptomycyny).

Przeprowadzone badanie mikrobiologiczne wykazało, że w nasieniu trutni występują: komórki drożdży z rodzaju *Candida*, sporocysty *Ascospheera apis*, ziarniaki i liczne Gram dodatnie i Gram ujemne laseczki z rodzaju *Bacillus*. Stosowane antybiotyki odznaczały się wysoką skutecznością antybakteryjną. Liczba kmórek *Bacillus sp.* ulegała znacznie większej redukcji w przypadku stosowania rifampicyny ($2,0 \times 10^2$) aniżeli streptomycyny ($7,0 \times 10^4$). Rifampicyna zapewnia zatem lepszą antybakteryjną ochronę dla pobieranego od trutni nasienia.

Słowa kluczowe: matka pszczela, inseminacja, antybiotyk, mikroflora nasienia.