السعودية المملكة العربية
العالي وزارة التعليم
فيصل جامعة الملك
البحث العلمي عمادة

Kingdom of Saudi Arabia
Ministry of Higher Education
King Faisal University
Deanship of Scientific Research

تقرير متابعة مشروع بحث سول

التقرير:

☐ الأول ☐ الثاني ☐ الثالث ☐ الرابع ☑ النهائي

١. الباحث الرئيس : د. عبدالقادر موسى حميده

- **الكلية : الطب البيطري والثروة الحيوانية**
- **القسم : وظائف الأعضاء والكيمياء الحيوية والأفربازين**

٢. عنوان المشروع :

تحديد فترة سحب أدوية السلفا في حليب النوق

٣. رقم المشروع : ٥١٠١ البحوث الغيرة

- **ميزانية المشروع: ١٧,٠٠٠**
- **المنصرف حتى تاريخ التقرير: ١٧,٠٠٠**
- **تاريخ البدء في المشروع : ١٤٢٥/٤/١هـ**
- **التاريخ المتوقع للانتهاء : ١٤٢٥/١٠/١٥هـ**

٤. النسبة التقريبية لما تم انجازة من المشروع:

☐ اقل من ١٠% ☐ بين ١٠% و٣٠% ☐ بين ٣٠ % و ٦٠ % ☐ أكثر من ٦٠ %

٥. صعوبات تواجه المشروع:

- لا توجد
-

٦. معد التقرير : د. عبدالقادر موسى حميده

التوقيع: ..... **التاريخ : ١٤٢٥/١٠/١٥هـ**

البريد الإلكتروني: .....

Final Report

Title of Project :

Establishment of Withdrawal Time of Sulfa Drugs in Camel Milk

Investigator :

Abdel Gadir Musa Homeida

Supported by :

Deanship of Scientific Research , KFU

Project No : 5101

Date : 21.10.1425

1. Literature Review

Sulphonamidine (SDM) is one of the most extensively used drugs in veterinary medicine for prophlaxis and therapeutics . The metabolic pathway of the drug involves acetylation of the paraamino group (N_4-acetylation), hydroxylation of heterocylic ring, glucuronidation (N_1-position), sulphate conjugation and presumably other minor pathways (Gilman et al, 1991) . Marked species differences in the metabolism of SDM have been reported (Yuan and Fung, 1990) . Ruminants, monkeys, birds , pigs , primates, horses , camels and man are able to acetylate sulphonamide drugs (Vree and Hekster, 1985; Nouwas et al, 1983; Younan et al, 1989) .

Camel's milk is less affected by storage and transportation than cow's milk . It has been reported that the acidity increases slowly at 30^{o}C with little or no change in the taste (Knoess 1982) . The general practice in Saudi Arabia is to consume the milk fresh . However , most Bedouin families pool the surplus milk with goat's milk and convert it to a dry fermented product called "Uqt" .

Passage of Antimicrobial Agents into Milk :

Studies of the penetration of antimicrobial agent's from the systemic circulation into milk indicate that the mammary gland epithelium behaves as a lipoidal membrane which separates blood of pH 7.4 from milk . Which has a somewhat lower pH value (normal pH range is 6.5 to 6.8) (Baggot 1983) . The passage of each drug into milk is determined by the extent of binding to plasma albumin . the pK value and the degree of lipid solubility .

It has been shown that only the hpid-soluble . nonionized moiety of an organic electroyte in the water phase of blood plasma diffuses into milk (Rasmussen . 1966). The binding of sulfonamides to milk proteins varies from zero to 40 per cent

depending on the derivative. In normal lactating cows. Weak acids give milk ultrafiltrate-to-piasma ultrafiltrare concentration ratios less than or equal to 1:

SDM residues have been found in milk following prophylactic and therapeutic use for mastitis, follwing intrauterine administration for treatment of metritis , or form contamination of milking equipment (Pugh et al., 1977; Oliver et al., 1984; Egan and O Connor, 1983 ; Slee and Brightling . 1981 ; Egan and Meaney , 1985) .

Risks to human health :

Possible risk to man of SDM residues in milk includes :

1. SDM – induced allergic reactions .
2. Alteration of the gastro – intestinal flora .
3. Prevalence of antmicrobial resistance among bacteria .
4. Carriage by man of antimicrobial – resistant – bacteria of animal origin .

Withdrawal Periods

Probably the most controversial area associated with drugs use in farm animals is the adherence or otherwise to stated withdrawal periods . These are imposed in an endeavour to prevent any significant amount of drugs being present in meat , milk or animal products destined for human consumption .

The term “withdrawal period” or “withdrawal time” is generally recognized as the period of time that must elapse between the last use of the drug preparation and the collection of meat or milk for human consumption (Oliver et al 1990) . Generally speaking , adherence to withdrawal times presents a variety of problems for the stockman . These may range from the impracticability of changing over to non-

medicated feed shortly before disposal to the obvious difficulties presented by the random selection of animals from different pens for slaughter . Probably the only way to ensure a general adherence to withdrawal periods is to penalize those users who consistently fail to comply . In case of milk , certain countries introduce a blue dye with mastitis preparation so as to preclude blue-stained milk being forwarded for manufacture ; this would also provide a simple and rapid colorimetric screening test instead of a biological test for the presence of inhibitory substances . Unfortunately a problem with using such a dye is that no one substance would be excreted at the same rate as all the currently used antibiotics .

b. Objectives

1- The purpose of this study is to establish the withdrawal period of some common antibiotic preparations frequenthy used to freat dairy camel , in Saudi Arabia .

2- To test whether SDM metabolites are excreted in milk .

The antibacterial treatment of mastitis in lactating animals is of considerable regulatory concern because of the possibility of antibacterial residues in milk[1]. Although intramammary (i.m.m) infusion has been recognized as the route of choice for treating mastitis[2], other investigators have recommended the use of combination of routes for administration of antibacterial agents[1,3]. Over 16% of milk samples collected 96h post-treatment were positive for antibiotic[3].

Sulphadimidine (SDM) is one of the most extensively used drugs in veterinary medicine for prophylaxis and therapeutics[4]. In camels, SDM is eleminated slowly from the body and the main SDM metabolite detected in plasma is N_4-acetyl

derivative (N_4-acetyl)[4, 5]. The withdrawal period for SDM and its metabolite is not prescribed in milk of lactating camel, therefore the objective of the present study were to deterime the concentration of SDM and its metabolite in milk after administration as a single or repeated-dose of treatments and follow the depletion of SDM in milk until its concentration dropped below the maximum residue limit.

2. MATERIALS and METHODS

Animals and treatments: Eight lactating one-humped camels, 4-5 years old, weighing 200-250 kg and representing various levels of milk production were used. Animals had free access to hay and water *ad libitum*. The camels were individually milked twice daily at 12h interval. Sulphadimidine (33.3%, Bremer Pharma GmbH, Germany) as a single dose treatment of 50 mg kg^{-1} (group1, 4 animals) or repeated dose treatments of 50 mg kg^{-1} (group2, 4 animals) was given intravenously (IV) to animals. The repeated doses were administered daily after morning milking for 3 days.

Collection of plasma and milk samples: Milk samples were collected at every milking from each cow. The first milk samples was taken before injection of drug. Samples were stored in labelled plastic containers at –20 ^{o}C until analysis. Blood samples were collected from group1 and 2 animals into heparinizied tubes, centrifuged at 2000 × g and plasma was separated and stored at –20 ^{o}C until analysis.

Sulphadimidine and its metabolite measurment: The HPLC analysis of SDM and N_4-acetyl was previously described [5]. The mobile phase consisted of methanol, acetonitril, 0.02M Sodium acetate, 0.2M acetic acid and distilled water 15: 4: 27: 50: 3.6, respectively. The detection limit for SDM and N_4-acetyl

Was 0.1 μg ml^{-1}.

Protein binding: Ultrafilterate of selected plasma and milk samples were obtained with the reusable Micropartition System (Amicon Corp, Leyington, MA). The ultrafilterates obtained were measured by HPLC and percentage protein-binding was calculated as descriped previously[6]. Values were compared using Student's *t-test*[7]. The probability value $P < 0.05$ was considered significant

3. RESULTS

Sulphadimidine or its metabolite was not detected in any milk samples collected before dosing in single (group-1) and repeated dose (group-2) treatments. The mean concentration of SDM and N_4-acetyl in milk was detected at the first milking (12h) post dosing in both groups (Table-1). Repeated injection of sulphadimidine in group-2 increased ($P < 0.01$) the concentration of both SDM and N_4-acetyl in milk compared to group-1.

Depletion of SDM and N_4-acetyl to < 0.1 μg ml^{-1} occurred at 10^{th} (120h) and 4^{th} (48h) milking, respectively, in single-dose treatment. Depletion of SDM and N_4-

acetyl to < 0.1 μg ml^{-1} occurred at 16^{th} (192h) and 11^{th} (121h) milking, respectively, in repeated-dose treatment. SDM concentration were < 0.1 μg ml^{-1} at 120h after the last dose of treatment in both group.

Sulphadimidine and N_4-acetyl plasma protein binding was significantly ($P < 0.01$) in single dose treatment (Table-2) highers than in repeated dosetreatment. The binding of SDM and N_4-acetyl in milk protein was significantly ($P < 0.01$) lower than in plasma proteins, the ratio being < 1.

Table-1: Mean ±SD concentration (μg ml^{-1}) of Sulphadimidine and N_4-acetyl in milk of camels after intravenous administration of SDM as a single or repeated dose treatments at a dose of 50 mg kg^{-1} body weight. (n=4 each).

Milking No.	Single-dose treatment		Repeated-dose treatment	
	SDM	**N_4-acetyl**	**SDM**	**N_4-acetyl**
Before dosing	ND	ND	ND	ND
1	10.1 ± 2.1	0.3 ± 0.1	10.3 ± 2.0	0.41 ± 0.2
2	5.3 ± 1.1	0.2 ± 0.1	14.2 ± 2.0	0.71 ± 0.3
3	3.1 ± 0.6	0.1 ± 0.1	18.1 ± 2.5	0.73 ± 0.3
4	2.1 ± 0.5	ND	16.1 ± 2.2	0.68 ± 0.4
5	1.6 ± 0.4	ND	17.2 ± 2.5	0.70 ± 0.2
6	1.2 ± 0.4	ND	16.0 ± 2.5	0.69 ± 0.3
7	0.4 ± 0.2		12.3 ± 2.0	0.43 ± 0.2
8	0.26 ± 0.1		10.1 ± 2.1	0.39 ± 0.2
9	0.1 ± 0.1		6.2 ± 1.1	0.31 ± 0.1
10	ND		3.1 ± 0.6	0.23 ± 0.1
11	ND		2.3 ± 0.8	ND
12	ND		1.6 ± 0.4	ND
13			1.1 ± 0.4	ND
14			0.4 ± 0.1	
15			0.21 ± 0.1	
16			ND	
17			ND	
18			ND	

ND: not detected or below assay sensitivity.

Table-2: Percentage binding of Sulphadimidine and N_4-acetyl to plasma and milk proteins. (n=4 each).

Samples	Single-dose treatment		Repeated-dose treatment	
	SDM %	**N_4-acetyl %**	**SDM %**	**N_4-acetyl %**
Plasma	85 ± 3.1	83.1 ± 3.3	51.3 ± 2.1	46.4 ± 2.1
Milk	41.1 ± 1.6	16 ± 1.8	44.2 ± 2.0	19.9 ± 1.7

5. DISCUSSION

Intravenous single-dose treatment or repeated-dose treatments with sulphadimidine produced increased concentration of SDM and its metabolite N_4-acetyl in the milk of camel. Injection of sulphadimidine to cattle and sheep by different routes have also produced SDM in milk[8]. Since the sodium salts of SDM is basic in nature they tend to distribute more readily into milk due to pH □aximum□ng phenomenon[9]. The pH of milk is acidic (6.6), therefore, SDM will ionize and be excreted in milk.

Residues of SDM in milk must be controlled as recent evidence indicating that SDM may be carcinogenic in human consuming small amounts over long period of time[10]. The primary reason for the occurrence of SDM residues in milk were the failure to observe drug withdraural time when drug concentration is at □aximum residue limit (MRL). The MRL in milk was suggested to be 0.1 μg ml^{-1}[11]. Depletion of SDM to MRL of 0.1 μg ml^{-1} in this study occurred five days post injection in single-dose treatment or five days after the last injection in repeated-dose treatment suggesting that five days could be considered as Withdrawal period in milk of camels. For dairy cows a period of 3-4 days was suggested as Withdrawal period in milk[8].

In repeated-dose treatments the percentage protein binding for SDM and N_4-acetyl was relatively less than in single-dose treatment. Similar observations at high and low plasma concentration of SDM have been reported[12]. A saturation of protein binding sites has to be assumed, and SDM may compete with its metabolite for the same binding site[8]. The fact that metabolite concentrations in milk did not exceed those of parent drug and milk protein binding for metabolite was very low, suggests that monitoring of sulphadimidine in milk of camel could be limited to SDM alone.

ACKNOWLEDGEMENTS

The authors thanks the Deanship of Scientific Research, King Faisal University for financial support.

5. REFERENCES

[1] **Sandholm M., Kaartinen, L. and Pyorala S.** (1990). Bovine mastitis-why does antibiotic therapy not always work? An overview. J. Vet. Pharmacol. Therap., **13**: 248-260.

[2] **Soback S.** (1988).Therapeutic success or failure in mastitis therapy-pharmacologic approach. J. Vet. Med., **44**: 233-243.

[3] **Seymour E. M., Jones G. M. and Gilliard M. L.** (1988). Persistence of residues in milk following antibiotic treatment of dairy cattle. J. Dairy Sci., **71**: 2292-2296.

[4] **Younan W., Nouws J. F. M., Homeida A. M., Vree T. B. and Degen M.** (1989). Pharmacokinetics and metabolism of sulphadimidine in the camel. J. Vet. Pharmacol. Therap., **12**: 327-329.

[5] **Homeida A. M.** (2000). Phenotypic variation in sulphoramide acetylation. J. Camel Pract. Res., **7**: 109-111.

[6] **Al-Nazawi M. H.** (2005). Pharmacokinetics and tolerance of thiamphenicol in camels and sheep. Intl. J. Pharmacol.,. **1**: 25-28.

[7] **Kirkwood, B. R.** (1988). Essential of Medical Statistics. Blackwell Scientific Publications, Oxford.

[8] **Nouws J. F. M., Vree T. B., Breukink H. J., Baakman M., Driessens F. and Smulders A.** (1985).Dose dependent Disposition of sulphadimidine and its N_4-acetyl and hydroxy metabolites in plasma and milk of dairy cows. The Vet.

Quart., **7**: 177-186.

[9] **Riviere J. E. and Sundlof S. F.** (2001). Chemical residues in tissues of food animals. In: Veterinary Pharmacology and Therapeutics. H. R. Adams (Ed.), Iowa State University Press, Iowa, pp. 1167-1174.

[10] **Littlefield N. A., Sheldon W. G., Allen R. and Gaylor D. W.** (1990). Chronic toxicity/carcinogenicity studies of sulphamethazine in Fischer 334/N rats. Food Chem. Toxicol., **28**: 157-167.

[11] **Bevill R. F.** (1989). Sulfonamide residues in domestic animals. J. Vet. Pharmacol. Therap., **12**: 241-252.

[12] **Vree T. B. and Hekster Y. A.** (1985). Pharmacokinetics of sulphonamides revisited. Antibiotics and Chemotherapy, **34**: 201-208.