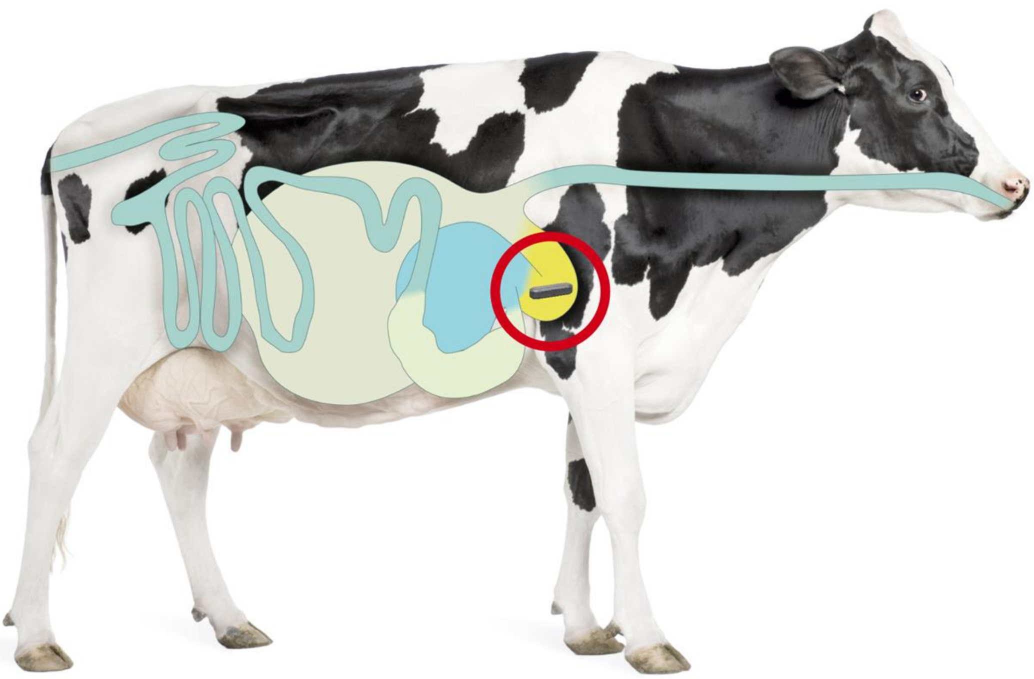
Economic effects of garlic bolus supplementation to dairy cows in the dry period



Research Project Veterinary Medicine University Utrecht

E.C. den Hartog

Student number: 3755258
Jan-Jun 2018

Supervisor:

Dr. Ir. W. Steeneveld

Department of Farm Animal Health
Faculty of Veterinary Medicine

Abstract

Due to global concerns about antimicrobial resistance and since preventive use of antimicrobials in the Netherlands is no longer allowed, Dutch dairy farmers are looking for alternatives for dry cow therapy. Recently, supplementing dry cows with garlic boluses, as an alternative for preventive antimicrobial therapy, has become more popular in the Netherlands. Because economic losses are one of the main factors for a farmer to improve mastitis management, it is important to know whether supplementing dry cows with garlic boluses is economically beneficial. The aim of this study was to calculate whether the supplementation of oral garlic boluses used as a preventive measure against mastitis in the dry period and the first 14 days of lactation could be economically profitable under Dutch circumstances, considering variation in parameters and probabilities. A stochastic Monte Carlo simulation model was developed simulating 10,000 multiparous cows in the dry period and the first 14 days of the successive lactation. Factors in the model included the probability of the cow developing mastitis in the dry period, at calving and or the first 14 days of lactation, the probability of bacteriological and or clinical cure after treatment, the probability of spontaneous cure and the physiological effects of the infection. Two scenarios were simulated, where in the first scenario the cow was supplemented with garlic boluses in the dry period and the second scenario where the cow was not supplemented. The consequences of supplementing or not supplementing garlic boluses were simulated and total average costs per scenario were calculated. Finally, total average costs were compared for the two scenarios. Some inputs for the model were based on literature and assumptions made by the author were used if no information from literature was available. The average costs of a non-supplemented cow were estimated to be €103 per cow, under default circumstances. On average, the total costs decreased with €5 when a cow was supplemented with garlic boluses in the dry period. The decrease in costs for factors such as milk production losses, discarded milk and culling did outweigh the additional costs of supplementing a cow with garlic boluses of €47 (bolus and labour costs). A sensitivity analyses showed that the profitability of supplementing a cow with garlic boluses depend on farm-specific factors (such as culling costs and milk price) and cow-specific factors (such as the probability of occurrence of mastitis during lactation). It also showed that costs made during lactation had the greatest influence on the total costs, so for supplementing the garlic boluses to become economically profitable, they should have a significant positive effect on the occurrence of mastitis during lactation.

Table of contents

Introduction

Mastitis is an endemic disease worldwide. It has great economic impact on dairy farms (Halasa et al., 2007). It impairs milk quality (Bezman et al., 2015), cow welfare (Kemp et al., 2008), causes irritation to the farmer (Jansen, 2010) and it is a hazard for public health. Mastitis is mainly treated with antibiotics (Royster and Wagner, 2015), which is a risk for antibiotic residues and may lead to the emergence of multiple resistant bacteria in dairy cows (Knappstein et al. 2005; Wendtland et al., 2013). All these reasons make it important to control mastitis on dairy farms.

Mastitis can be divided into subclinical mastitis (SCM) and clinical mastitis (CM). SCM is manifested by an increased somatic cell count (SCC) in the milk of a cow. CM is manifested by changes in the udder (redness, swelling, pain, heat), visible changes of the milk (colour, fibrin, clots) and could include systemic involvement (fever, anorexia, shock) (Smith, 2015). SCC is used as a parameter for SCM, and the incidence rate of CM is often used to express the occurrence of CM on a farm (Huijps et al., 2010). The thresholds in the Netherlands for SCM are a SCC ≥ 150,000 cells/mL for primiparous cows and a SCC ≥ 250,000 cells/mL for multiparous cows (Scherpenzeel et al., 2014).

In the Netherlands an incidence rate of CM of 28.1 cases per 100 cows per year and a prevalence of SCM of 22.2% (Proportion of cows with a composite SCC ≥200,000 cells/mL), were estimated by Lam et al. (2013) in 2009. Van Soest et al. (2016) found an incidence of CM of 27% and an average SCC of 192,000 cells/mL in 2012. And according to Santman-Berends et al. (2015), the mean incidence rate of CM in 2013 in the Netherlands was 32.1 cases per 100 cows per year. Although the incidence rates differ over time, CM and SCM remain a major problem on Dutch dairy farms.

Mastitis remains difficult to control for farmers, because it is a multifactorial disease, which means that there are many risk factors. Mastitis vaccines were supposed to become the solution for the mastitis problem. Although vaccination reduces clinical signs of mastitis, it does not decrease the incidence rate of CM and prevalence of SCM (Bradley et al., 2015) and thus does not live up to its expectations. Management measures that have proven to be an effective preventive measure against mastitis include post milking teat disinfection, optimizing feed, supplementing dry cows with minerals, prevent overcrowding and drying off. The most likely effects for the different management measures, given as a percentage decrease in incidence of CM for a 100% environmental and a 100% contagious situation, are respectively 36,51% and 37,15%, 17% and 16,48%, 14,98% and 14,27%, 12,06% and 8,75%, 11,75% and 14,02% (Huijps et al., 2010). All these measures are aimed at treating the disease, preventing transmission of the infection and or improve the immune system of the cow.

Recently, supplementing garlic (Allium sativum L.) as a therapeutic agent or as a preventive measure against mastitis has become more popular among dairy farmers in the Netherlands. Garlic is one of the most commonly used plants as a therapeutic agent (Suleria et al., 2015). It has been proven to have antimicrobial activity, have fungicidal properties, acts as an antioxidant, reduces cholesterol, lowers blood pressure and has immune-modulatory activity (Borlinghaus et al., 2014; Suleria et al., 2015). Several garlic boluses for treating or preventing mastitis and endometritis are on the market in the Netherlands, but little research has been done to the use of garlic as a therapeutic agent against endometritis and mastitis in cattle (Mandhwani et al., 2017; Masona et al., 2017). Because an important motivation for farmers is the economic damage of mastitis to reduce the prevalence and incidence of SCM and CM on their farm (Huijps et al., 2008), it is also important to know if it is economically beneficial to supplement all the dry cows on a farm with these garlic boluses.

The aim of this study was to calculate whether the supplementation of oral garlic boluses used as a preventive measure against mastitis in the dry period and the first 14 days of lactation could be economically profitable under Dutch circumstances, considering variation in parameters and probabilities.

Economics mastitis

Mastitis causes great economic losses on dairy farms (Halasa et al., 2007). The costs of mastitis can vary greatly between farms, because there is a large variation in the mastitis management per farm. Farms vary in structural factors such as age of the barn or breed of the cows, but variation in farms is mainly related to the management of the farmer and the precision with which the management is executed (Hogeveen et al., 2011). In this chapter, an overview will be given of the economic aspects of mastitis and its prevention, of the latest estimated costs of mastitis in the Netherlands and of the calculated economic losses for five different Dutch dairy farms.

Economic aspects of occurrence and prevention of mastitis

Costs caused by mastitis can be divided in failure costs and preventive costs. Failure costs are costs which are caused by the disease and are present on every farm, because of the direct relationship with the disease. Preventive costs are made taking preventive measures against mastitis and are different on every farm, depending on the farmers management (van Soest et al., 2016).

Failure costs

Failure costs consists of the following factors:

- Milk production losses
- Veterinary visits
- Drugs
- Discarded milk
- Labour
- Culling
- Feed

Milk production losses

During an intramammary infection, bacteria cause damage to mammary epithelium and thus to the mammary gland. Therefore, milk production will decrease, and the somatic cell count will increase (Zhao and Lacasse, 2008). Milk production loss occurs with both CM and SCM and is a direct cost of mastitis. It varies under influence of the current milk market price in a non-quota system. Decreased milk production due to mastitis means that less milk will be delivered to the factory, so less will be paid by the factory.

Veterinary visits

In some cases of mastitis, the veterinarian must pay a visit.

Drugs

Drugs are necessary to treat cows with mastitis and are a direct cost of mastitis. The costs of drugs vary due to different treatments for different mastitis pathogens and different veterinary practices. Mastitis is mainly treated intramammary or systemic with antibiotics and Non-Steroidal Anti-Inflammatory Drugs (NSAID's) (Royster and Wagner, 2015; Leslie and Petersson-Wolfe, 2012)

Discarded milk

Like milk production loss, discarded milk is a direct cost of mastitis. The milk produced in the treatment period of mastitis and in the withdrawal period, will be discarded and not delivered to the factory. This results in less payment by the factory.

Labour

Labour as failure costs is the time spent by the farmer on treating a cow with mastitis. Labour costs is calculated by multiplying the labour time with the hourly wage of the farmer.

Culling

Culling is a difficult cost to estimate, because it is difficult to determine the optimal moment of replacement of the cow. Culling costs are influenced by factors such as the parity, milk production, lactation stage, pregnancy status and the pathogen causing the mastitis (which influences the probability of culling). Culling consists of direct costs, namely the rearing costs or the price of a heifer/cow as replacement, and indirect costs, namely possible reduced efficiency of milk production of the replacement heifer/cow. Heifers generally have a lower milk production than multiparous cows and produce less efficiently (Halasa et al., 2007). Indirect costs vary due to variation in milk production, parity and lactation stage of the replacement cow. However, a benefit of culling is the slaughter value, which returns when the cow is slaughtered.

Feed

Feed costs are a benefit in the costs of mastitis. When a cow has CM, it will eat less and thus less feed costs will be made compared to a healthy cow.

Preventive costs

Preventive costs generally consist of extra labour and investment in materials, such as wearing milking gloves, use teat disinfectant and fixate cows after milking. (Hogeveen et al., 2011). Not only cause these preventive measures extra costs, but they also cause reduced losses by reducing the incidence rate of CM and prevalence of SCM. The decision of implementing preventive measures against mastitis on a farm should be influenced by the nett benefit of the measure. Unfortunately, farmers are often advised based on the assumed effect of the measure or the costs of the measure. Management measures with the highest reduction of CM and SCM do not necessarily have the highest cost efficiency (Huijps et al., 2010).

Table 1 shows estimated costs and benefits of preventive management measures against mastitis. It was assumed that the preventive measure was not carried out yet and would be carried out precisely in the new situation. Blanket dry-cow therapy, keep cows standing after milking, use milkers' gloves, use of a treatment protocol and washing dirty udders had a positive nett benefit for the assumed farm (Hogeveen et al., 2011).

TABLE 1: NETT BENEFIT (€/COW/YEAR) OF IMPLEMENTING 18 MASTITIS PREVENTION MEASURES FOR A DAIRY FARM OF 65 COWS IN THE NETHERLANDS WITH AN AVERAGE 305-DAY MILK PRODUCTION OF 8,500 KG/YEAR, AN AVERAGE BULK TANK SOMATIC CELL COUNT OF 200,000 CELLS/ML, AN INCIDENCE OF CLINICAL MASTITIS (CM) OF 30% PER YEAR (65% ENVIRONMENTAL AND 35% CONTAGIOUS), AND A MILKING PARLOUR WITH 12 STANDS. OBTAINED FROM HOGEVEEN ET AL., 2011, 26-3-2018

	€/COW/YEAR		
MANAGEMENT MEASURE	Additional expenditure	Reduced losses	Nett benefit
MILK COWS WITH CM LAST	37	16	-21
MILK COWS WITH SUBCLINICAL MASTITIS LAST	104	20	-84
USE SEPARATE CLOTH	26	9	-17
WASH DIRTY UDDERS	3	9	6
PRE-STRIPPING	34	9	-25
USE MILKERS' GLOVES	1	9	8
POSTMILKING TEAT DISINFECTION	31	31	0
RINSE CLUSTERS WITH CM COW	1	11	10
RINSE CLUSTERS WITH SUBCLINICAL MASTITIS COW	123	15	-108
REPLACE TEAT CUP LINERS IN TIME	13	11	-2
USE OF A TREATMENT PROTOCOL	7	15	8
BLANKET DRY-COW THERAPY	9	36	27
KEEP COWS STANDING AFTER MILKING	2	12	10
FEED DRY COW MINERALS	13	13	0
PREVENT OVERCROWDING	23	13	-10
CLEAN CUBICLES	54	15	-39
CLEAN YARDS	51	8	-43
OPTIMIZE FEED RATION	24	13	-11

Latest estimated costs of mastitis in the Netherlands

Several studies have recently estimated the costs of mastitis per case or per average cow on the farm. In Table 2 several published cost estimates from Dutch studies, are summarised. The different studies used an economic model to simulate a dairy farm and to calculate milk production losses for CM and SCM, veterinary visits, drugs, discarded milk, labour and culling. Below, the different studies will be discussed.

Huijps et al. 2008 developed a tool, based on a model that calculates the economic consequences of mastitis, to calculate the farm-specific economic losses due to mastitis. Farm specific input data, such as number of clinical cases, distribution of pathogens, the distribution of the occurrence of mastitis in lactation, the effect of pathogens on milk production loss and different distributions of SCC depending on the BTSCC, can be entered in the tool. However, when input information is not known, default settings can be used. The default situation was a farm with 65 cows, a milk production per cow of 8500 kg/305 days, a BTSCC of 200,000 cells/ml and an incidence of CM of 30% per year (65% environmental and 35% contagious). The total calculated costs of mastitis in the default situation was €140 per cow per year.

Huijps et al. 2009 calculated the costs of mastitis in heifers on both farm and heifer level. They developed a Monte Carlo simulation model, which is a stochastic model. In this model an elevated SCC in early lactation (>200.000 cells/ml) either cures, remains elevated or is associated with a case of CM. Costs of heifer mastitis were calculated as costs per average farm with 20 heifers and per average heifer present on the farm. Every heifer was assigned to one of the five production levels and one of the four SCC groups with a probability distribution. The probability of a heifer developing SCM were related to the different SCC levels and the probability of CM as a flare up of SCM was related to the SCC. Production losses were related to the SCC groups and SCC level. The total calculated costs of heifer mastitis were €31 per heifer per year.

Van Soest et al. 2011 estimated farm-specific costs of mastitis for Dutch dairy farms, with questionnaire data, milk production recording data and data of CM from 120 different farms. Calculations were carried out according to Huijps et al. 2008. The applied preventive management measures per farm were taken into account in the model, in contrast of the study by Huijps et al. 2008. The average total calculated costs of mastitis were €164 per average cow on the farm per year.

Van Soest et al. 2016 estimated the total costs of mastitis as a construct of failure costs and preventive costs. They used questionnaire data, milk test-day records and data on CM, of 108 Dutch dairy herds. Milk production losses due to SCM were based on the test-day records of the individual farm. The difference between total realized and potential milk production per farm was assumed to be the milk production loss due to SCM. Failure costs of CM were based on the cost calculations of Huijps et al. 2008. Preventive costs were based on preventive measures taken against mastitis on the farm and the precision by which the preventive measures were taken by the farmer. They estimated the total costs of mastitis to be €240 per lactating cow per year.

TABLE 2: OVERVIEW OF RECENTLY PUBLISHED ESTIMATES OF ECONOMIC LOSSES DUE TO MASTITIS IN THE NETHERLANDS.

	VAN SOEST ET AL. 2016	VAN SOEST ET AL. 2011	HUIJPS ET AL. 2009	HUIJPS ET AL. 2008
CATEGORY	€ average per lactating cow per year	€ average per cow per year	€ per heifer per year	€ per cow per year
MILK PRODUCTION LOSSES				
- CLINICAL	32	17	2,94	23
- SUBCLINICAL	37	14	6,47	77
VETERINARY VISITS	0,30	0	0,16	0,30
DRUGS	6	7	4,43	6
DISCARDED MILK	20	9	2,09	9
LABOUR	4	5	0,74	3,30
CULLING	20	24	14,37	22
TOTAL FAILURE COSTS	120	76	31	140
TOTAL PREVENTIVE COSTS	120	88	-	-
TOTAL	240	164	31	140
- CLINICAL	83	62	13,49	63
- SUBCLINICAL	37	14	17,85	77

Table 2 shows great variation in total costs and cost factors for both CM and SCM. The distinctive lower costs of mastitis estimated by Huijps et al. 2009 compared to the other studies, can be explained by the lower incidence rate and prevalence of CM and SCM in heifers.

Notable in table 2 is the great variation in costs due to milk production losses, between van Soest et al. 2011, 2016 and Huijps et al. 2008. This could be explained by the different methods of calculating milk production losses and the milk price that was used. Huijps et al. 2008 used a distribution of SCC depending on BTSCC and they said every doubling of the SCC above 50.000 cells/ml results in milk production losses of 0.4 kg milk/d for primiparous cows and 0.6 kg milk/d for multiparous cows. Van Soest et al. 2011 used SCC measurements of individual cows for each farm and allocated them to different SCC levels. Milk production losses were calculated using milk production losses depending on different SCC levels, according to Halasa et al. 2009b. Van Soest et al. 2016 based the milk production losses on the potential and realised milk production of each farm.

Huijps et al. 2008 used a milk price of €0,12 per kg for both production losses and discarded milk. Van Soest et al. 2011 used a milk price of €0,12 per kg milk production loss and €0,17 per kg discarded milk. Van Soest et al. 2016 used a milk price of €0,41 per kg for both milk production loss and discarded milk. The studies from Huijps et al. 2008 and van Soest et al. 2011 were performed under Dutch quota circumstances.

Calculated economic losses for five different Dutch dairy farms

Data from five specific farms with different structural and management factors, were inserted in the updated economic tool from Huijps et al. 2008. Table 3 shows the divergent input data of the five different farms. Input data related to CM and SCM, such as occurrence of veterinary visits, treatment time and costs of drugs, corresponded for the different farms and were generally corresponding with the default values, thus not mentioned in Table 3. In this tool the costs and benefits of farm specific management measures were not taken into account.

TABLE 3: OVERVIEW OF THE DIVERGENT INPUT DATA OF FIVE DIFFERENT FARMS INSERTED IN THE ECONOMIC TOOL.

	FARM 1	FARM 2	FARM 3	FARM 4	FARM 5
NUMBER OF COWS	550	170	37	110	110
BTSCC (CELLS/ML)	205.000	109.000	375.000	170.000	150.000
NUMBER OF CLINICAL MASTITIS CASES PER YEAR	85	45	10	41	12
NUMBER OF CLINICAL MASTITIS CASES FIRST THREE MONTHS OF LACTATION	39	22	2	21	6
NUMBER OF CLINICAL MASTITIS CASES REST OF LACTATION	46	23	8	20	6
NUMBER OF COWS WITH SOMATIC CELL COUNT <=50	257	95	0	41	50
NUMBER OF COWS WITH SOMATIC CELL COUNT 50-100	126	47	4	34	19
NUMBER OF COWS WITH SOMATIC CELL COUNT 100-150	54	9	2	11	3
NUMBER OF COWS WITH SOMATIC CELL COUNT 150-200	17	3	4	8	4
NUMBER OF COWS WITH SOMATIC CELL COUNT 200-250	19	2	3	5	2
NUMBER OF COWS WITH SOMATIC CELL COUNT 250-300	14	1	5	1	1
NUMBER OF COWS WITH SOMATIC CELL COUNT 300-350	10	1	4	2	0
NUMBER OF COWS WITH SOMATIC CELL COUNT 350 - 400	6	0	2	1	3
NUMBER OF COWS WITH SOMATIC CELL COUNT >400	47	12	6	7	3
NUMBER OF CULLED CASES	8	2	4	6	6
MILK PRICE (€/KG MILK)	0,38	0,38	0,38	0,45	0,38
COSTS VETERINARY VISISTS (€/VISIT)	70	70	70	40	50
FEED COSTS (€/KG MILK)	0,11	0,11	0,11	0,15	0,12
CULLING COSTS (€/CASE)	480	480	480	200	1300

Farm 1 is a dairy farm of 550 cows in the Netherlands with an average 305-day milk production of 9.583 kg/year, an average bulk tank somatic cell count of 205.000 cells/ml, an incidence of CM of 15,45% per year (60% environmental and 40% contagious) and a milking parlour with 20 stands. The calculated costs of mastitis of this dairy farm are €96,07 per cow present on the farm and €350,02 per clinical case.

Farm 2 is a dairy farm of 170 cows in the Netherlands with an average 305-day milk production of 7.500 kg/year, an average bulk tank somatic cell count of 109.000 cells/ml, an incidence of clinical mastitis of 26,47% per year (70% environmental and 30% contagious) and a milking parlour with 24 stands. The calculated costs of mastitis of this dairy farm are €94,07 per cow present on the farm and €268,15 per clinical case.

Farm 3 is a dairy farm of 37 cows in the Netherlands with an average 305-day milk production of 10.515 kg/year, an average bulk tank somatic cell count of 375.000 cells/ml, an incidence of clinical mastitis of 27,03% per year (30% environmental and 70% contagious) and a milking parlour with 8 stands. The calculated costs of mastitis of this dairy farm are €242,47 per cow present on the farm and €526,52 per clinical case.

Farm 4 is an organic dairy farm of 110 cows in the Netherlands with an average 305-day milk production of 6.500 kg/year, an average bulk tank somatic cell count of 170.000 cells/ml, an incidence of clinical mastitis of 37% per year (70% environmental and 30% contagious) and a milking parlour with 18 stands. The calculated costs of mastitis of this dairy farm are €148,26 per cow present on the farm and €305,23 per clinical case.

Farm 5 is a dairy farm of 110 cows in the Netherlands with an average 305-day milk production of 8.996 kg/year, an average bulk tank somatic cell count of 150.000 cells/ml and an incidence of clinical mastitis of 10,90% per year. The calculated costs of mastitis of this dairy farm are €129,48 per cow present on the farm and €993,40 per clinical case.

TABLE 4: OVERVIEW OF THE RESULTS OF THE COSTS OF MASTITIS FOR THE FIVE DIFFERENT FARMS.

	FARM 1	FARM 2	FARM 3	FARM 4	FARM 5
MILK PRODUCTION LOSSES CLINICAL MASTITIS (€/YEAR)	14.255	5.906	1.840	5.996	2.051
MILK PRODUCTION LOSSES SUBCLINICAL MASTITIS (€/YEAR)	23.089	3.924	3.706	3.794	2.188
DISCARDED MILK (€/YEAR)	8.600	3.563	1.110	3.670	1.534
VETERINARY VISISTS (€/YEAR)	40	40	40	40	30
MEDICINES (€/YEAR)	1.870	990	220	1.025	309
LABOUR (€/YEAR)	1.147	607	135	553	331
CULLING (€/YEAR)	3.840	960	1.920	1.230	7.800
TOTAL (€/YEAR)	52.841	15.991	8.971	16.308	14.242

It is clear that the costs per clinical case of mastitis vary greatly between farms. This variation is mainly caused by differences in culling costs. The costs of mastitis per cow present on the farm are between €90 and €150 with an outlier of €242,47. The variation is mainly caused by differences in number of cows, bulk tank somatic cell count, incidence rate of CM and culling costs.

Garlic

Garlic (*Allium sativum*) is a plant species of the genus *Allium* and closely related to onion, chive and shallot. Garlic is believed to be originated 6000 years ago in Central Asia, has been used for over 3000 years in Chinese medicine and is prescribed for a number of applications in ancient medical texts from Egypt, China, India, Greece and Rome (Suleria et al., 2015; Yun et al., 2014). The composition of garlic and the presumptive pharmaceutical effects of garlic that might be important in the treatment of bovine mastitis, will be discussed in this chapter.

Components of garlic

A garlic bulb consists of the organosulfur compounds alliin, γ-glutamyl-S-allylcysteine, S-methylcysteine sulfoxide, S-trans-1-propenylcysteine sulfoxide, S-2-carboxypropylglutathione and S-allylcysteine (SAC) (Yun et al., 2014). The chemical composition of garlic changes with temperature and age and the content of organosulfur compounds change during cultivation and storage. For example, the organosulfur compound thiacremonone (2,4-dihydroxy-2,5-dimethyl-thiophene-3-one) was found in heated garlic (Yun et al., 2014). Alliin is the major substrate in garlic. After cutting, crushing or grinding garlic, the vacuolar enzyme alliinase is released, which catalyses alliin to allicin (Figure 1). The organosulfur compounds and allicin, are mainly responsible for the medicinal properties and pharmacological effects of garlic (Suleria et al., 2015; Yun et al., 2014). The odour and flavour of garlic is also attributed to the organosulfur compounds (Suleria et al., 2015; Tsai et al., 2012).

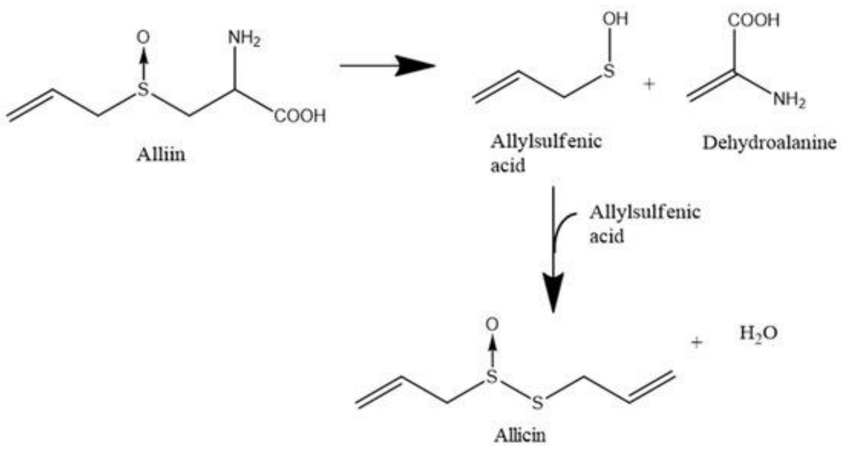


FIGURE 1: BIOSYNTHESIS OF ALLICIN. THE NONPROTEINOGENIC AMINO ACID ALLIIN IS CONVERTED BY THE ACTIVITY OF THE ALLIINASE ENZYME TO ALLYL SULFENIC ACID AND DEHYDROALANINE. TWO MOLECULES OF ALLYL SULFENIC ACID CONDENSE SPONTANEOUSLY TO ONE MOLECULE OF ALLICIN. OBTAINED FROM GRUHLKE ET AL. 2017, 15-03-2018

Effects of garlic on cholesterol and blood pressure

Studies indicate that garlic has inhibitory effects on cholesterol synthesis and lowers blood cholesterol levels. In vitro studies show that garlic inhibits key enzymes that are involved in cholesterol and fatty acid synthesis in cultured rat hepatocytes and human HepG2 cells (Yun et al., 2014). It is believed that garlic reduces low-density lipoprotein (LDL) cholesterol largely, due to inhibition of hepatic hydroxymethylglutaryl-CoA reductase activity by alliin and allicin (Tsai et al., 2012). Another health-promoting property of garlic is its ability to lower both systolic and diastolic blood pressure (Suleria et al., 2015). According to Borlinghaus et al. 2014 the decomposing of allicin to its degradation products, by a complex reaction cascade with thiols, results in the release of hydrogen sulphide (H_2S). H_2S causes relaxation of smooth-muscle cells from blood vessels, so they can expand, which results in a lower blood pressure.

Antioxidant effects of garlic

Garlic and mainly the water-soluble organo-sulphur compounds of garlic, such as SAC and S-allylmercaptocysteine (SAMC), have great antioxidant potential. Especially aged garlic has a high content of these compounds and thus has a higher antioxidant potential (Suleria et al., 2015). SAC has been proven to scavenge reactive oxygen species (ROS), withhold oxidative LDL from damaging endothelial cells and protect PC12 neuron cells from injury by hydrogen peroxide (Tsai et al., 2012). Garlic increases the activity of several anti-oxidic enzymes, such as GSH reductase, superoxide dismutase, g-glutamate cysteine ligase, and GST (Tsai et al., 2012).

Immune-Modulatory effects of garlic

It is suggested that garlic has anti-allergic properties. It was shown to reduce histamine release, suppression of IgE-mediated antigen-specific reaction in murine model and that garlic could beneficially balance, or modify the function of mast cells, basophile, and activated T lymphocyte factors. All the above play a key role in inflammation and allergic reactions (Yun et al., 2014). It is observed that allicin inhibits the migration of neutrophilic granulocytes into epithelia and thus has an anti-inflammatory effect. Furthermore, it has been shown that allicin inhibits the SDF1α-chemokine-induced chemotaxis of T-cell lymphocytes, which is correlated with an impaired dynamic of the actin-cytoskeleton. Allicin also inhibits the trans endothelial migration of neutrophils (Borlinghaus et al., 2014). Other immune-modulatory effects of garlic are the inhibitory effect of allicin on the release of TNFα-dependent pro-inflammatory cytokines in intestinal epithelia, a TNFα-stimulating effect of allicin in LPS-stimulated cells, inhibitory effect of allicin on phosphatase-activity correlated with an enhanced phosphorylation of ERK1/2 and the supressing effect of allicin on the release of reactive nitrogen species (RNS) by LPS-stimulated macrophages (Borlinghaus et al., 2014).

Antifungal effects of garlic

Allicin has antifungal properties, by inhibiting spore germination and hyphal growth. It acts synergistically with other antifungal substances, such as copper and amphotericin B. This suggest that allicin has an impact on the fungal plasma membrane. Allicin could also cause an oxidation of glutathione, which results in induction of apoptosis after a shift of the cellular redox-potential. This makes it possible to use allicin for eradication of fungi in agriculture and medicinal therapy (Borlinghaus et al., 2014).

Antimicrobial effects of garlic

Probably the best-known property of garlic is the antimicrobial activity. It has antimicrobial activity against both gram-positive and gram-negative bacteria. Bioactive compounds that have antibacterial activity are dialildisuphide, dialiltrisulphide and mainly allicin (Safithria et al., 2011). Allicin diffuses easily across artificial and natural phospholipid membranes and thus into cells. In the cell allicin reacts with every cysteine residue, if the -SH-group is freely available. It reacts with the sufhydryl-group of cysteine via a disulphide exchange-like reaction. And it was found that allicin inhibits several (thiol-containing) enzymes irreversibly (Borlinghaus et al., 2014).

Effect of supplemented garlic on cattle

No research has been done to the effect of garlic on the occurrence of mastitis in dairy cows. Therefore, research was used that has been done to post ruminal physiological effects of garlic in dairy cattle, intrauterine treatment with garlic in dairy cattle, *in-vitro* antibacterial activity of garlic against mastitis bacteria of cows and camels and to the effect of intramammary infusion of garlic extract in camels (Oh et al. 2013; Mason et al., 2017; Sarkar et al., 2006; Tuteja et al., 2013; Safithri et al, 2011). These studies will be discussed below.

Oh et al. 2013 investigated the post ruminal physiological effects of supplemented phytonutrients, including garlic, on nutrient utilization, gut microbial ecology, immune response and productivity of lactating dairy cows. Eight dairy cows were pulse dosed daily with 2 g/cow of garlic extract for 9 days. Effects on dry matter intake (DMI), milk yield, feed efficiency, milk composition, rumen fermentation variables, blood chemistry and immune response were measured.

Milk composition, rumen fermentation variables, and blood chemistry were not affected by treatment with garlic. DMI tended to be lower for the garlic treatment, which caused a greater feed efficiency, but also a slightly decreased milk yield. Alanine aminotransferase and cholesterol concentrations were slightly elevated in all cows, except for the cows treated with garlic. Garlic increased the proportion of total CD4+ cells and total CD4+ cells that co-expressed the activation status signal and CD25 in blood and increased 8-isoprostane levels. CD25 is considered an indicator of the activation status of bovine T lymphocytes (Oh et al., 2013). The effects observed in this study should be interpreted with caution because of the short treatment duration with garlic.

Mason et al. 2017 investigated the plasma and tissue pharmacokinetics of thymol, carvacrol and diallyl disulphide (garlic). 7 healthy cows were dosed orally or intravaginally with 3 ml garlic every 12 hours for six days. Plasma, fat, kidney and liver samples were collected from each animal and the withholding time of diallyl disulphide was estimated. No detectable levels of diallyl disulphide were found in any tissue or plasma samples of the cows. They report that there is a zero-day withhold time for garlic.

In the study of Sarkar et al. 2006, they examined the therapeutic effects of garlic extract and prostaglandin F_2 alpha on the recovery and hormonal changes in cows with endometritis. 10 cows with endometritis were intrauterine infused, with 10 ml garlic extract mixed with 90 ml normal saline. They were infused three times with a 12-hour interval, starting on the day of oestrus. Cervico-vaginal mucus (CVM) was collected from each animal before and after treatment oestrus and subjected to white side test, pH determination and total bacterial load. The clinical recovery of cows was assessed by negative white side test reaction, pH value and total bacterial count of CVM at subsequent oestrus. During subsequent standing oestrus the cows were inseminated twice.

In 70% of the cows treated with garlic the CVM turned clear, compared to 33.3% of the control group. There was a higher negative response to the white side test in the garlic treated cows. Treatment groups also showed a significant decline in mean pH and bacterial load (98% reduction). Among the bacterial species found in CVM of treated and control animals were, *E. coli* (24.6%), *Staphylococcus aureus* (21.5%), *Ps. aeruginosa*. (15.4%), *Bacillus sp*. and *Streptococcus sp*. (9.2% each), *Proteus sp.* (7.7%), *A. pyogenes* (6.2%) and *Klebsiella sp*. (6.2%). It also revealed that the overall conception rate increased by 50% after treatment with garlic. This study indicates the therapeutic effectiveness of garlic extract on endometritis in cows.

The antimicrobial activity of garlic against microorganisms causing intramammary infections in camels was studied by Tuteja et al. 2013. They measured the *in-vitro* effect of fresh juices of garlic bulb on seventy-four bacterial isolates, *Staphylococcus aureus* (24), *Staph epidermidis* (35), *Bacillus* spp. (5), *Corynebacterium* spp. (3) and *Micrococcus* spp. (7), obtained from intramammary infections in camels. They also measured the *in-vivo* effect of the infusion of garlic extract in seven infected quarters of 4 camels. 72 hours after infusion, quarter milk samples were taken and subjected to bacteriological examination.

In-vitro sensitivity evaluation showed that garlic caused inhibition zones of 21-30 mm and >30mm in 10 and 11 cultures of *Staph. epidermidis,* 6 and 16 cultures of *Staph. aureus*, 4 and 1 cultures of *Micrococcus* spp., 3 and 0 cultures of *Bacillus* spp., and in 1 and 0 cultures of *Corynebacterium* spp., respectively.

The clearance of infection after garlic infusion was 57,14%. Two out of three quarters infected with *Staph. aureus,* one out of two quarters infected with *Staph. epidermidis,* one out of one quarter infected *with Streptococcus* spp. and one out of one quarter infected with Bacillus spp. were cured 72 hours after treatment. In the rest of the treated quarters reduction in colony count was observed.

These results indicate the potential of garlic as an antimicrobial agent, which could be uses as treatment for mastitis. Results should be interpreted with caution, because of the lack of significance of the results.

Safithri et al. 2011 examined the *in-vitro* antibacterial activity of garlic extracts (5%, 10%, 20%, 30%, 40%) against the mastitis bacteria *Streptococcus agalactie, E. coli* and *Staphylococcus aureus* of dairy cattle.

TABLE 5: DIAMETER OF INHIBITION ZONE OF GARLIC WATER EXTRACT AND GARLIC ETHANOL EXTRACT AGAINST MASTITIS BACTERIA. OBTAINED FROM: SAFITHRI ET AL. 2011, 22-03-2018

GARLIC CONCENTRATION (% B/V)	INHIBITION ZONE (MM) GARLIC WATER EXTRACT			INHIBITION ZONE (MM) GARLIC ETHANOL EXTRACT		
	S. agalactie	*S. aureus*	*E. coli*	*S. agalactie*	*S. aureus*	*E. coli*
5	9.17±0.23^{fg}	7.30±0.26^{h}	7.73±0.71^{gh}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}
10	12.50±0.15^{de}	9.83±0.51^{f}	9.80±1.65^{f}	10 1.80±0.09^{c}	0.00±0.00^{d}	1.50±0.20^{cd}
15	14.17±0.06^{abcd}	12.13±0.15^{a}	12.97±0.65^{cde}	15 2.73±0.216^{c}	0.00±0.00^{d}	1.97±0.25^{c}
20	14.58±0.44^{abc}	14.00±0.46^{abcd}	14.70±0.46^{abc}	20 2.70±0.306^{c}	0.00±0.00^{d}	2.58±0.03^{bc}
25	15.63±0.06^{a}	14.53±0.06^{abc}	14.83±0.64^{ab}	25 3.67±1.85^{b}	0.00±0.00^{d}	3.03±0.06^{bc}
AMPICILLIN 0.01%	14.33±0.15^{abcd}	14.87±1.23^{ab}	13.72±0.85^{bcde}	14.33±0.15a	14.33±0.15a	13.72±0.85a

NOTE: MEANS IN THE SAME COLUMN AND ROW WITH DIFFERENT SUPERSCRIPT DIFFER SIGNIFICANTLY ($p<0,05$).

Table 5 shows the results of the inhibition zone measurements. The higher the concentration of garlic extract, the larger the inhibition zone, which indicates that the fewer bacteria could grow. The antibacterial activity of garlic water extract against *S. agalactia, S. aureus* and *E. coli* did not differ significantly with ampicillin from concentration of 10% b/v, 20% b/v and 15% b/v respectively. Garlic ethanol extract did not inhibit *S. aureus* at all concentrations. The results indicate that fresh garlic can be used to inhibit growth of mastitis bacteria.

Results from the studies above indicate the potential of garlic to use as a therapeutic agent against mastitis, due to its antibacterial activity, immune modulatory effects and the zero-day withhold time in cattle.

Materials and methods

Model development

Using a cow level stochastic Monte Carlo simulation model, the economic effects of the usage of oral garlic boluses was calculated. A stochastic model developed for a comparable drug was adjusted and used for this study. It was built and adjusted using Microsoft Excel with @Risk add-in software (Palisade, 2002).

A stochastic Monte Carlo method is a simulation technique in which the output of a model is simulated under repeated random samples, each under different conditions. The result of this collection of simulations is a distribution function that reflects the entire range of possible outcomes.

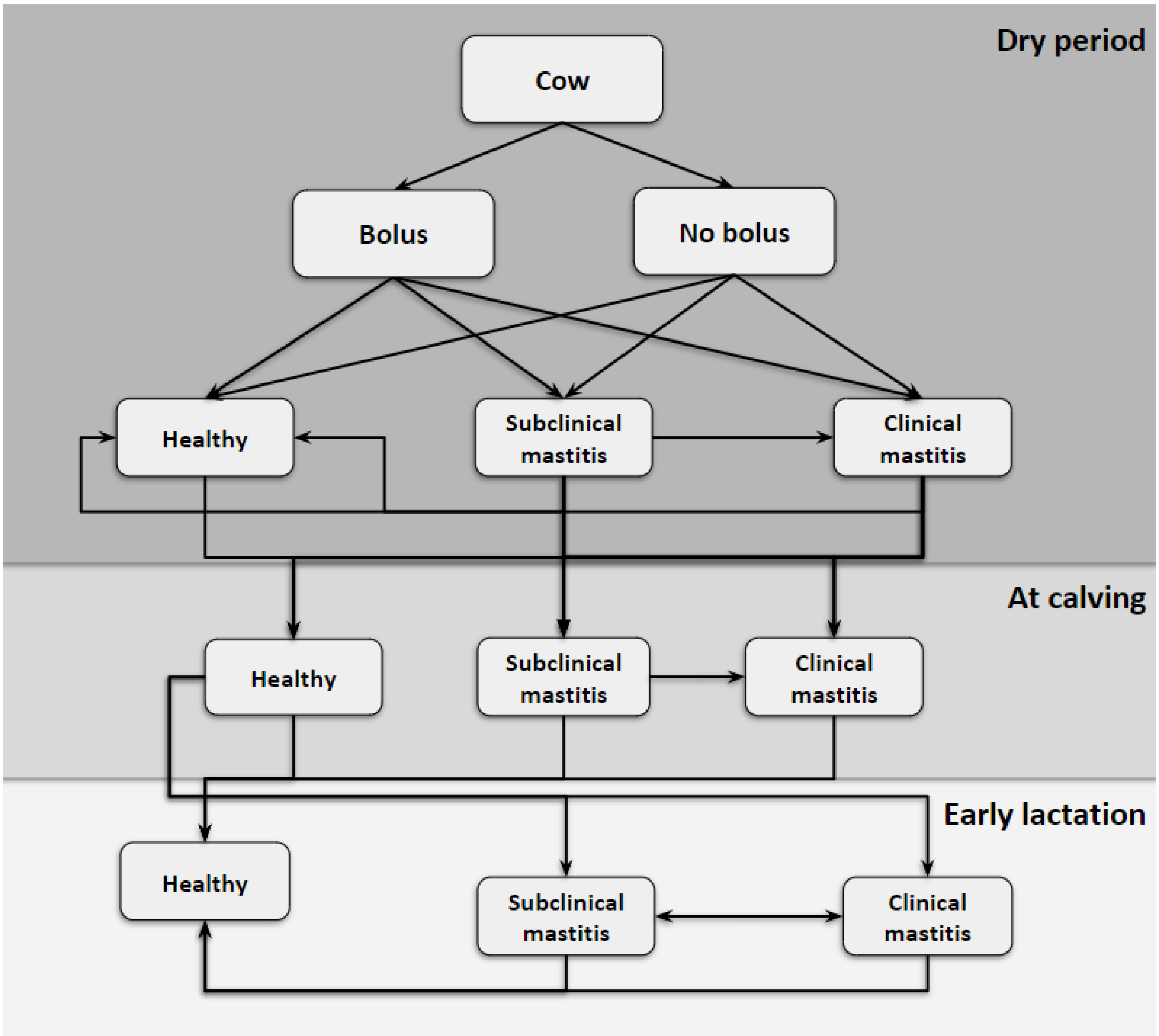


Figure 2: Graphical representation of the simulation of a cow provided with or without garlic boluses.

Model description

The model outcomes were generated in two parts. Part one consists of the dynamics of mastitis, where every iteration (10.000) during the simulation process created a specific cow with or without CM or SCM. The model simulates the dynamics of a healthy, subclinical infected or clinically infected cow in the dry period, at calving and the first 14 days (d) (early lactation) of lactation. In part two, the economic effects of having or not having CM or SCM were calculated for that specific cow. Two scenarios were simulated: A scenario with preventive treatment by supplementing a garlic bolus two times in the dry period to all multiparous cows and a scenario with no preventive treatment to all multiparous cows (Figure 2). All discrete events and variability regarding the modelled mastitis cases were triggered stochastically, using random numbers drawn from distributions. With this technique, variance of parameters can be specified, so model behaviour can be controlled by a set of decision variables that describe the cow characteristics.

Input data

All input data were based on data found in literature or assumptions made by experts. Literature was found through Scopus, PubMed, Google Scholar and Utrecht University Library. Input data for the dynamics of mastitis, such as probabilities of getting mastitis, milk production losses and culling are given in Table 6. Input data for the economic effect of mastitis, such as milk price, veterinary costs, labour costs and culling costs are given in Table 7.

Simulation of a cow (with mastitis)

A cow was simulated in the dry period and in the successive lactation.

The probability of getting CM or SCM, the probability of a clinical flare up, the probability of bacterial cure of CM and the probability of spontaneous cure of SCM in the dry period were described by a discrete probability distribution. The probabilities of occurrence with or without boluses of CM or SCM, clinical flare up, bacterial cure of CM and spontaneous cure of SCM during the dry period are shown in Table 6.

The milk production kg/305-d (MP) was assumed to have a normal distribution with an average and a standard deviation.

MP = Normal(9706;500)

During lactation the daily milk production was estimated using the Wood lactation curve (Wood, 1967). The length of the calving interval (CI) has a Pert distributed value with a minimum of 336 d, a most likely value of 414 d, and a maximum of 556 d (CRV, 2017). With a dry period of 60 d, the length of lactation (LL) was naturally 60 d shorter than the CI and is also the day of drying off (DDO).

CI = Pert(336; 414; 556)

LL or DDO = CI-60

TABLE 6: INPUT DATA OF THE STOCHASTIC SIMULATION MODEL FOR THE DYNAMICS OF MASTITIS, CLINICAL MASTITIS (CM) AND SUBCLINICAL MASTITIS (SCM).

	VALUE	REFERENCE
PROBABILITY OF OCCURRENCE* (NO BOLUSES)		
- **MASTITIS DRY PERIOD**	0,200	Adapted from Scherpenzeel et al. 2016
- **SCM DRY PERIOD**	0,198	Expert opinion
- **CM DRY PERIOD**	0,002	Expert opinion
- **CLINICAL FLARE UP DRY PERIOD**	0,260	Adapted from Halasa et al. 2010
- **CURE CM/CLNICAL FLARE UP DRY PERIOD**	0,874	Adapted from Halasa et al. 2010
- **SPONTANEOUS CURE SCM DRY PERIOD**	0,473	Adapted from Halasa et al. 2010
- **MASTITIS AT CALVING**	0,084	Adapted from Halasa et al. 2010
- **SCM AT CALVING**	0,048	Adapted from Halasa et al. 2009a
- **CM AT CALVING**	0,036	Adapted from Halasa et al. 2009a
- **MASTITIS FIRST 14 DAYS LACTATION**	0,628	Scherpenzeel et al. 2016
- **SCM FIRST 14 DAYS LACTATION**	0,483	Scherpenzeel et al. 2016
- **CM FIRST 14 DAYS LACTATION**	0,145	Scherpenzeel et al. 2016
- **CLINICAL FLARE UP LACTATION**	0,104	Adapted from Halasa et al. 2009a
- **BACTERIAL CURE CLINICAL CASES LACTATION**	0,530	Adapted from Steeneveld et al. 2011
- **CLINICAL CURE OF NON-BACTERIAL CURED CASES LACTATION**	0,900	Adapted from Steeneveld et al. 2011
PROBABILITY OF OCCURRENCE* (BOLUSES)		
- **MASTITIS DRY PERIOD**	0,100	Authors expertise
- **SCM DRY PERIOD**	0,099	Authors expertise
- **CM DRY PERIOD**	0,001	Authors expertise
- **CLINICAL FLARE UP DRY PERIOD**	0,200	Authors expertise
- **CURE CM/CLINICAL FLARE UP DRY PERIOD**	0,874	Adapted from Halasa et al. 2010
- **SPONTANEOUS CURE SCM DRY PERIOD**	0,473	Adapted from Halasa et al. 2010
- **MASTITIS AT CALVING**	0,045	Authors expertise
- **SCM AT CALVING**	0,026	Authors expertise
- **CM AT CALVING**	0,019	Authors expertise
- **MASTITIS FIRST 14 DAYS LACTATION**	0,300	Authors expertise
- **SCM FIRST 14 DAYS LACTATION**	0,231	Authors expertise

- **CM FIRST 14 DAYS LACTATION**	0,069	Authors expertise
- **CLINICAL FLARE UP LACTATION**	0,104	Adapted from Halasa et al. 2009a
- **BACTERIAL CURE CLINICAL CASES LACTATION**	0,530	Adapted from Steeneveld et al. 2011
- **CLINICAL CURE OF NON-BACTERIAL CURED CASES LACTATION**	0,900	Adapted from Steeneveld et al. 2011
MILK PRODUCTION LOSSES PER DAY (%)		
- **CM**	5	Van Soest et al. 2016
DURATION OF TREATMENT (D)	3	Van Soest et al. 2016
DURATION OF TOTAL WITHDRAWAL TIME OF MILK (D)	7	Van Soest et al. 2016
LABOUR TIME FOR TREATMENT (MIN/CASE)	45	Van Soest et al. 2016
VETERINARY VISITS (% OF CASES)	5	Van Soest et al. 2016
PROBABILITY OF CULLING COW		
- **CM**	0,15	Van Soest et al. 2016
- **SCM**	0,12	Swinkels et al. 2005, Steeneveld et al. 2007
LABOUR TIME FOR SUPPLEMENTING BOLUSES (MIN/CASE)	20	Expert opinion

*In the first 14 days of lactation.

The parity of a cow was described by a discrete probability distribution. The probability of a cow being in parity 2 was 0.29, in parity 3 was 0.29, in parity 4 was 0.29 and in parity 5 was 0.13.

The probabilities of getting CM or SCM, at calving or in early lactation were described by a discrete probability distribution. The probability of occurrence of CM and SCM at calving or in early lactation with or without boluses are shown in Table 6. A cow could only have CM or SCM at calving when the cow had CM or SCM in the dry period, which did not cure clinically and/or bacteriologically in the dry period.

The probabilities of clinical flare up, bacterial cure of CM or clinical flare up, clinical cure of non-bacterial cured cases during lactation were described by a discrete probability distribution. The probabilities of occurrence of clinical flare up, bacterial cure and clinical cure with or without boluses are shown in Table 6.

It was assumed that a cow can only have one case of mastitis at a time.

The day of diagnosis of CM and SCM at calving was set on the first day of lactation.

The days of diagnosis of CM (DD_{cm}) and SCM (DD_{scm}) in early lactation were assumed to be at any day during the first 14 days of lactation with equal probability and were described by a uniform probability distribution, with a minimum day and a maximum day.

DD_{CM} = Uniform(1;14)

DD_{SCM} = Uniform(1;14)

The day of diagnosis of a clinical flare up (DD_{CF}) was assumed to be at any day during lactation with equal probability and was described by a uniform probability distribution, with a minimum and maximum day.

DD_{CF} = Uniform(1;LL)

TABLE 7: INPUT DATA OF THE STOCHASTIC SIMULATION MODEL FOR THE GENERAL AND ECONOMIC INPUT OF MASTITIS

	VALUE	REFERENCE
MILK PRODUCTION KG/305-D	9.706	Adapted from CRV 2017
FAT %	4,35	CRV 2017
PROTEIN %	3,55	CRV 2017
CALVING INTERVAL	414	CRV 2017
LIFE SPAN COW (LACTATIONS)	5	Expert opinion
MILK PRICE (€/KG OF MILK)	0,35	Dutch market price March 2018 (https://www.boerderij.nl/Markt/)
PRICE OF FEED (€/KVEM)	0,16188	Remmelink et al. 2012 and Vermeij 2012
COST OF ANTIBIOTICS FOR TREATMENT (€/TREATMENT)	22	Van Soest et al. 2016
COST OF VETERINARY VISITS (€/VISIT)	40	Expert opinion
COST OF LABOUR (€/H)	20	Van Soest et al. 2016
REARING COSTS (€)	1567	Mohd Nor et al. 2012
COSTS BOLUSES (€)	40	Own estimation

When a cow got SCM at calving or during early lactation the length of SCM (L_{scm}) was modelled assuming a pert distribution.

L_{SCM} = Pert(1;126;DDO – DD_{SCM})

The mean length of SCM was calculated by multiplying the prevalence distribution of the major pathogens causing SCM (Schepers et al. 1997) with the mean length of SCM per pathogen according to Lam et al. 1997 (Table 8). Pathogen distribution of SCM and CM was calculated by means of results from Sampimon et al. 2009 and is presented in Table 8.

The length of SCM after a non-bacteriological cure of CM or a clinical flare up was modelled assuming a uniform distribution from de day of diagnosis of CM or clinical flare up, until the end of lactation.

The end of SCM was estimated as the length of SCM, when it was smaller than the LL. Otherwise the end of SCM was estimated as the LL.

Milk production losses due to SCM were modelled as a function of SCC. The SCC of a cow was described by a pert distribution with a minimum of 50.000 cells/ml, a most likely of 258.558 cells/ml (Table 8) and a maximum of 3.287.500 cells/ml. Quarter milk can reach 13 000 000 cells/ml without clinical symptoms (Pyörälä and Mattila, 1987). Therefore, the maximum cow SCC was 3.287.500 cells/ml ((13.000.000+3*50.000)/4).

SCC = Pert(50.000;258.559;3.287.500)

Based on the results of Hortet and Seegers (1998), a cow with an SCC of 50.000 cells/ml was assumed to be healthy and to have no production losses. The milk production loss in kg/d (milk loss) for multiparous cows, due to an elevated cow SCC above 50.000 cells/ml, was calculated with the following formula obtained from Hortet et al. 1999:

Milk loss = $(0,00001*LL^2+0,0015*LL+0,3341) * (\text{Log}(SCC) – \text{Log}(50.000))$

TABLE 8: CALCULATED PREVALENCE DISTRIBUTION, LN SOMATIC CELL COUNT (SCC), CALCULATED MEAN QUARTER SOMATIC CELL COUNT, CALCULATED MEAN COW SOMATIC CELL COUNT, CALCULATED TOTAL COW SOMATIC CELL COUNT AND MEAN DURATION OF SUBCLINICAL MASTITIS FOR THE MAJOR PATHOGENS CAUSING SUBCLINICAL MASTITIS.

MAJOR MASTITIS PATHOGENS	PREVALENCE *	LN SCC**	MEAN QUARTER SCC[1] (CELLS/ML)	MEAN COW SCC[2] (CELLS/ML)	COW SCC[3] (CELLS/ML)	MEAN DURATION SCM*** (DAYS)
COAGULASE-NEGATIVE STAPHYLOCOCCI	0,46	4,22	16596	41649	5831	128
STREPTOCOCCUS UBERIS	0,09	6,72	5248075	1349519	121457	99
STAPHYLOCOCCUS AUREUS	0,18	5,98	954993	276248	49725	115
CORYNEBACTERIUM BOVIS	0,14	3,96	9120	39780	5569	187
STREPTOCOCCUS DYSGALACTIAE	0,13	6,34	2187762	584440	75977	86
TOTAL	1				258559	126

1: Mean Quarter SCC = $10^{\text{Ln SCC}}$

2: Mean Cow SCC = (Mean Quarter SCC + (3*50.000))/4

3: Total cow SCC = Mean Cow SCC * Prevalence pathogens

* Sampimon et al. 2009

**Schepers et al. 1997

***Lam et al. 1997

Decrease in daily milk production due to CM during lactation was set on 5% (Table 6). The withdrawal period was estimated to be 7 days and it was assumed that milk production loss remained until the end of lactation. The daily milk production from the start of CM or clinical flare up and the remaining milk production after CM was calculated by multiplying the daily milk production with 0,95.

Veterinary visit for clinical cases, in the dry period and during lactation, was calculated by multiplying a discrete distribution of veterinary visit with veterinary costs.

Veterinary visit = Discrete(1:0;0,05:0,95)

The probability of culling a cow with CM (CUL_{cm}) during lactation or SCM (CUL_{scm}) during lactation (Table 6) was described with a discrete distribution or a cow was culled immediately when a cow did not cure clinically and bacteriologically after treatment of CM during lactation. When a cow cured bacteriologically after treatment of

CM during lactation, the day of culling ($DCUL_{cm}$) was described with a uniform probability distribution, with as minimum the day of diagnosis of CM or clinical flare up and as maximum the day of drying off. When a cow has SCM during lactation ($DCUL_{scm}$) the day of culling was described with a uniform probability distribution, with as minimum the day of diagnosis of SCM and as maximum the last day of SCM. When a cow did not cure clinically and bacteriologically after CM during lactation ($DCUL_{im}$) the day of culling was set on the day of diagnosis of CM plus the withdrawal period.

CUL_{cm} = Discrete(1:0;0,15;0,85)

CUL_{scm} = Discrete(1:0;0,12;0,88)

$DCUL_{cm}$= Uniform(DD_{CM} or DD_{CF} ; DDO)

$DCUL_{scm}$= Uniform(DD_{SCM} ; L_{SCM} + DD_{SCM})

$DCUL_{im}$= DD_{CM} or DD_{CF} + 7

Calculation of costs and revenues

Costs due to mastitis in the dry period consisted of medicines, labour and veterinary visits and during lactation it consisted of medicines, labour, veterinary visits, production losses, discarded milk and culling (Table 7).

Costs of medicines in the dry period were the same as during lactation (Table 7). Costs of labour in the dry period was calculated by multiplying the treatment time in the dry period, with the hourly rate of the farmer. Cost of labour during lactation was determined by multiplying treatment time during lactation with the hourly rate of the farmer. Costs of veterinary visits were the same for CM in the dry period and CM during lactation (Table 7). Costs of production losses was determined by multiplying production losses due to CM or SCM during lactation with the milk price and costs of total discarded milk was determined by multiplying milk withdrawals with the milk price. When a cow with CM does not cure bacteriologically, there is a chance that the cow will have a repeated case of CM during lactation, thus costs related to a non-bacteriologically cured CM case during lactation were multiplied with 1,5. These costs included costs for medicines, labour, veterinary visits and discarded milk. The cost of culling was calculated by subtracting the parity of a cow from the average life span of a cow and multiply this with the rearing costs minus the slaughter value that is divided by the average life span of a cow (Table 7). When a cow had parity 5 the culling costs were zero.

$COSTS_{CUL}$ = (5-parity)*((1567-500)/5)

Costs of SCM consisted of production losses due to SCM, which was determined by multiplying production losses due to SCM with the milk price, and culling costs.

The (reduction in) feed costs was calculated. A reduction might occur because cows with mastitis will produce less and therefore would eat less. The feed costs were determined by calculating the Fat Protein Corrected Milk (FPCM) and the VEM, a Dutch parameter indicating the (nett) energy content of a feed product for milking cows (CVB, 2012).

FPCM = 0,337+0,116*%fat+0,06*%protein*kg milk

VEM = 5.323+440*FPCM+0,73*$(FPCM)^2$

Where after the VEM was corrected for parity and pregnancy (CVB, 2012). The corrected VEM was multiplied with the price of kVEM. The calculated feed costs were subtracted from feed costs calculated for the same cow but without any mastitis, which resulted in reduced feed costs.

Costs of supplementing garlic boluses consisted of the costs of the boluses and costs of labour to supplement the boluses.

Lastly, the total costs were calculated. The total costs consisted of the costs of mastitis in the dry period and during lactation, reduced feed costs and costs of supplementing garlic boluses.

Sensitivity analysis

A sensitivity analysis was conducted to verify the values of the input parameters. The results of sensitivity analysis on each parameter were compared to the results of the model outcome in the default situation to assess the impact of each parameter on the costs of mastitis per cow in the two different scenarios. Values for input variables in the sensitivity analysis are based on information in the literature and assumptions made by experts. When sensitivity analysis was carried out on one parameter, the other parameters were retained at default values. The sensitivity analysis was performed for probability of occurrence of SCM, CM and clinical flare up in the dry period, at calving and in early lactation, the probability of bacteriological and/or clinical cure of CM, and costs of the boluses, in cows with garlic boluses. And it was performed for milk price and rearing costs for cows both with or without boluses.

Results

Model outcomes of case-specific parameters under default circumstances are shown in table 9. Supplementation with boluses resulted in about half fewer cases of CM, SCM and clinical flare up cases during the dry period, at calving and during lactation.

TABLE 9: MODEL OUTCOMES OF MASTITIS SPECIFIC PARAMETERS UNDER DEFAULT CIRCUMSTANCES.

	BOLUSES	NO BOLUSES
CLINICAL MASTITIS CASES DRY PERIOD (%)	0,1	0,2
SUBCLINICAL MASTITIS CASES DRY PERIOD (%)	9,8	19,8
CLINICAL FLARE UP DRY PERIOD (%)	1,9	5,3
CLINICAL MASTITIS CASES AT CALVING (%)	2	3,6
SUBCLINICAL MASTITIS CASES AT CALVING (%)	2,3	4,5
CLINICAL MASTITIS CASES EARLY LACTATION (%)	6,3	13,4
SUBCLINICAL MASTITIS CASES EARLY LACTATION (%)	22,4	44,4
CLINICAL FLARE UP LACTATION (%)	2,6	5,2

Costs for supplemented and non-supplemented dried off cows are presented in Table 10. Costs for non-supplemented cows were on average €103 and consisted of costs for medicines, veterinarian and labour during the dry period and costs for medicines, veterinarian, labour, milk production losses, discarded milk, culling and reduced feed during lactation. Costs during lactation had the greatest influence on the total costs for cows supplemented with or without boluses. Without boluses, costs for milk production losses (€45) and culling (€35) during lactation had the highest contribution to the total average costs of dried off cows. Cows with boluses gave lower costs for medicines, labour, veterinary visits, milk production losses, discarded milk and culling in comparison with cows without boluses. These decreases did outweigh the costs for the boluses (€47) and reduced feed (€5) for cows with boluses. Therefore, supplementation of boluses gave on average lower costs than without boluses and thus makes the supplementation of boluses to cows in the dry period profitable. In Table 10, the variability in outcomes is shown using 5th and 95th percentiles. The range in outcomes was larger for no boluses than for boluses (€0 – 432 and €47 – 369, respectively).

TABLE 10: ECONOMIC CONSEQUENCES (€/COW DRIED OFF) OF MASTITIS PER AVERAGE COW DRIED OFF ON A DAIRY FARM SUPPLEMENTED WITH OR WITHOUT BOLUSES FROM THE DRY PERIOD UNTIL THE FIRST 14 DAYS OF LACTATION UNDER DEFAULT CIRCUMSTANCES. AVERAGE IS GIVEN FOR BOLUSES AS WELL AS NO BOLUSES, WITH 5% AND 95% PERCENTILES BETWEEN BRACKETS.

COST FACTOR	BOLUSES	NO BOLUSES	DIFFERENCE
MEDICINES	3 (0 – 22)	6 (0 - 33)	-3
LABOUR	2 (0 – 15)	4 (0 – 23)	-2
VETERINARY VISITS	0,3 (0 – 0)	0,5 (0 – 0)	-0,2
MILK PRODUCTION LOSSES	22 (0 – 172)	45 (0 – 182)	-23
DISCARDED MILK	11 (0 – 99)	23 (0 – 129)	-12
CULLING	18 (0 – 0)	35 (0 – 427)	-17
BOLUSES	47 (47- 47)	-	47
REDUCED FEED	5 (0 – 41)	11 (0 – 44)	-6
TOTAL COSTS	98 (47 – 369)	103 (0 – 432)	-5

Results of the sensitivity analysis with parameters for the dynamics of infection are given in Table 11. If the probability of mastitis in the dry period increases or decreases with about 10% the average costs will increase or decrease with respectively €6 and €7. However, an increase of occurrence of CM in the dry period results in slightly lower average costs. A change in occurrence of clinical flare up in the dry period does not have an effect on the average costs. Also, an alteration of the probabilities of cure of CM, clinical flare up or SCM have a small effect on the average costs. A change in occurrence of mastitis or CM during the first 14 days of lactation has the greatest effect on the average costs. With a decrease and increase of 10% of mastitis the first 14 days of lactation a decrease and increase of respectively €15 and €13 occur. A decrease of 3% and an increase of 6% in probability of occurrence of CM in the first 14 days of lactation, result in a decrease and increase with €8 and €13 of the average costs respectively. The average costs for cows with boluses were €8 higher than for cows without boluses. An alteration of the probability of clinical flare up during lactation caused a small effect on the average costs as well as an alteration of the probability of bacterial cure of non-bacterial cured cases during lactation. Also, the probability of bacterial cure of clinical cases did not have a significant effect on the average costs.

TABLE 11: SENSITIVITY OF THE TOTAL COSTS (€/COW DRIED OFF) OF AN AVERAGE COW DRIED OFF SUPPLEMENTED WITH GARLIC BOLUSES FOR FACTORS ON THE DYNAMICS OF THE INFECTION. AVERAGE IS GIVEN FOR BOLUSES AS WELL AS NO BOLUSES, WITH 5% AND 95% PERCENTILES BETWEEN BRACKETS.

VARIABLE	VALUE	DEFAULT VALUE	BOLUSES	DEFAULT SITUATION	DIFFERENCE
PROBABILITY OF MASTITIS DRY PERIOD	0,001	0,100	92 (47 – 369)	98 (47 – 369)	-6
	0,200		105 (47 – 378)	98 (47 – 369)	7
PROBABILITY OF CLINICAL MASTITIS DRY PERIOD	0,0005	0,001	98 (47 – 367)	98 (47 – 369)	0
	0,002		99 (47 – 372)	98 (47 – 369)	1
	0,004		97 (47 – 371)	98 (47 – 369)	-1
PROBABILITY OF CLINICAL FLARE UP DRY PERIOD	0,100	0,200	99 (47 – 373)	98 (47 – 369)	1
	0,300		99 (47 – 374)	98 (47 – 369)	1
PROBABILITY OF CURE CLINICAL MASTITIS OR CLNICAL FLARE UP DRY PERIOD	0,750	0,874	95 (47 – 362)	98 (47 – 369)	-3
	0,950		98 (47 – 372)	98 (47 – 369)	0
PROBABILITY OF SPONTANEOUS CURE SUBCLINICAL MASTITIS DRY PERIOD	0,370	0,473	98 (47 – 371)	98 (47 – 369)	0
	0,570		96 (47 – 366)	98 (47 – 369)	-2
PROBABILITY OF CLINICAL MASTITIS AT CALVING	0,013	0,019	96 (47 – 370)	98 (47 – 369)	-2
	0,024		98 (47 – 353)	98 (47 – 369)	0
PROBABILITY OF MASTITIS FIRST 14 DAYS LACTATION	0,200	0,300	83 (47 – 328)	98 (47 – 369)	-15
	0,400		111 (47 – 387)	98 (47 – 369)	13
PROBABILITY OF CLINICAL MASTITIS FIRST 14 DAYS LACTATION	0,030	0,069	90 (47 – 346)	98 (47 – 369)	-8
	0,120		111 (47 – 385)	98 (47 – 369)	13
PROBABILITY OF CLINICAL FLARE UP LACTATION	0,004	0,104	94 (47 – 371)	98 (47 – 369)	-4
	0,200		101 (47 – 369)	98 (47 – 369)	3
PROBABILITY OF BACTERIAL CURE CLINICAL CASES LACTATION	0,400	0,530	98 (47 – 375)	98 (47 – 369)	0
	0,650		97 (47 – 363)	98 (47 – 369)	-1
PROBABILITY OF CLINICAL CURE OF NON-BACTERIAL CURED CASES LACTATION	0,800	0,900	101 (47 – 382)	98 (47 – 369)	3
	0,990		96 (47 – 365)	98 (47 – 369)	-2

Results of the sensitivity analyses for economic parameters are given in Table 12. In the default situation the economic value for milk loss and discarded milk was €0,35. An increase in milk price caused an increase of the average costs for cows both with or without boluses. In a situation with an economic value of milk of €0,35 the costs for cows with boluses became higher than for cows without boluses. When rearing costs were €1000 the average costs per cow were €5 higher for cows with boluses. The average costs increased with €36 for cows without boluses when rearing costs increased, in contrast to the increase of €14 for cows with boluses. With a price for boluses of €50 the total costs were higher for cows with boluses than for cows without boluses.

TABLE 12: SENSITIVITY OF TOTAL COSTS (€/COW DRIED OFF) OF SUPPLEMENTED WITH OR WITHOUT GARLIC BOLUSES OR NO BOLUSES TO AN AVERAGE COW DRIED OFF FOR ECONOMIC FACTORS. AVERAGE IS GIVEN FOR BOLUSES AS WELL AS NO BOLUSES, WITH 5% AND 95% PERCENTILES BETWEEN BRACKETS.

VARIABLE	VALUE	DEFAULT VALUE	BOLUSES	NO BOLUSES	DIFFERENCE
DEFAULT SITUATION			98 (47 – 369)	103 (0 – 432)	-5
MILK PRICE (€/KG OF MILK)	0,25	0,35	89 (47 – 283)	81 (0 – 421)	8
	0,45		107 (47 – 454)	120 (0 – 457)	-13
REARING COSTS (€)	1000	1567	89 (47 – 350)	84 (0 – 332)	5
	2000		103 (47 – 369)	120 (0 – 606)	-17
COSTS BOLUSES (€)	30	40	91 (32 – 366)	103 (0 – 432)	-12
	50		106 (62 – 377)	103 (0 – 432)	3

Discussion

Results from this study are highly influenced by the assumptions used. Multiple assumptions had to be made, because of a lack of detailed information from literature. Therefore, the results are uncertain and can vary between farms and between different circumstances. The biggest assumptions made, include the pathogen distribution used and the probabilities of prevention and cure of mastitis in the dry period and in early lactation due to supplementing dry cows with garlic boluses. The pathogen distribution used and probabilities of occurrence of mastitis in the dry period and early lactation for the scenario without boluses were based as much as possible on information in the peer-reviewed literature (Scherpenzeel et al., 2016; Halasa et al., 2010; Halasa et al., 2009a; Steeneveld et al., 2011; Sampimon et al. 2009). The effect of supplementing cows with garlic boluses on mastitis is however uncertain, and not described in literature. Therefore, expertise from experts and authors were used, and a sensitivity analysis was performed.

In this study no difference has been made between pathogen distribution in the dry period and in early lactation. Bradley et al. 2015 however shows that there is a difference in quarter prevalence of mastitis pathogens before drying of, during the dry period and post calving. Thus, the pathogen distribution used in this model might not completely represent a normal pathogen distribution for the dry period. In this model the pathogen distribution influences the probability of clinical flare up in the dry period and during lactation, cure of clinical flare up in the dry period and bacterial cure of CM during lactation, spontaneous cure of SCM in the dry period, clinical mastitis at calving and the mean duration of SCM during lactation. Pathogen-specific probabilities of clinical flare up, cure of clinical flare up and spontaneous cure of SCM in the dry period were available for *Staphylococcus aureus, Streptococcus uberis and Streptococcus dysgalactiae* (Halasa et al., 2010), but for Coagulase-negative staphylococci and *Corynebacterium bovis* assumptions had to be made based on results from Halasa et al., 2010. Furthermore, it was necessary to make assumptions of the pathogen-specific probability of bacterial cure of CM and clinical cure of CM during lactation for Coagulase-negative staphylococci and *Corynebacterium bovis* based on Steeneveld et al., 2011. In addition, pathogen-specific probabilities for Coagulase-negative staphylococci and *Corynebacterium bovis* for CM at calving and clinical flare up during lactation had to be estimated based on Halasa et al. 2009a. Therefore, the probabilities of mastitis might not be completely representable for a normal situation.

No research has been done on the effect of garlic on the occurrence of mastitis in dairy cattle. It is not clear what the therapeutic effect of garlic is in cows. Research has shown that garlic has an antimicrobial effect on mastitis pathogens (Borlinghaus et al., 2014; Safithria et al., 2011). Therefore, it is likely that garlic has an antimicrobial effect in the udder of the cow and thus a curing effect. It was assumed that the garlic bolus acts more preventive than curative in the stochastic model of this study, because the bolus is used as preventive treatment. It seems likely that a proportion of the bacteria that enter the udder are eradicated before they can infect the udder, due to an assumed constant level of garlic in the blood and udder cells. Therefore, the occurrence of CM and SCM will decrease and less damage will be caused by the bacteria in the udder. Assumptions made by the author were used to estimate all missing probabilities of occurrence of mastitis and its cure in the boluses scenario. The assumed probabilities of the occurrence of mastitis in the dry period and early lactation with boluses, were adapted based on the probabilities of occurrence of mastitis during dry period and early lactation without boluses and based on garlic studies related to cattle and or mastitis (Sarkar et al., 2006; Tuteja et al., 2013; Safithri et al., 2011). A lot of uncertainty existed about the used probabilities of mastitis and cure, and thus the sensitivity analysis was important to verify the estimates. The probabilities of mastitis and its cure influenced the total costs per cow. Especially increasing the probabilities of mastitis during lactation resulted in higher total costs per cow and had the greatest effect on the average costs. Increasing the probability of CM in the dry period, however, did result in lower total costs per cow. When the probability of CM increases, the probability of SCM decreases in the dry period (Table 11). This implies that the occurrence of SCM in the dry period has a greater impact on the total costs per cow. During lactation however, it seems like the occurrence of CM has a greater impact on the total costs per cow. This could be explained by the higher costs of production losses, discarded milk and culling during lactation. Using the defined values on the probabilities of mastitis and cure, supplementing cows with boluses is economically beneficial. Nevertheless, this conclusion may change when results of future clinical trials on the effect of garlic boluses on mastitis prove differently.

Another effect of the garlic boluses might be a decrease in occurrence of other infectious disease in the periparturient period of the cow, such as (endo)metritis. Due to a constant level of garlic in the blood of the cow in the dry period and early lactation, the bacterial load in the uterus will for example decline and the overall conception rate might increase (Sarkar et al., 2006), which would be economically beneficial (Inchaisri et al. 2010). This shows that the decrease in occurrence of other disease due to supplementing garlic boluses would be extra economically beneficial.

Results of the sensitivity analyses for economic parameters show that the milk price has an evident impact on the average costs. This suggests that costs due to milk production losses and discarded milk are notable part of the average costs. Also culling costs account for a considerable share of the average costs per cow. This implies that costs made during lactation (milk production losses, culling) are of greatest impact on the average costs. So, for the garlic boluses to become profitable economically, they should have a significant positive effect on the occurrence of mastitis during lactation, which the results of the sensitivity analyses with parameters for the dynamics of the infection showed as well.

Mastitis does not only affect the total costs per cow directly, but also has indirect beneficial effects on social and economic factors on dairy farms. Job satisfaction, overall situation on the farm and economic losses are the main factors influencing the decision of Dutch farmers to improve mastitis management (Valeeva et al., 2007). Job satisfaction implies the pleasure a farmer will encounter, when applying good mastitis management, which is experienced by farmers as a reward. The factor overall situation on the farm reflects the pleasant aspects of improved mastitis management on a farm, such as fewer problems with other cow diseases, a more efficient milking process and a lower animal daily dose of antibiotics (Valeeva et al., 2007). So, when the number of CM cases decrease due to supplementing garlic boluses to dry cows on a farm, the job satisfaction of the farmer might increase. This increased job satisfaction will in turn trigger the farmer to optimize his management and thereby improving the health status of his herd regarding mastitis but also other diseases, such as ketosis and endometritis. When a farmer decides to start using garlic boluses to improve the udder health on his farm, he changed his attitude towards udder health. Van den Borne et al. 2014 found that farmers' change in attitude and knowledge and farmers' change in behaviour explained 24% and 5% of the variance in the decreased incidence rate of CM, respectively. However, they did not observe a change in management factors related to mastitis control. This indicates that incidence rate of CM decreases due to improving the quality of mastitis management by the farmer, rather than changing the mastitis management. Thus, the occurrence of mastitis might not only decrease due to the garlic boluses, but also due to a change in the farmers' attitude towards udder health, by wanting to reduce the incidence of mastitis.

Not only the farmers mindset has influence on the health status of the herd, also the mastitis incidence is related to the health status of the herd regarding other diseases than mastitis. Mastitis is also known to be predisposing for displaced abomasum and ketosis. Both displaced abomasum and ketosis cause milk production losses and increase the risk of culling (Gröhn et al., 2003). So, by reducing the mastitis incidence, the overall health status of cows on a farm will improve, which would also be economically beneficial.

Moreover, the occurrence of mastitis has an effect on the reproductive performance of a dairy cow. Mastitis in a cow disrupts the endocrine and immune system, resulting in oestrous cycle abnormalities, ovarian disorders, metritis and early embryo death (Gröhn et al., 2003; Siatka et al., 2018) It causes a longer interval from calving to conception, lower conception rates, more services per conception rate and a higher risk of embryo loss (van Soest et al. 2017). Impaired reproductive performance causes economic losses on a farm (Inchaisri et al. 2010; Rutten et al., 2014). Inchaisri et al. 2010 calculated annual net economic losses of €34 and €120 for an "average" and "poor" reproductive performance scenario on a farm. Thus, by supplementing cows with garlic boluses and thereby reducing the occurrence of mastitis, reproductive performance of these cows will improve, and economic losses will decrease.

Besides the economic benefits for the farmer to reduce the occurrence of mastitis on its farm, the importance of milk quality has started to play an important in Dutch society the past few years and thus started to play a role on Dutch dairy farms. Aspects of milk quality do not only concern, the percentage fat and protein, SCC and bacterial count, absence of antibiotics, but also the wish of consumers that the milk they are buying is produced conforming to certain ethical standards, such as animal welfare and environmental concerns (Hogeveen et al., 2011). Mastitis is a painful disease and it could be an animal welfare problem, when there is a high incidence on a farm (Kemp et al., 2008; Hogeveen et al. 2011). Usually dry cow therapy with antibiotics is used to decrease mastitis incidence during the dry period and early lactation. However, the usage of antibiotics should be reduced as much as possible, to minimize the risk of the emergence of multiple resistant bacteria (Royster and Wagner, 2015; Knappstein et al. 2005; Wendtland et al., 2013). Garlic boluses could be used as a replacement for dry cow therapy to reduce the use of antibiotics on dairy farms.

Conclusion

The developed stochastic simulation model for this study, is a distribution function that describes the entire range of economic outcome for a dairy cow. Results suggest that, on average and under default circumstances, supplementing cows with boluses in the dry period might be profitable economically. Under the default conditions, the average economic costs of a cow due to mastitis was estimated to be €103 per cow. On average, the total costs decrease by €5 when a cow is supplemented with garlic boluses in the dry period. The decrease in costs for factors such as milk production losses, discarded milk and culling did outweigh the additional costs of supplementing a cow with garlic boluses of €47 (boluses and labour). Sensitivity analysis showed that the average costs were most sensitive to the probabilities of occurrence of mastitis and CM in the first 14 days of lactation and to the milk price and rearing costs. Thus, the garlic boluses should reduce the probability of occurrence of mastitis in the first 14 days significantly to make it economically profitable to supplement them. Because the greatest uncertainty in this model exists in the effect of the garlic boluses on the probabilities of occurrence of CM, SCM and clinical flare up in the dry period and in early lactation, further research should be done to the effect on garlic boluses on the prevalence of SCM and the incidence rate of CM in the dry period and early lactation.

References

Bezman, D., Lemberskiy-Kuzin, L., Katz, G., Merin, U., and Leitner, G. (2015). Influence of intramammary infection of a single gland in dairy cows on the cow's milk quality. *Journal of Dairy Research*, 82: 304–311

Borlinghaus, J., Albrecht, F., Gruhlke, M.C.H., Nwachukwu, I.D., and Slusarenko, A.J. (2014). Allicin: Chemistry and Biological Properties. *Molecules*, 19: 12591-12618

Bradley, A.J., Breen, J.E., Payne, B., White, V., and Green, M.J. (2015). An investigation of the efficacy of a polyvalent mastitis vaccine using different vaccination regimens under field conditions in the United Kingdom. *Journal of Dairy Science*, 98: 1706–1720

CRV. (2017). https://www.crv4all.nl/ Accessed February 28, 2018.

CVB. (2012). *Tabellenboek Veevoeding 2012: Voedernormen Landbouwhuisdieren en Voederwaarde Veevoeders.* Den Haag, Nederland: CVB

Gröhn, Y.T., Rajala-Schultz, P.J., Allore, H.G., DeLorenzo, M.A., Hertl, J.A., and Galligan, D.T. (2003). Optimizing replacement of dairy cows: modelling the effects of diseases. *Preventive Veterinary Medicine,* 61: 27-43

Gruhlke, M.C.H., Nicco, C., Batteux, F., and Slusarenko, A.J. (2017). The Effects of Allicin, a Reactive Sulfur Species from Garlic, on a Selection of Mammalian Cell Lines. *Antioxidants,* 6(1): 1

Halasa, T., Huijps, K., Østerås, O., and Hogeveen H. (2007). Economic effects of bovine mastitis and mastitis management: A review. *Veterinary Quarterly*, 29(1): 18-31

Halasa, T., Nielen, M., Huirne, R.B.M., and Hogeveen, H. (2009a). Stochastic bio-economic model of bovine intramammary infection. *Livestock Science*, 124: 295–305

Halasa, T., Nielen, M., de Roos, A.P.W., Van Hoorne, R., de Jong, G., Lam, T.J.G.M., van Werven, T., and Hogeveen, H. (2009b). Production loss due to new subclinical mastitis in Dutch dairy cows estimated with a test-day model. *Journal of Dairy Science*, 92: 599–606

Halasa, T., Nielen, M., van Werven, T., and Hogeveen, H. (2010). A simulation model to calculate costs and benefits of dry period interventions in dairy cattle. *Livestock Science,* 129: 80-87

Hogeveen, H., Huijps, K., and Lam, T.J.G.M. (2011). Economic aspects of mastitis: New developments. *New Zealand Veterinary Journal*, 59(1): 16-23

Hortet, P., Beaudeau, F., Seegers, H., and Fourichon, C. (1999). Reduction in milk yield associated with somatic cell counts up to 600 000 cells/ml in French Holstein cows without clinical mastitis. *Livestock Production Science*, 61: 33–42

Hortet, P., and Seegers, H. (1998). Calculated milk production losses associated with elevated somatic cell counts in dairy cows: review and critical discussion. *Veterinary Research*, 29(6): 497-510.

Huijps, K., Hogeveen, H., Lam, T.J.G.M., and Oude Lansink, A.G.J.M. (2010). Costs and efficacy of management measures to improve udder health on Dutch dairy farms. *Journal of Dairy Science*, 93: 115–124

Huijps K., De Vliegher, S., Lam, T.J.G.M., and Hogeveen, H. (2009). Cost estimation of heifer mastitis in early lactation by stochastic modelling. *Veterinary Microbiology*, 134: 121–127.

Huijps, K., Lam, T.J.G.M., and Hogeveen, H. (2008). Costs of mastitis: facts and perception. *Journal of Dairy Research*, 75: 113–120.

Inchaisri, C., Jorritsma, R., Vos, P.L.A.M., van der Weijden, G. C., and Hogeveen, H. (2010). Economic consequences of reproductive performance in dairy cattle. *Theriogenology*, 74: 835–846.

Jansen, J. (2010). *Mastitis and farmer mindset: Towards effective communication strategies to improve udder health management on Dutch dairy farms.* PhD Thesis. Wageningen University, Wageningen, the Netherlands.

Kemp, M.H., Nolan, A.M., Cripps, P.J., Fitzpatrick, J.L. (2008). Animal-based measurements of the severity of mastitis in dairy cows. *Veterinary Record*, 163: 175-179

Knappstein, K., Suhren, G., Walte, H.G. (2005). Influences on excretion of antibiotic residues in milk with special emphasis on milking frequency. *Mastitis in Dairy Production: Current Knowledge and Future Solutions*, 677-683

Lam, T.J.G.M., van den Borne, B.H.P., Jansen, J., Huijps, K., van Veersen, J.C.L., van Schaik, G., and Hogeveen, H. (2013). Improving bovine udder health: A national mastitis control program in the Netherlands. *Journal of Dairy Science*, 96: 1301–1311

Lam, T.J.G.M., van Vliet, J.H., Schukken, Y.H., Grommers, F.J., van Velden-Russcher, A., Barkema, H.W., and Brand, A. (1997). The effect of discontinuation of postmilking teat disinfection in low somatic cell count herds. II. Dynamics of intramammary infections. *Veterinary Quarterly*, 19(2): 47-53

Leslie, K.E., and Petersson-Wolfe, C.S. (2012). Assessment and Management of Pain in Dairy Cows with Clinical Mastitis. *Veterinary Clinics of North America - Food Animal Practice,* 28(2): 289-305

Mandhwani, R., Bhardwaz, A., Kumar, S., Shivhare, M., and Aich, R. (2017). Insights into bovine endometritis with special reference to phytotherapy. *Veterinary World,* 10(12): 1529-1532.

Mason, S.E., Mullen, K.A.E., Anderson, K.L., Washburn, S.P., Yeatts, J.L., and Baynes, R.E. (2017). Pharmacokinetic analysis of thymol, carvacrol and diallyl disulfide after intramammary and topical applications in healthy organic dairy cattle. *Food Additives and Contaminants: Part A*, 34(5): 740-749

Mohd Nor, N., Steeneveld, W., Mourits, M.C.M., and Hogeveen, H. (2012). Estimating the costs of rearing young dairy cattle in the Netherlands using a simulation model that accounts for uncertainty related to diseases. *Preventive Veterinary Medicine*, 106: 214-224

Oh, J., Hristov, A.N., Lee, C., Cassidy, T., Heyler, K., Varga, G.A., Pate, J., Walusimbi, S., Brzezicka, E., Toyokawa, K., Werner, J., Donkin, S.S., Elias, R., Dowd, S., and Bravo, D. (2013). Immune and production responses of dairy cows to postruminal supplementation with phytonutrients. *Journal of Dairy Science,* 96 :7830–7843

Palisade (2004). Guide to Using @Risk. Palisade Corporation, Newfield, NY, USA

Pyörälä, S., and Mattila, T. (1987). Inflammatory changes during experimental bovine mastitis induced by Staphylococcus aureus, Streptococcus dysgalactiae and Streptococcus uberis. *Journal of Veterinary Medicine,* A, 34: 574–581

Remmelink, G.J., Blanken, K., van Middelkoop, J.C., Ouweltjes, W., and Wemmenhove, H. (2012). *Handboek Melkveehouderij 2012 (Handbook of Dairy Farming).* Livestock Research, Wageningen UR, Lelystad, the Netherlands.

Royster, E., and Wagner, S. (2015). Treatment of Mastitis in Cattle. *Veterinary Clinics of North America - Food Animal Practice*, 31: 17–46

Rutten, C. J., Steeneveld, W., Inchaisri, C., and Hogeveen, H. (2014). An ex ante analysis on the use of activity meters for automated estrus detection: To invest or not to invest? *Journal of Dairy Science,* 97: 6869–6887.

Safithria, M., Bintanga, M., and Poeloenganb, M. (2011). Antibacterial Activity of Garlic Extract Against some Pathogenic Animal Bacteria. *Media Peternakan,* 34(3): 155-158

Sampimon, O., Barkema, H.W., Berends, I., Sol, J., and Lam, T. (2009). Prevalence of intramammary infection in Dutch dairy herds. *Journal of Dairy Research*, 76: 129–136

Santman-Berends, I.M.G.A., Lam, T.J.G.M., Keurentjes, J., and van Schaik, G. (2015). An estimation of the clinical mastitis incidence per 100 cows per year based on routinely collected herd data. *Journal of Dairy Science*, 98: 6965–6977

Sarkar, P., Kumar, H., Rawat, M., Varshney, V.P., Goswami, T.K., Yadav, M.C., and Srivastava, S.K. (2006). Effect of administration of garlic extract and PGF2α on hormonal changes and recovery in endometritis cows. *Asian Australasian Journal of Animal Science.,* 19(7): 964-969.

Schepers, A.J., Lam, T.J.G.M., Schukken, H., Wilmink, J.B.M., and Hanekamp, W.J.A. (1997). Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. *Journal of Dairy Science*, 80(8): 1833-1840

Scherpenzeel, C.G.M., den Uijl, I.E.M., van Schaik, G., Olde Riekerink, R.G.M., Hogeveen, H., and Lam, T.J.G.M. (2016). Effect of different scenarios for selective dry-cow therapy on udder health, antimicrobial usage, and economics. *Journal of Dairy Science,* 99: 3753-3764

Scherpenzeel, C.G.M., den Uijl, I.E.M., van Schaik, G., Olde Riekerink, R.G.M., Keurentjes, J.M., and Lam, T.J.G.M. (2014). Evaluation of the use of dry cow antibiotics in low somatic cell count cows. *Journal of Dairy Science*, 97: 3606–3614

Siatka, K., Sawa, A., and Krężel-Czopek, S. (2018). Effect of postpartum endocrine function, metabolism, and mastitis on fertility in high-yielding cows: A review. *Annals of Animal Science,* 18(2): 351-59

Smith, B.B. (2015). *Large Animal Internal Medicine, Fifth Edition.* Missouri, MO: Elsevier

Van Soest, F.J.S., Abbeloos, E., McDougall, S., and Hogeveen, H. (2017). Addition of meloxicam to the treatment of bovine clinical mastitis results in a net economic benefit to the dairy farmer. *Journal of Dairy Science*, 101: 3387-3397

van Soest, F.J.S., Huijps, K., Dohmen, W., Olde Riekerink, R., Santman-Berends, I.M.G.A., Sampimon, O.C., Lam, T.J.G.M., and Hogeveen, H. (2011). Costs and benefits of mastitis management measures on individual dairy farms. *Udder Health and Communication,* 213-220

van Soest, F.J.S., Santman-Berends, I.M.G.A., Lam, T.J.G.M., and Hogeveen, H. (2016). Failure and preventive costs of mastitis on Dutch dairy farms. *Journal of Dairy Science*, 99: 8365–8374

Steeneveld, W., Swinkels, J.M., and Hogeveen, H. (2007). Stochastic modelling to assess economic effects of treatment of chronic subclinical mastitis caused by *Streptococcus uberis. Journal of Dairy Research,* 74: 495-467

Steeneveld, W., van Werven, T., Barkema, H. W., and Hogeveen, H. (2011). Cow-specific treatment of clinical mastitis: An economic approach. *Journal of Dairy Science,* 94: 174-188

Suleria, H.A.R., Butt, M.S., Khalid, N., Sultan, S., Raza, A., Aleem, M., and Abbas, M. (2015). Garlic (Allium sativum): diet based therapy of 21st century –A review. *Asian Pacific Journal of Tropical Disease*, 5(4): 271-278

Swinkels, J.M., Rooijendijk, J.G.A., Zadoks, R.N., and Hogeveen, H. (2005). Use of partial budgeting to determine the economic benefits of antibiotic treatment of chronic subclinical mastitis caused by Streptococcus uberis or Streptococcus dysgalactiae. *Journal of Dairy Research,* 72: 75–85.

Tuteja, F.C., Dixit, S.K., and Patil, N.V. (2013). Antibacterial activity of vegetable herbs against intramammary infections in camels. *Veterinary Practitioner,* 14(1): 143-145

Tsai, C.-W., Chen, H.-W., Sheen, L.-Y., and Lii, C.-K. (2012). Garlic: Health benefits and actions. *Biomedicine,* 2: 17-29

Valeeva, N.I., Lam, T.J.G.M., and Hogeveen, H. (2007). Motivation of dairy farmers to improve mastitis management. *Journal of Dairy Science,* 90: 4466–4477

Vermeij, I. (2012). *Handboek Kwantitatieve Informatie Veehouderij, KWIN-V 2012–2013, Handboek 23 (Quantitative Information Livestock Farming 2012–2013, Handbook 23)*. Livestock Research, Wageningen UR, Lelystad, the Netherlands.

Wendlandt, S., Schwarz, S., and Silley, P. (2013). Methicillin-Resistant Staphylococcus aureus: A Food-Borne Pathogen? *Annual Review Food Science Technology*, 4: 117–39

Wood, P.D.P. (1967). Algebraic model of the lactation curve in cattle. *Nature,* 216: 164–165.

Yun, H.-M., Bana, J.O., Park, K.-R., Lee, C.K., Jeong, H.-S., Han, S.B., and Hong, J.T. (2014). Potential therapeutic effects of functionally active compounds isolated from garlic. *Pharmacology & Therapeutics*, 142: 183–195

Zhao, X., and Lacasse, P. (2008). Mammary tissue damage during bovine mastitis: Causes and control. *Journal of Animal Science,* 86(13): 57-65