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ORIGINAL ARTICLE

Efficacy of a galactogogue containing silymarin-phosphatidylserine and galega in mothers of preterm infants: a randomized controlled trial

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BACKGROUND/OBJECTIVES: Human milk (HM) is the best possible food for all infants, especially for preterm ones, but lactation and breastfeeding are very difficult for mothers of preterm babies and high rates of breastfeeding difficulties have been reported. Our aim was to investigate the efficacy of a galactogogue containing silymarin-phosphatidylserine and galega in increasing milk production during the first month after delivery in a population of mothers of preterm infants.

SUBJECTS/METHODS: Mothers of infants with gestational age (GA) between 27^{+0} and 32^{+6} weeks were enrolled in this prospective, double-blind, randomized trial and were randomly allocated to receive either the galactogogue containing silymarin-phosphatidylserine and galega, 5 g/day (galactogogue group, GG), or a placebo, 5 g of lactose per day (placebo group, PG) from the 3rd to the 28th day after delivery.

RESULTS: Fifty mothers were included in each group. General characteristics of mothers and pregnancies were similar. Milk production was significantly greater in the GG at the 7th day of life and at the 30th day of life. Daily milk production from the 7th to the 30th day of life was 200 (110–380) ml in the GG vs 115 (60–245) ml in the PG ($P < 0.0001$). The total production of milk during the study period was significantly higher in the GG (6523 ± 5298 ml vs 4136 ± 4093 ml; $P < 0.02$). At the end of the study, 45 mothers of the GG were able to reach the target of milk supply of 200 ml/day compared with 25 mothers of the PG ($P < 0.01$). No adverse reactions were noticed in the study groups.

CONCLUSIONS: Silymarin-phosphatidylserine and galega increased milk production in mothers of preterm infants without any significant side effects.

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INTRODUCTION

Human milk (HM) is important for all infants, especially for those born premature. It is well known that HM reduces the risk of short-term and long-term morbidities with a combination of nutritional, anti-infective, anti-inflammatory, anti-oxidative and epigenetic actions.[1–3] Many studies suggested that administration of HM to preterm infants improves feeding tolerance and reduces infections and late-onset sepsis.[4–8] However, breastfeeding can be very difficult for mothers of babies born preterm. High rate of breastfeeding failure is reported worldwide. One of the most important factors affecting HM availability is the delayed onset of lactation (>72 h) among mothers of preterm neonates. Sometimes even if an effective and regular sucking is established, a small amount of milk is produced for several days after birth.[1,8–11] For these reasons, donor milk is often used in the Neonatal Intensive Care Units as a substitute for mother's milk, especially during the first days of life.[12–14] Improving human lactation is therefore one of the goals of neonatal nutrition, and this is particularly important for preterm neonates. The use of evidence-based lactation technologies, such as breast pumps, specialized breast-pump suction patterns and nipple shields, helps the clinician to improve HM production.[1]

Medications or other products are often requested by mothers in order to increase milk production.[15–17] Galactogogues are substances that promotes lactation in humans. It may be a pharmaceutical drug or a herbal supplement. In the United States, it is estimated that 15% of breastfeeding women use herbal galactogogues, whereas in a Norwegian study this estimate is 43%.[18–20]

In recent years, silymarin, a component of milk thistle (silybum marianum), has been reported to have a galactogogic effect.[21–24] Silymarin includes four flavonolignans: silybin (65%), silychristin (20%), silydianin (10%) and isosilybin (5%). The flavonolignans are bioflavonoid phytoestrogens. Flavonolignans stimulate lactation directly, but they could act on estrogen receptors (ER2) by limiting the endogenous receptor antagonism of milk production.[21,25] Galactogogic effect of milk thistle has been reported in cows[22,26] and in rats;[27] few data showed similar effects in humans as well.[28,29] A recent systematic review concluded that well-designed and well-conducted clinical trials are needed to generate evidence for recommendations about the use of herbs as galactogogues.[30]

Herbal extracts are usually characterized by low solubility and thus poor bioavailability. To increase their absorption, a new formulation was developed and used as a delivery system: a phytosome composed of silymarin and phospholipids (phosphatidylserine). In addition, phosphatidylserine has antioxidant effects and acts as a regulator of cortisol levels.[31] It acts as a carrier for insoluble molecules, promoting intestinal absorption. A recent scientific paper demonstrated that phytosome containing silymarin is able to clearly improve the bioavailability of silymarin

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when compared with normal (pure) silymarin and micronized silymarin.[31]

This double-blinded, randomized trial investigated the efficacy of a galctogogue containing silymarin-phosphatidylserine and galega in mothers of premature infants.

METHODS

Design and settings

This prospective, randomized, clinical trial was conducted between 15th November 2013 and 31st May 2015, in the Neonatal Intensive Care Unit of our university hospital. The study was approved by our institutional review board. Mothers of infants born at a gestational age (GA) between 27^{+0} and 32^{+6} weeks, aiming to breastfeed, were considered eligible for the study. Women with contraindication to breastfeeding and those with lactose intolerance were excluded. Enrollment was performed into the first 48 h of life. Before enrollment, mothers were informed about the advantages of breastfeeding. It was explained that the study was approved by an ethics committee and that all available measures to encourage and improve breastfeeding would be provided in both the arms of the study. Mothers included in the study were assigned randomly to the galactogogue group (GG) or to the placebo group (PG) within the first 72 h of life. The allocation sequence was generated by using Stata 10 (Stata, College Station, TX, USA). Two investigators enrolled participants and assigned them to study groups by opening sealed envelopes.

Outcomes and sample size calculation

The primary outcome was the milk production. A retrospective review of our database showed that the mean daily production of breast milk during the first month of life was 152 ± 63 ml. Calculations gave a sample size of 50 mothers in each group to yield 80% power (with $\alpha = 0.05$) in order to detect a 25% increase in milk production from the 7th to the 30th day after the delivery. The secondary outcome was to evaluate the safety of the intervention by monitoring undesired side effects.

Intervention protocol

Silymarin-phosphatidylserine and galega (a daily single dose of 5 g) were given to the mothers assigned to the GG, whereas a placebo (a daily single 5 g dose of lactose) was given to those assigned to the PG. Treatment and placebo forms had indistinguishable shape, color, taste and flavor. The medication was administered from the 3rd to the 28th day after the delivery, and the amount of milk produced was recorded. At enrollment, we explained to the mother to pump regularly every 2–3 h during the day for 15 min for each breast. A dedicated room, supplied with an electric breast pump, was available in the neonatal ward in order to encourage mothers to express their milk. At home, the mothers were advised to pump the milk in a sterile bottle, store it in the refrigerator and bring it to the ward. We suggested following a balanced Mediterranean diet taking at least 1500 ml/day of fluids and avoiding substances potentially dangerous for the child. A chart was provided to all mothers in order to record the consumption of medication, day, time, duration and amount of obtained milk, and any side effect. The number of pumping sessions was not recorded. Monitor sessions were performed by one of the investigators at days 14, 21 and 28 after delivery.

Statistical analyses

Statistical analyses were performed with Microsoft Excel 2007 (Microsoft, Redmond, WA, USA) and SPSS for Windows 17.0 (SPSS, Chicago, IL, USA). Comparison was made using the Student's *t*-test for normally distributed continuous variables and Fisher's exact test for categorical variables. The Mann–Whitney *U*-test was used to analyze the differences when the variables were not normally distributed. A P-value of 0.05 was considered statistically significant. The D'Agostino-Pearson test was performed in order to assess the normality. Coefficients of skewness and kurtosis were calculated in order to further characterize the distribution.

RESULTS

In the study period, 118 mothers were considered eligible, 18 of them refused to participate and 100 were enrolled and completed the study. There was no loss to follow-up (Figure 1).

Table 1 shows that baseline characteristics of the GG and the PG were similar.

No other medications that affect milk production were consumed by the mothers throughout the study. All the mothers started the medication 72 h after delivery. Significant milk production was not reported during the first 2 days. By the 3rd day, only few mothers reported a significant amount of milk expression, whereas at the 7th day of life all the mothers included in the study recorded some milk production.

Figure 2 shows the amount of milk produced in the studied groups. Median and interquartile ranges were used because milk production was not normally distributed. The coefficient of skewness was 1.46, and the coefficient of kurtosis was found to be 2.35. The amount of milk produced was significantly higher in the GG at the 7th and at 30th day after the delivery ($P < 0.05$).

Mean daily milk production from the 7th to the 30th day after the delivery was 200 (110–380) ml in the GG vs 115 (60–245) in the PG ($P < 0.0001$). The total production of milk during the study period was also significantly higher in the GG (6523 ± 5298 ml vs 4136 ± 4093 ml; $P < 0.02$). Only eight mothers in the GG produced less than 2500 ml of total milk production vs 21 mothers in the PG ($P < 0.02$). At the 7th day after delivery, 29 mothers in the GG achieved a daily milk production higher than 200 ml compared with 15 in the PG ($P < 0.05$). At the end of the study, the number of mothers able to produce a daily amount of milk greater than

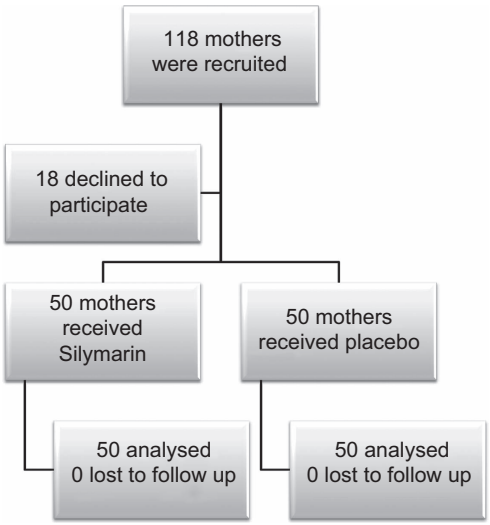

Figure 1. Flow chart of the study.

Table 1. Baseline characteristics of the GG and PG

	GG (n = 50)	*PG (n = 50)*	P-*value*
Maternal age, years, mean ± s.d.	34 ± 5	34 ± 6	n.s.
Primigravida, *n* (%)	17 (34.0)	20 (40.0)	n.s.
Primipara, *n* (%)	32 (64.0)	39 (78.0)	n.s.
Pregnancy pathology, *n* (%)	34 (68.0)	25 (50.0)	n.s.
Hypertension	10 (20.0)	6 (12.0)	
Diabetes	9 (18.0)	6 (12.0)	
Hypothyroidism	4 (8.0)	4 (8.0)	
Coagulopathy	6 (12.0)	4 (8.0)	
Other	5 (10.0)	5 (10.0)	
Singleton, *n* (%)	39 (78.0)	38 (76.0)	n.s.
Vaginal delivery, *n* (%)	7 (14.0)	9 (18.0)	n.s.
Gestational age, week, mean ± s.d.	31.5 ± 1.5	30.6 ± 1.9	n.s.
Birth weight, g, mean ± s.d.	1468 ± 361	1391 ± 420	n.s.
Small for gestational age, *n* (%)	6 (12.0)	8 (16.0)	n.s.

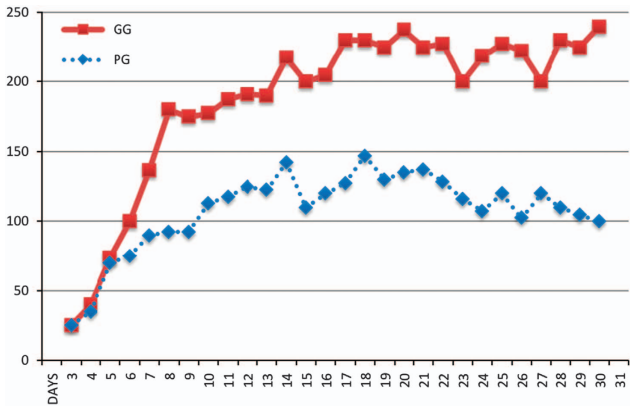

Figure 2. Milk yield in the GG and the PG. The median was significantly higher in the treatment group from the 7th to the 30th day after the delivery ($P < 0.05$).

200 ml was also significantly different (45 in the GG vs 25 in the PG, $P < 0.001$). No adverse reactions were noted in this study.

DISCUSSION

HM is the best available nutrient for all infants and is especially important for preterm neonates.[1,32] Early initiation of enteral feeding with HM has been universally advised, but several mothers are not able to produce enough milk soon after a preterm delivery. HM is also the first choice for the minimal enteral feeding that occurs during the first 3–5 days in extremely preterm babies. HM stimulates the normal gastrointestinal growth and the development of a better intestinal microbioma.[2,3] In the first days after birth preterm infants receiving HM show less gastric residual, less vomiting and lower incidence of abdominal distention when compared with formula-fed infants.[33] The benefit of HM on feeding tolerance improves the achievement of full enteral feeding, which is one of the main goals in the management of preterm neonates.[4,5,32] Furthermore, recent observations suggest that the administration of small amount of colostrum reduces the number of late-onset sepsis.[34] Establishing enteral feeding with own maternal milk can also improve the bonding between the mother and the baby born preterm. Preterm delivery is a risk factor for delayed and/or insufficient lactation because of maternal and infant risk factors that impair the complex interplay in lactation hormones and changes in mammary epithelial cells. Mothers often worry whether they will be able to produce enough milk for their child, and this can have a negative impact on lactation.[1]

Strategies need to be established as soon as possible after the delivery in order to stimulate lactation and promote breastfeeding. These strategies include (A) frequent breast pumping, (B) allow the mother to stay at the bedside, (C) breastfeed the baby or feed the baby with the expressed milk as soon as possible, (D) adequate intake of fluid and nutrients, (E) skin-to-skin contact and (F) kangaroo care. All those strategies improve the bonding between the mother and the baby, emphasizing the importance of her milk for the baby's health.[15–20]

This randomized clinical trial aimed to evaluate the efficacy and the safety of a galactogogue containing silymarin-phosphatidylserine and galega on milk production during the first month of life in mothers of preterm infants. In our study, the number of mothers producing some milk at the 7th day of life is similar in both groups, but the GG produced a significantly higher amount of milk. There is also a significantly higher number of mothers in the GG producing more than 200 ml of milk per day. Our data show that breastfeeding can be successfully promoted even in mothers of baby born preterm. In fact, mothers enrolled in PG produced enough milk by the 7th day after the delivery. However, it is noteworthy that the difference on milk production is evident during the first 2 weeks after the delivery and remained for the whole study period. This study demonstrated that the medication was effective from the beginning of the treatment. A satisfying milk production was maintained throughout the first month after the delivery in almost all the mothers enrolled in the treatment group, whereas only 1/3 of the PG mothers were able to produce more than 200 ml of milk per day at 7th day after the delivery and only half of the PG mothers reached the threshold of 200 ml/day by the end of the study.

The possible mechanisms of action of silymarin are not completely understood. Silymarin includes four flavonolignans that are bioflavonoid phytoestrogens, and their structure can explain the effects reported in the literature: hepato-protection, antioxidant properties, antitoxin action, antiviral and anti-inflammatory effects and anticancer activities.[35,36] The ability to increase milk production of silymarin could be related to its weak anti-estrogenic property and to its ability to enhance prolactin secretion. The flavonoids and saponins stimulate the release of prolactin (with a mechanism similar to the micronized silymarin).[20] Galega, on the other hand, increases milk production with silymarin improving the circulation of the mammary gland and therefore enhancing its oxygenation.[15,18,21,25] Our data confirm the positive effect of this galactogogue on milk production in mothers of baby born preterm, as previously reported for mothers of term babies.[28,29] Side effects reported in other studies, such as allergic reactions, diarrhea or gastrointestinal problems, were not detected in our study, probably because of the lower dose.[37] However, we did not check for liver enzymes that are reported to be elevated during chronic treatment with some galactogogues.[15] We did not observe any significant side effect on infants of treated mothers, and this observation seems to be consistent with the observation that silymarin does not pass into HM at a lower dose (600 mg/3 times a day).[28]

The strengths of our study are that it is a double-blind RCT, involving a consistently high number of mothers, all the subjects adhered to the protocol, and all the treated mothers had some amount of milk production at the end of the study. However, there are some limitations that should be emphasized. First of all, the amount of milk production was checked by nurses during hospitalization, but after discharge the amount of milk was measured by the mothers themselves at home, making some error possible. Second, daily number of pumping sessions was rarely reported by the mothers, even if recommended; hence, we cannot be absolutely sure that a regular pumping pattern occurred overnight. One could assume that this bias was equally distributed in both the study groups because of the correct randomization, but different numbers of pumping sessions could have influenced milk production. Third, we found some patient-to-patient variability in the daily milk production as it is evident from the median, the interquartile range, and coefficient of skewness and kurtosis, but this was something expected for the feature of the variables studied. We did not perform a qualitative evaluation of milk, but some studies showed no changes in milk composition during treatment with galactogogues.[21] Side effects of galactogogues observed in adults, such as diarrhea and gastrointestinal upset, were not reported in mothers and babies of our population. However, safety data on neonatal and pediatric subjects are not available in the literature, and our study was not targeted to assess this outcome; hence, safety remains to be determined. Finally, we did not evaluate the effect of treatment on growth because this was not one of the study aims.

Despite its limits, our study demonstrates that administration of a galactogogue containing silymarin-phosphatidylserine and galega improves milk production in mothers of preterm neonates during the first month of life.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

EZ designed and conducted the study and wrote the final manuscript. AAZ generated the allocation sequence and performed the statistical analysis. AD and LG enrolled participants and assigned them to study groups. ET and TP gave the intervention to the women and collected the data. CR designed the study and revised the final manuscript.

REFERENCES

1 Meier PP, Engstrom JL, Patel AL, Jegier BJ, Bruns NE. Improving the use of human milk during and after the NICU stay. *Clin Perinatol* 2010; **37**: 217–245.
2 Jeurink PV, van Bergenhenegouwen J, Jimenez E, Knippels LM, Fernández L, Garssen J *et al.* Human milk: a source of more life than we imagine. *Benef Microbes* 2013; **4**: 17–30.
3 Riskin A. Changes in immunomodulatory constituents of human milk in response to active infection in the nursing infant. *Pediatr Res* 2012; **71**: 220–225.
4 Levy I, Comarsca J, Davidovits M, Klinger G, Sirota L, Linder N. Urinary tract infection in preterm infants: the protective role of breastfeeding. *Pediatr Nephrol* 2009; **24**: 527–531.
5 Schanler RJ, Shulman RJ, Lau C. Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk vs preterm formula. *Pediatrics* 1999; **103**: 1150–1157.
6 Sisk PM, Lovelady CA, Dillard RG, Gruber KJ, O'Shea TM. Early human milk feeding is associated with a lower risk of necrotizing enterocolitis in very low birth weight infants. *J Perinatol* 2007; **27**: 428–433.
7 Furman L, Taylor G, Minich N, Hack M. The effect of maternal milk on neonatal morbidity of very low-birth-weight infants. *Arch Pediatr Adolesc Med* 2003; **157**: 66–71.
8 Hurst NM. Recognizing and treating delayed or failed lactogenesis II. *J Midwifery Womens Health* 2007; **52**: 588–594.
9 Dewey KG, Nommsen-Rivers LA, Heinig MJ, Cohen RJ. Risk factors for suboptimal infant breastfeeding behavior, delayed onset of lactation, and excess neonatal weight loss. *Pediatrics* 2003; **112**: 607–619.
10 Nommsen-Rivers LA, Dolan LM, Huang B. Timing of stage II lactogenesis is predicted by antenatal metabolic health in a cohort of primiparas. *Breastfeed Med* 2012; **7**: 43–49.
11 Nommsen-Rivers LA, Chantry CJ, Peerson JM, Cohen RJ, Dewey KG. Delayed onset of lactogenesis among first-time mothers is related to maternal obesity and factors associated with ineffective breastfeeding. *Am J Clin Nutr* 2010; **92**: 574–584.
12 Carroll K, Herrmann K. Introducing donor human milk to the NICU: lessons for Australia. *Breastfeed Rev* 2012; **20**: 19–26.
13 Boyd CA, Quigley MA, Brocklehurst P. Donor breast milk versus infant formula for preterm infants: a systematic review and meta-analysis. *Arch Dis Child Fetal Neonatal Ed* 2007; **92**: F169–F175.
14 Arslanoglu S, Ziegler EE, Moro GE. WAPM Working Group on Nutrition Donor human milk in preterm infant feeding: evidence and recommendations. *J Perinat Med* 2010; **38**: 347–351.
15 Zuppa AA, Sindico P, Orchi C, Carducci C, Cardiello V, Romagnoli C. Safety and efficacy of galactogogues: substances that induce, maintain and increase breast milk production. *J Pharm Pharmaceut Sci* 2010; **13**: 162–174.
16 Mannion C, Mansell D. Breastfeeding self-efficacy and the use of prescription medication: a pilot study. *Obstet Gynecol Int* 2012; **2012**: 562704.
17 Marasco L. Increasing your milk supply with galactogogues. *J Hum Lact* 2008; **24**: 455–456.
18 Academy of Breastfeeding Medicine Protocol Committee. ABM Clinical Protocol #9: Use of galactogogues in initiating or augmenting the rate of maternal milk secretion (first revision, January 2011). *Breastfeed Med* 2011; **6**: 41–49.
19 Nordeng H, Havnen GC. Use of herbal drugs in pregnancy: a survey among 400 Norwegian women. *Pharmacoepidemiol Drug Saf* 2004; **13**: 371–380.
20 Puschner B, Galey FD, Holstege DM, Palazoglu M. Sweet clover poisoning in dairy cattle in California. *J Am Vet Med Assoc* 1998; **212**: 857–859.
21 Carotenuto D, Di Pierro F. Studio sulla tollerabilità ed efficacia della silimarina BIO-C® (Piùlatte®) micronizzata come galattagogo. *Acta Neonatol Pediatr* 2005; **4**: 393–340.
22 Vojtisek B. Milk Thistel (silybum marianum) in the feed of ketotik cow. *Vet Med (Praha)* 1991; **36**: 321–330.
23 Kren V, Walterova D. Silybin and Silymarin – new effects and applications. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2005; **149**: 29–41.
24 Pradhan SC, Girish C. Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine. *Indian J Med Res.* 2006; **124**: 491–504.
25 Saxena BN, Shrimanker K, Grudzinskas JG. Levels of contraceptive steroids in breast milk and plasma of lactating women. *Contraception* 1977; **16**: 605–613.
26 Garavaglia L, Galletti S, Tedesco D. Silymarin and lycopene administration in periparturient dairy cows: effects on milk production and oxidative status. *NZ Vet J* 2015; **63**: 313–318.
27 Capasso R. Effect of Silitidil, a standardized extract of milk thistle, on the serum prolactin levels in female rats. *Nat Prod Commun* 2014; **9**: 943–944.
28 Di Pierro F, Callegari A, Carotenuto D, Mollo Tapia M. Clinical efficacy, safety and tolerability of BIO-C (micronized Silymarin) as a galctogogue. *Acta Biomed* 2008; **79**: 205–210.
29 Castoldi F, Pivetti V, Moiraghi L, Marangione P, Lista G. Sylimarin/galega administration in term and preterm mothers to sustain breast feeding: an observational study. *Minerva Pediatr* 2014; **66**: 375–378.
30 Mortel M, Mehta SD. Systematic review of the efficacy of herbal galactogogues. *J Hum Lact* 2013; **29**: 154.
31 Wilinska M, Schleußner E. Galactogogues and breastfeeding. Focus on new natural solutions for hypogalactia. *Nutrafoods* 2015; **14**: 119–125.
32 Ahrabi AF, Schanler RJ. Human milk is the only milk for premies in the NICU! *Early Hum Dev* 2013; **89**: S51–S53.
33 Schanker RJ. The use of human milk for premature infants. *Pediatr Clin North Am* 2001; **48**: 207–219.
34 Lee J, Kim H-S, Jung YH, Choi KY, Shin SH, Kim E-K *et al.* Oropharyngeal colostrum administration in extremely premature infants: an RCT. *Pediatrics* 2015; **135**: e357.
35 Tedesco D, Tava A, Galletti S, Tameni M, Varisco G, Costa *et al.* Effects of silymarin, a natural hepatoprotector, in periparturient dairy cows. *J Dairy Sci* 2004; **87**: 2239–2247.
36 Siegel AB, Stebbing J. Milk thistle: early seeds of potential. *Lancet Oncol* 2013; **14**: 929–930.
37 Mayer KE, Myers RP, Lee SS. Silymarin treatment of viral hepatitis: a systematic review. *J Viral Hepatitis* 2005; **12**: 559–567.