

## ***Eimeria* spp. (Coccidia, Protozoa) infections in a flock of sheep in Iceland: Species composition and seasonal abundance**

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### ABSTRACT

The *Eimeria* spp. and their seasonal infection pattern in three replacement ewe lambs (REL)/young ewes on the Fossárdalur sheep farm in East Iceland were characterized. Ten species were identified; *Eimeria ovinoidalis* predominated in all seasons with a relative abundance of 40.7%, followed by *E. bakuensis* (18.9%), *E. weybridgeensis* (11.1%), *E. granulosa* (8.2%), *E. parva* (6.7%), *E. ahsata* (5.6%), *E. faurei* (4.2%), *E. intricata* (1.6%), *E. pallida* (1.6%) and *E. crandallis* (1.4%). On average 7.4 species (range 5-9) were found in each sample. The eleventh species reported in Central and Western Europe, *E. marsica*, has also been identified in Iceland but in a different flock of sheep. Seasonal abundance differences were mainly observed for *E. ovinoidalis*, *E. bakuensis*, *E. weybridgeensis* and *E. granulosa*. Spring and summer coccidiosis was rarely observed, probably due to the early releasing of ewes and their lambs to sparsely oocyst-contaminated grazing areas. The faeces of ten REL lambs were studied specially in autumn and they got coccidiosis, usually with severe diarrhoea, approximately three weeks after their return from the summer rangelands in adjacent mountains. Intermittent oocyst excretion peaks were observed during winter. Oocyst excretion did not markedly increase during the periparturient period in spring. Comparison of the *Eimeria* spp. composition in ewes when they were 14-15 month old and their six-week-old lambs indicated similar abundance values except for *E. faurei*, which was absent in the ewes but quite often identified in their lambs.

**Keywords:** *Eimeria* spp., Iceland, identifications, lambs, seasonal occurrence, sheep

### YFIRLIT

*Tegundasamsetning og árstíðasveiflur hnísla í ásetningsgimbrum á fjárbúinu í Fossárdal*

Tegundasamsetning og árstíðasveiflur hnísla (*Eimeria* spp.) voru rannsakaðar í ásetningsgimbrum á fjárbúinu í Fossárdal í Berufirði frá september 2002 til október 2003. Einnig var gerður samanburður á hníslasýkingum gemsanna og lamba þeirra í júlí áður en þeim var sleppt á fjall úr stórrí girðingu þar sem fé hafði verið beitt mjög dreift. Alls fundust tíu tegundir hnísla. *Eimeria ovinoidalis* var langsamlega algengasta tegundin á öllum árstímum með 40,7% hlutdeild. Tegundin er vel þekktur sjúkdómsvaldur en meinvirkni margra hinna tegundanna er yfirleitt lítið þekkt. Næstalgengust var *E. bakuensis* (18,9%) en síðan komu *E. weybridgeensis* (11,1%), *E. granulosa* (8,2%), *E. parva* (6,7%), *E. ahsata* (5,6%), *E. faurei* (4,2%), *E. intricata* (1,6%), *E. pallida* (1,6%) og *E. crandallis* (1,4%). Að meðaltali fundust 7.4 tegundir (5-9) í hverju sýni. Ellefta tegundin sem vitað er um í sauðfé í Evrópu, *E. marsica*, fannst ekki í Fossárdalshjörðinni en hún var staðfest um svipað leyti í lömbum á Gerpissvæðinu. Árstíðasveiflur komu fram einkum hjá tegundunum *E. ovinoidalis*, *E. bakuensis*, *E. weybridgeensis* og *E. granulosa*. Hnísasótt virðist sjaldgæf að vori og sumri á bænum, sennilegast vegna þess hversu fé er sleppt fljótt eftir burð á beitiland með litlu hníslasmiti. Á hinn bóginn fá lömb í

sig hnísla strax og þau koma af fjalli niður á láglandi á haustin. Tíu lömb sem athuguð voru sérstaklega voru komin með hníslasótt eftir um þrjár vikur og einni til tveimur vikum síðar náði hníslafjöldi í saur hámarki. Nokkrir lægri hníslatoppar greindust að vetrinum. Hníslafjöldi í saur virtist ekki hækka að neinu marki í kring um sauðburð og reyndist vera lítil en stöðugur frá miðjum vetri allt fram á haust þegar 17 mánaða aldri var náð. Sömu hníslategundir fundust í gemsunum og sex vikna gömlum lömbum þeirra í byrjun júlí og voru tegundirnar í svipuðum hlutföllum, ef undan er skilin *E. faurei* sem einungis fannst í lömbunum.

## INTRODUCTION

Oocysts of *Eimeria* spp. are normally present in small numbers in the faeces of healthy sheep of all ages. Disease outbreaks, referred to as coccidiosis, occur when susceptible animals are exposed to infection with pathogenic species. The severity of signs depends on the size of the infecting dose and the susceptibility of the host (Gregory 1989, Rommel 2000). The major cause of ovine coccidiosis is *E. ovinoidalis* McDougald. *E. ahsata* Honess, *E. bakuensis* Musaev, *E. crandallis* Honess and *E. parva* Kótlán, Móscy & Vajda have also been suggested to be associated with this disease (Catchpole et al. 1976, Catchpole & Gregory 1985, Gregory 1989, Gregory et al. 1989, Mahrt & Sherrick 1965, Rommel 2000, Venkataratnam & Hafeez 1985). The pathogenic influence of the remaining species, *E. faurei* (Moussu & Marotel) Martin, *E. granulosa* Christensen, *E. intricata* Spiegl, *E. pallida* Christensen, *E. marsica* Restani and *E. weybridgeensis* Norton, Joyner & Catchpole, is either mild or unknown (Rommel 2000).

Morphological studies of sporulated oocysts of a few lambs in Iceland (Reginsson & Richter 1997) confirmed the occurrence of 9 of the 11 *Eimeria* species that are usually reported from sheep in Western and Central Europe (Eckert et al. 1995, Rommel 2000).

Approximately three weeks after the return of four-month-old lambs on the Fossárdalur sheep farm in Eastern Iceland from the mountainous summer rangelands to the lowland severe coccidiosis usually starts to affect lambs on the farm. The diarrhoea lasts for one or more weeks and sometimes ends fatally (Skirnisson & Hansson 2006). However, at the same time, older sheep on the farm do not develop clinical coccidiosis, indicating that adult sheep have at least some immunity to the disease.

The species composition and the importance of each species of *Eimeria* on the farm were unknown. Therefore, it was of interest to evaluate their abundance in the replacement ewe lambs (REL). Here, the results on the *Eimeria* species composition are presented, not only during the coccidiosis phase of the replacement ewe lambs in autumn but also in other seasons of the year. Furthermore, comparisons were made on the *Eimeria* species composition in the young ewes and their approximately six-week-old lambs in early July.

## MATERIALS AND METHODS

### *The lambs and sampling*

In the second half of September every year, soon after the return of sheep to the lowland, replacement ewe lambs are weaned from their mothers on the Fossárdalur sheep farm (64°45'13.47 N, 14°31'03.81 W) and kept grazing in a flock on home pastures until the middle of October when hay feeding starts in a sheep house. In late May the one-year-old ewes usually give birth to one lamb and are released within a few days to extended home pastures where they graze for approximately one month before being released on extensive summer rangelands in the adjacent mountains. The young ewes that give birth to twins are kept longer on the farm, usually for an additional two to three weeks, and fed with concentrates supplementary to hay to increase their milk production.

The farmers collected rectal faecal samples (wearing disposable gloves) from ten replacement ewe lambs (REL 1-10). This was done at four-day intervals from late September to early November 2002 (13 occasions in 50 days), again in late November and after that at the beginning of each month until July 2003.

The last sampling was performed in early October, 2003, after the return of the sheep from the summer rangelands. Thus in total sampling of faeces occurred on 23 occasions during 54 weeks. Actually, the "June" collection occurred in late May, when the REL flock was still kept indoors. In early July, when releasing the young ewes (REL) to the summer rangelands in the adjacent mountains, each ewe with an approximately six-week-old lamb (except REL 1 that had born twins), faecal samples were also collected from six of their six-week-old lambs.

Each sample was put in an 80 ml plastic container with a screw cap. The samples were sent overnight to the laboratory at Keldur where examination was usually carried out on the next day or two.

#### *Laboratory methods and identification*

To obtain quantitative estimates of the total *Eimeria* spp. oocyst excretion samples were examined with the McMaster method (Anonymous 1986). The minimum number of detectable oocysts per gram (opg) of faeces was 50. Sporulation of oocysts followed at room temperature for 10 days after submerging the faecal sample in 3%  $K_2Cr_2O_7$ , following which samples were stored at 4°C. The sporulation rate was not measured.

To evaluate the species composition and the seasonal occurrence of *Eimeria* spp. three of the four-month-old replacement ewe lambs (REL 1, 6 and 7) were further examined until July of the following year. During September to November 2002 two faecal samples were analysed each month and after that one sample per month. On average 87 oocysts (range 50-150) were identified to species level from each lamb each month, in total 860 oocysts from REL 1, 1070 from REL 6 and 920 from REL 7.

To compare the mother-offspring infections the species composition was examined in early July 2003 in five of the 14-15-month-old mothers (REL 1, 4-6 and 8, altogether 250 oocysts, 50 from each sheep) and their approximately six-week-old lambs (altogether 300 oocysts

50 from four individuals and 100 oocysts from one lamb).

After sedimentation of faeces in a centrifugation tube at 800 g for two minutes whereby the supernatant was decanted and refilled with Parasitol (specific density 1.27 g ml<sup>-1</sup>, Meku®, DK 7171, Denmark) the sporulated oocysts were accumulated (Anonymous 1986). Morphological examination was performed at a 1250x magnification under a Leitz Laborlux K microscope. Differentiation followed according to descriptions given by Barutzki & Gothe (1988), Eckert et al. (1995), Gregory et al. (1987), Joyner et al. (1966), Norton et al. (1974), O'Callaghan et al. (1987), Rommel (2000) and Shah (1963).

## RESULTS

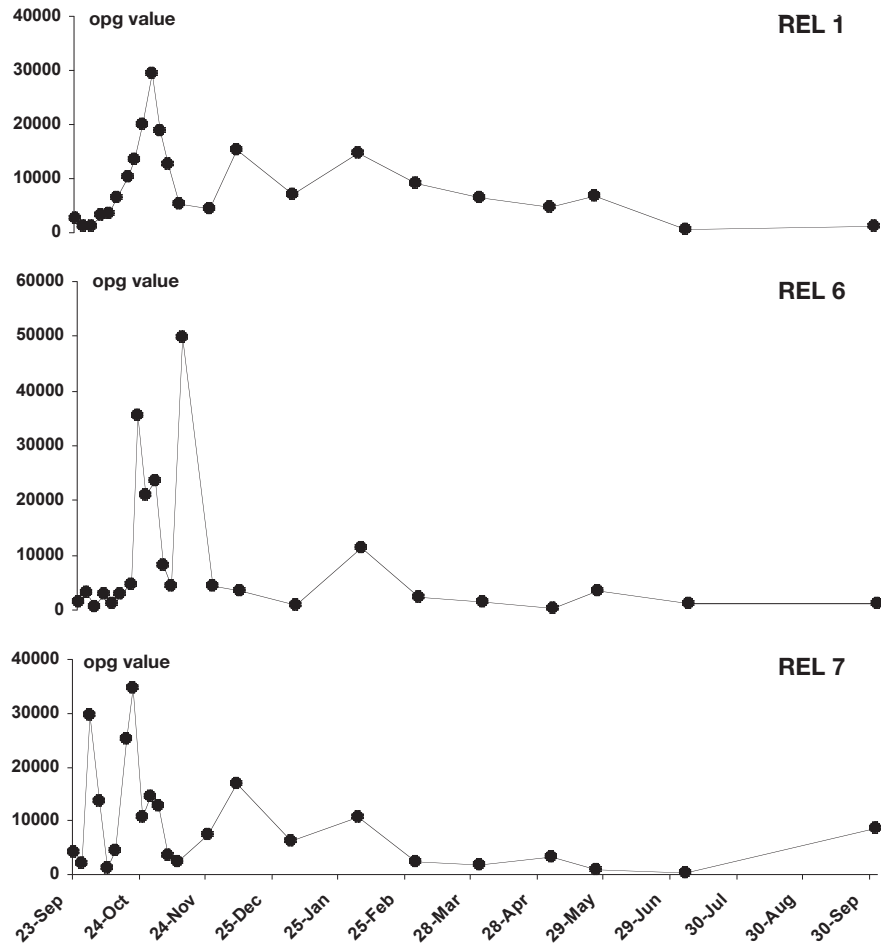
### *Total oocyst counts*

Total excretion of eimerian oocysts was low in ten lambs on returning from the summer rangelands in late September but within two to three weeks all had come down with coccidiosis with diarrhoea. Subsequently opg values peaked. These results have already been published (Skirnisson & Hansson 2006). During winter one or more smaller opg peaks were noticed. However, no marked periparturient increase in the total oocyst output was observed in late May when the young ewes were about to give birth to their lambs.

Focusing on three of these lambs, one (REL 1) or two (REL 6, REL 7) prominent opg peaks were observed during autumn (Figure 1). Maximum opg values reached 29,400, 49,800 and 34,800, respectively in REL 1, 6 and 7. In February smaller peaks were noticed (opg values 14,700, 11,400 and 10,800) but after that total oocyst counts remained low until summer with minimum opg values of only 300 to 600. In October 2003, slightly increased oocyst excretion was exclusively noted in REL 7.

### *Species composition*

In total 10 *Eimeria* spp. were identified in the flock (Figure 2, Table 1). The predominant species was *E. ovinoidalis* (the average relative abundance was 40.7%), followed by



**Figure 1.** Total oocyst excretion (oocyst numbers per gram faeces, opg) of *Eimeria* spp. in three replacement ewe lambs (REL)/young ewes on 23 occasions from September 2002 to October, 2003 at the Fossárdalur sheep farm.

**Table 1.** Relative abundance (%) of *Eimeria* spp. identified once a month from September 2002 to July 2003 from faeces of three replacement ewe lambs/young ewes at the Fossárdalur sheep farm. The overall average value is shown in the last column.

	REL 1 (%)	REL 6 (%)	REL 7 (%)	Average (%)
<i>E. crandallis</i>	1.64	1.00	1.55	1.39
<i>E. pallida</i>	2.36	1.45	1.00	1.61
<i>E. intricata</i>	0.18	2.18	2.55	1.64
<i>E. faurei</i>	6.18	2.27	4.00	4.15
<i>E. ahsata</i>	2.36	8.45	5.91	5.58
<i>E. parva</i>	7.45	6.18	6.55	6.73
<i>E. granulosa</i>	8.27	12.36	4.09	8.24
<i>E. weybridgeensis</i>	11.91	10.27	11.00	11.06
<i>E. bakuensis</i>	16.27	18.82	21.73	18.94
<i>E. ovinoidalis</i>	43.36	37.00	41.64	40.67

*E. bakuensis* (18.9%) and *E. weybridgensis* (11.1%). Four species had a relative abundance of 4.2% to 8.2% (*E. faurei*, *E. ahsata*, *E. parva* and *E. granulosa*), and the remaining species (*E. crandallis*, *E. pallida* and *E. intricata*) varied between 1 and 2%.

Usually, the relative abundance of *Eimeria* spp. showed similar values in REL 1, 6 and 7, respectively (Table 1). Most deviations (5.5-8.3%) were noted for *E. granulosa*, *E. ovinoidalis*, *E. bakuensis* and *E. ahsata*. Exclusively *E. crandallis*, *E. granulosa* and *E. weybridgensis* showed some periparturient increase (Figure 2).

#### Proportion of pathogenic eimerids

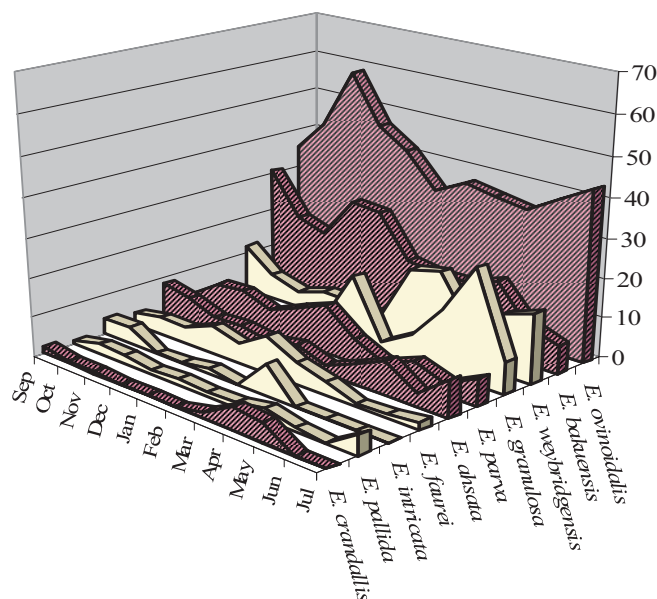
A total of 73.3% of the oocysts are known sheep pathogens (*E. ovinoidalis*) or species that are suggested to be associated with this disease (*E. ahsata*, *E. bakuensis*, *E. crandallis* and *E. parva*). The most pathogenic species, *E. ovinoidalis*, clearly predominated (Table 1).

#### Number of *Eimeria* spp. per sample

All samples were positive for *Eimeria* spp. oocysts, confirming the 100% prevalence of infection. Moreover, multiple infections were confirmed in every sample. On average 7.4 species were identified per sample. The highest species number in a sample was nine (occurring in 18.2% of the samples) and the lowest species number identified was five (9.1%). A similar proportion of samples yielded seven (30.3%) and eight (33.3%) *Eimeria* spp. respectively.

#### Seasonal variation of *Eimeria* spp.

*E. ovinoidalis* was by far the most common



**Figure 2.** Relative abundance (%) of ten *Eimeria* spp. in faeces of three replacement ewe lambs (REL)/young ewes (average values) during their lowland stay from late September 2002 to early July 2003 at the Fossárdalur sheep farm. Species that are known to cause coccidiosis (*E. ovinoidalis*) or have been suggested to be associated with this disease (*E. bakuensis*, *E. parva*, *E. ahsata* and *E. crandallis*) are presented with hatched graphics.

eimerid in the lambs in all seasons and marked seasonal abundance was observed (Figure 2). The abundance peaked in autumn and early winter, concomitant with the high oocyst excretion during the coccidiosis phase (Figure 1).

The relative abundance of *E. bakuensis* gradually dropped from early winter to July. An opposite tendency was noted for *E. weybridgensis* and *E. granulosa*, whereas *E. parva* and *E. ahsata* appeared to be similarly abundant throughout the study period (Figure 2). The remaining species were rarely identified and no clear changes in seasonal abundance were noted.

#### Mother-offspring comparisons

A total of nine *Eimeria* spp. were found in the young ewes and their offspring in early July (Table 2). Total oocyst excretion was low, not only in the young mothers (opg value on average 1,620, range 600-3,000) but also in their

**Table 2.** Relative abundance (%) of *Eimeria* spp. of five 14-15-month-old ewes and their six-week-old offspring on 6 July, 2003 on the Fossárdalur sheep farm.

	Mothers (%)	Offsprings (%)
<i>E. crandallis</i>	1.5	2.0
<i>E. pallida</i>	6.8	3.0
<i>E. intricada</i>	0	0
<i>E. faurei</i>	0	10.2
<i>E. ahsata</i>	6.4	2.2
<i>E. parva</i>	13.3	11.8
<i>E. granulosa</i>	2.2	0
<i>E. weybridgensis</i>	19.3	14.5
<i>E. bakuensis</i>	8.8	4.3
<i>E. ovinoidalis</i>	41.7	52.0

single offspring (opg value on average 3,675, range 2,400-4,800). However, the only twin lamb examined had an opg value of 47,600.

Usually a similar abundance was observed for the respective eimerids in the ewes and their offspring, with the exception of *E. faurei* and *E. ovinoidalis*, which were both more abundant in the lambs (Table 2).

The proportion of the species that has been associated with coccidiosis (*E. ahsata*, *E. bakuensis*, *E. crandallis*, *E. ovinoidalis* and *E. parva*) was almost the same in the young ewes (71.7%) as in their lambs (72.3%).

## DISCUSSION

Previously nine *Eimeria* spp. had been identified from lambs in Iceland (Reginsson & Richter 1997). In the present study all these species were identified again, but in addition the previously questionable *E. granulosa*. *E. marsica*, the eleventh species known to infect sheep in Central and Western Europe (Eckert et al. 1995, Rommel 2000) was not detected on the Fossárdalur farm but was commonly found in lambs that grazed during the summer of 2003 in Sandvík, 55 km northeast of the Fossárdalur sheep farm. Therefore, all sheep eimerids reported on the continent of Europe have also been found in Iceland.

*E. ovinoidalis* clearly predominated in the Fossárdalur farm in all seasons. Similar findings have been reported in other studies on

ovine coccidians. For example *E. ovinoidalis* was the predominant species in surveys that have been carried out recently in Germany (Gauly et al. 2001, Reeg et al. 2005), Gahna (Agyei 2003) and Brazil (Bresciani et al. 2002). In Turkey, however, *E. crandallis* (Kaya 2004) and *E. parva* (Gul & Deger 2002) were the dominating species, but in both these surveys *E. ovinoidalis* was the second most abundant species.

In the study of Reginsson & Richter (1997) on one-month-old lambs in Iceland *E. ovinoidalis* was also the second most predominant eimerid but the species that was most commonly found was *E. crandallis*. This is comparable to the already mentioned results of Kaya (2004). Other studies where *E. crandallis* was found to dominate were carried out by Amarante & Barbosa (1992) and Joyner et al. (1966), among others. Exactly the opposite was observed on the Fossárdalur farm where *E. crandallis* appeared to be the rarest eimerid in the flock (Table 1).

*E. bakuensis* and *E. weybridgensis* were very common on the Fossárdalur farm; *E. bakuensis* was more abundant during autumn and winter than in spring and summer, but *E. weybridgensis* showed clearly increasing abundance from mid-winter until summer. Both species are usually listed among the most common ovine eimerids in other studies (Agyei 2003, Bresciani et al. 2002, Gauly et al. 2001, Gul & Deger 2002, Kaya 2004, Reeg et al. 2005, Rommel 2000).

Ewes act as a reservoir of eimerid infection and shed low numbers of oocysts, particularly around the periparturient period (Rommel 2000). In the present study no increased oocyst excretion was noticed during the periparturient period in May. Exclusively, the usually rarely occurring *E. crandallis*, *E. granulosa* and *E. weybridgensis* seemed to become relatively more abundant prior to the lambing season.

In the present study comparison was made of the *Eimeria* spp. composition in 14-15-



month-old ewes (REL) and their six-week-old offspring in early July. Usually, quite similar values were noted for the nine *Eimeria* spp. identified in the two groups. The main deviation was observed in the case of *E. faurei*, which was absent in the mothers but was regularly identified in their lambs (every tenth oocyst). *E. intricata* was totally absent in both the ewes and their lambs this time (Table 2).

Low opg values in single lambs in early July were assumed to be mainly influenced by their quite early removal from the oocyst contaminated areas around the farm. The relocation occurred when the lambs were approximately one week old and the young ewes and their lambs were transferred to extended, sparsely grazed, home fields. There, they grazed for more than a month prior to the sampling date in early July. Interestingly, the only twin lamb examined this time had a considerably (13x) higher opg value than the single lambs, probably due to the fact that twin lambs are usually kept approximately two weeks longer on the farm than single lambs. These findings support the assumption that the commonly observed spring and summer coccidiosis in Icelandic sheep (Richter 1974, Richter & Eydal 1985, Richter et al. 1983) can be more or less avoided by an early removal of ewes and lambs from the infection source. But, as a consequence, young lambs on the farm do not have much opportunity to develop resistance to coccidiosis and are therefore highly susceptible to coccidiosis when they return to lowland from the summer rangelands in autumn when they are approximately four month old (Richter & Eydal 1985, Skirnisson & Hansson 2006).

Little is known about the pathogenicity of ovine eimerids in Iceland. However, it is pointed out that *E. ovinoidalis*, the best known cause of ovine coccidiosis, together with the other eimerids that have been suggested to be associated with the disease (*E. ahsata*, *E. bakuensis*, *E. crandallis* and *E. parva*) (Catchpole et al. 1976, Catchpole & Gregory 1985, Gregory 1989, Gregory et al. 1989, Mahrt & Sherrick 1965, Rommel 2000, Venkataratnam & Hafeez

1985), predominated on the Fossárdalur sheep farm in all seasons of the year.

#### ACKNOWLEDGEMENTS

The farmers Guðný Gréta Eyjólfsdóttir and Hafliði Sævarsson are thanked for collecting the samples and for delivering information about farming practices and clinical symptoms of the lambs. Thanks are also due to Berglind Guðmundsdóttir for work on the preparation of the samples and species identifications and to Hákon Hansson for initiating the project and valuable discussions. The Icelandic Agricultural Production Fund partly supported the studies. Sigurður H. Richter and an anonymous referee provided for valuable comments on the manuscript.

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Manuscript received 5 May 2007

Accepted 27 September 2007