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# EVALUATION OF SALTING AS A HAY PRESERVATIVE AGAINST FARMER'S LUNG DISEASE AGENTS

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**Abstract:** Salting is a traditional, empirical practice used commonly in dairy farming regions to prevent moulding and heating in hay. Our aim was to evaluate the effect of salting hay on the proliferation of microorganisms, particularly thermophilic actinomycetes and moulds involved in farmer's lung disease. Fifty-one pairs of salted and unsalted hay bales from 14 farms were produced during the haymaking season between March and July. Both the salted and the unsalted bales came from the same field, and were packed and stored under identical conditions. Sampling was performed by microbiological analysis including 6 culture media during the winter following salting (January-February). The use of salt did not significantly decrease the amount of *Saccharopolyspora rectivirgula*, the actinomycetes most commonly involved in farmer's lung disease, or that of *Absidia corymbifera*, *Eurotium amstelodami* and *Wallemia sebi*, three moulds responsible for farmer's lung disease in eastern France. Our results are important in that they can inform farmers and dispel the false sense of security induced by salting, which is reinforced by the misconception that palatable hay is healthy hay.

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## INTRODUCTION

Farmer's lung disease (FLD) is a pulmonary affection caused by an allergic reaction to the inhalation of microorganisms [1, 13]. This pathology has been shown to be a problem in dairy farming regions [3, 5]. The microbial agent classically reported to induce FLD is a thermophilic actinomycete: *Saccharopolyspora rectivirgula* (*Micropolyspora faeni*). In eastern France, recent studies showed three moulds to be the probable main causes of FLD: *Absidia corymbifera*, *Eurotium amstelodami* and *Wallemia sebi* [15]. The amount of these microorganisms is higher in hay that contains soil or that has been harvested wet due to bad climatic conditions [8, 16]. The use of hay-drying systems decreases the quantity of microorganisms in hay [4], but the cost of purchasing and operating such systems is often prohibitive. An alternative could be the use of commercial chemical additives, such as propionic acid, or inoculation of bacteria such as *Pediococcus pentosaceus* or *Lactobacillus buchneri*. Propionic acid, effective in high rates of application, could be corrosive and support the growth of some

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**Table 1.** Storage conditions and salting procedures used in the 14 dairy farms.

| Farm number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Altitude (m) | 850 | 750 | 630 | 840 | 900 | 900 | 600 | 240 | 220 | | 940 | 730 | 1,000 | 900 | 900 |
| Grassland surface (ha)[c] | 73 | 70 | 140 | 75 | 90 | 100 | 70 | 60 | 92 | | 73 | 230 | 58 | 90 | 104 |
| Livestock (number of cows) | 90 | 80 | 150 | 110 | 35 | 50 | 90 | 80 | 40 | | 90 | 50 | 15 | 150 | 95 |
| Haymaking technique[a] | B | R | R | R | R | R | R | R | R | C | R | R | C | R | R |
| Salting procedure[b] | G | G | G | G | G | G | G | B | B | B | B | B | G | G | B |
| Amount of salt (kg/ha)[c] | 25 | 40 | 40 | 25 | 40 | 50 | 50 | 120 | 80 | 80 | 40 | 80 | 15 | 50 | 80 |
| Number of paired hay[d] | 3 | 4 | 7 | 4 | 4 | 3 | 2 | 2 | 4 | 2 | 2 | 4 | 2 | 6 | 2 |

[a]in bulk (B), large high-density round bales (R), medium density cubic bales (C); [b]during the gathering process (G) or before baling (B); [c]ha: hectare; [d]a pair of bales was one salted hay paired to one unsalted hay.

microorganisms less susceptible to this acid [9, 11, 14]. *Pediococcus pentosaceus* failed to prevent deterioration in hay and induced a significant inflammatory response in the lung in mice [6, 7]. *Lactobacillus buchneri* seems to be effective in silage [12]. For a long time, farmers have empirically applied salt to hay stored in the barn in order to prevent mould and heating. The advantage of this technique is that it is well accepted by farmers and increases the palatability of the hay. To our knowledge, this technique has never been evaluated in the field. Our aim was to evaluate the effects of salting the hay on the proliferation of microorganisms which are responsible for FLD, particularly moulds and actinomycetes.

## METHODS

**Selection and sampling of hay bales.** Fourteen dairy farms were randomly selected among all dairy farms in the Doubs region in France. These farms, located at an altitude of 220 to 1,000 metres, were described in Table 1. Hay was packed in bulk, either large high-density round bales (200 kg) or medium density cubic bales (40 kg), and stored in closed barns. Six of the farmers usually salted their hay and 8 did not. Fifty-one pairs of hay bales were produced from these 14 farms during the haymaking season between March and July. Each pair of bales was composed of 1 salted hay and 1 unsalted control hay, both of which came from the centre of the same field. The procedure was as follows: 3 salted bales were made, and while still in the field, the middle one was labelled as salted with red ribbon so as to avoid partially salted bales. After salting, the machine was removed and 3 more bales were made. The middle bale was marked in blue as non-salted on the spot, so as to avoid contamination by salt.

The number of pairs of bales per farm varied from 2–7 in order to take the various harvesting conditions into account for each farm (field exposure, soil, and meteorological conditions). Fine white sea salt was used (Salin du midi, Aigues Mortes, France). Two methods of salting were accepted: 1) before baling: salt was applied on the grass 3 days before it was baled. In this case, the amount of salt recommended by the manufacturer was 80 kg per hectare (kg/ha); 2) during the gathering process: salt was applied directly on the hay before it was stored with the amount of salt recommended by the manufacturer as 40 kg/ha. Bales in each group were stored under identical conditions: among other hays from the farm, neither on the ground nor at the edge of the barn, in closely stacked piles for round and cubic bales, or in boxes with separate, labelled layers for bulk. All barns were separate from the cowshed. Farms with barns located above the cowshed were excluded from the study to avoid added humidity and increased temperature in hay due to cows' breathing and digestion. Sampling was performed during the winter following hay treatment, between January and March. The amounts of microorganisms in hay were highest at this time [16]. Two samples per bale were collected in sterile bags. The sampling method was standardised: for round bales, samples were taken from 20 cm below top of the bale, ¼ of the way in, and for bulk, samples were taken 40 cm below the top of the stack. Data related to salting methods and the amount of salt actually used was collected (Tab. 1) by means of a standardised questionnaire which included crossed questions concerning the method of salting (before baling or during the gathering process), the amount of salt used (expressed by the farmer and noted by the investigator with respect to the amount of salt remaining), the number of salted and unsalted bales, the date of harvest and meteorological conditions during harvest. This questionnaire was developed with an agricultural technician and administered by the head author, accustomed to agricultural practices.

**Microbiological analysis.** Each sample was frozen at -18°C overnight to kill mites. Samples were weighed, rinsed with 20 ml of sterile distilled water, shaken vigorously for 1 min, and cultured on petri dishes. Culture media included the following: Dichloran-Glycerol (Oxoid, Unipath, Basingstoke, UK) with 0.5% chloramphenicol (Merck, Darmstadt, Germany) at 30ºC for mesophilic mould isolation, 3% malt-agar (Oxoid, Unipath, Basingstoke, UK) with 10% salt and 0.5% chloramphenicol at 20ºC for osmophilic fungal species, actinomycete isolation agar Bacto medium (Difco, Detroit, MI, USA) at 30ºC for mesophilic actinomycetes

and at 52ºC for thermophilic actinomycetes, R8 medium [2] at 52ºC and Muller-Hinton (Becton Dickinson®, Cockeysville, MD, USA) at 37ºC for thermotolerant aerobic bacteria. Results were expressed in colony-forming units per gram of hay (cfu/g). A dilution of the sample was carried out when the colony count per dish was greater than 50. Two samples were analysed for each bale. The mean value of the number of colonies obtained from both samples was taken into account for analysis.

**Statistical analysis.** The concentration of microorganisms in salted hay was compared to the concentration in unsalted hay. Environmental data are known not to show normal distribution, so the Wilcoxon signed ranks test for paired samples was used to compare matched series. We used Statview 5.0 software (SAS, North Carolina, USA). Probability values less than 0.05 were considered significant.

## RESULTS

The total concentrations of microorganisms were $1.7 \times 10^7$ cfu.g$^{-1}$ in salted hay and $1.5 \times 10^7$ cfu/g in unsalted hay for the 51 pairs of bales. The same species were found, but in different proportions: 77% of fungi, 5% of actinomycetes and 18% of thermotolerant bacteria for salted hay, *vs* 67% of fungi, 11% of actinomycetes and 22% of thermotolerant bacteria for unsalted hay.

With regard to the total fungi count, the species most present were: *W. sebi* (64.5%), *E. amstelodami* (22.5%), *Eurotium umbrosum* (6%), and *A. corymbifera* (1.8%). More than 15 others species were present at less than 1%, including: *Aspergillus* spp., *Fusarium* spp., *Alternaria* spp., *Cladosporium* spp., *Penicillium* spp., *Blastobotrys nivea*, and yeasts. The number of cfu per gram of hay was not significantly lower in salted hay than in unsalted hay, either for the 3 moulds involved in farmers' lung disease in Doubs (*A. corymbifera*, *E. amstelodami*, *W. sebi*) or for other fungi (Tab. 2). Similar results were found for mesophilic and thermophilic actinomycetes including *S. rectivirgula*. Bacterial flora was essentially composed of *Bacillus* spp., *Corynebacterium* spp., and Gram-positive cocci. Low amounts of enterobacteria or Gram-negative bacteria were isolated (less than 1% of total bacteria). Table 2 shows that salting did not significantly reduce the amount of bacteria in hay.

When we studied 14 pairs of bales which had been salted equal to or more than 80 kg/ha (Tab. 1), no significant differences were observed between salted and unsalted hay for concentrations of total microorganisms (p=0.64).

Salting showed no effect either for high or low contaminated bales in any of the 51 pairs of bales (Fig. 1). The concentration of total microorganisms was not lower in salted hay than in unsalted hay, either for hay salted during the gathering process (n=35, p=0.91) or for hay salted before baling (n=16, p=0.58).

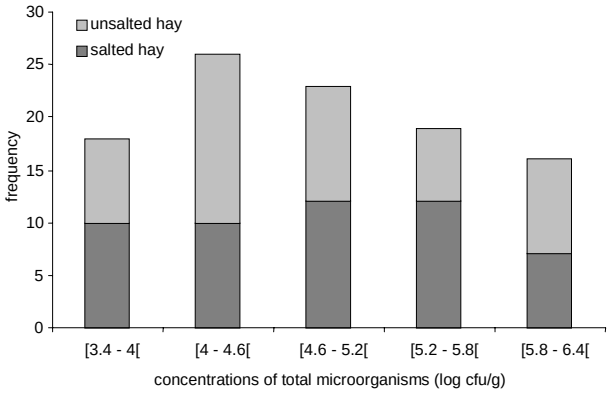


**Figure 1.** Grouped frequency distribution of concentrations for total microorganisms (log cfu/g) on salted and unsalted hay (n=102).

**Table 2.** Comparison between salted (n=51) and unsalted hay (n=51) for concentrations of microorganisms ($10^3$ cfu/g).

| | Salted hay | Unsalted hay | p-values |
|---|---|---|---|
| *Absidia corymbifera* | | | |
| mean (SD) | 5 (17.3) | 3.2 (13.2) | NS |
| median (range) | 0.3 (0–86) | 0.3 (0–92.9) | |
| *Eurotium amstelodami* | | | |
| mean (SD) | 55.4 (152.7) | 48.7 (135.2) | NS |
| median (range) | 2.4 (0–1,013) | 4 (0–800) | |
| *Wallemia sebi* | | | |
| mean (SD) | 175.2 (399) | 122.9 (313.3) | NS |
| median (range) | 4 (0–2,320) | 1.5 (0–1,651) | |
| Other fungi | | | |
| mean (SD) | 28.6 (63.5) | 23.7 (47.1) | NS |
| median (range) | 4.8 (0–402) | 4.4 (0–244) | |
| Mesophilic actinomycetes | | | |
| mean (SD) | 7.7 (25.5) | 8.5 (20.3) | NS |
| median (range) | 0.8 (0–164) | 0.8 (0–100) | |
| *Saccharopolyspora rectivirgula* | | | |
| mean (SD) | 1.3 (4.9) | 12.2[a] (84.4) | NS |
| median (range) | 0 (0–29) | 0 (0–603) | |
| Other thermophilic actinomycetes | | | |
| mean (SD) | 6.5 (19.5) | 10.7 (38.4) | NS |
| median (range) | 1.1 (0–106) | 1.4 (0–260) | |
| Thermotolerant bacteria | | | |
| mean (SD) | 62.8 (287.8) | 64.2 (245) | NS |
| median (range) | 3.3 (0–2,012) | 6.4 (0–1,500) | |

[a] This high mean was due to the existence of 1 bale which presented more than 600,000 cfu/g of *S. rectivirgula*; SD - standard deviation, NS - not significant.

## DISCUSSION

Our results did not allow to show an effect of salting on the proliferation of microorganisms involved in FLD, notably *S. rectivirgula*, *A. corymbifera*, *E. amstelodami* and *W. sebi*. The same negative results were obtained for the other fungi, mesophilic and thermophilic actinomycetes and thermotolerant bacteria.

FLD is a frequent and disabling lung disease. Because no effective treatment exists, prevention measures are all the more important. Hay handling is the main source of microorganism exposure for farmers in Doubs region, where cows are fed mainly with hay (7–20 kg/day per cow). Therefore, a decrease in the incidence of FLD necessarily implies a decrease in moulds and actinomycetes in hay. Different techniques can be used to preserve hay: hay-drying systems, but the cost is often prohibitive [4]; commercial chemical additives or inoculation of bacteria, but these more or less effective techniques are forbidden in Doubs region, where the origin and quality of cheese is controlled [6, 7, 9, 10, 11, 12, 14] as well as salting, but this technique has been studied relatively little to date.

Salting hay is common in the Doubs region. A study of 42 farms from a stratified sampling representative of agricultural practices in Doubs province showed that 30% of the farms salt their hay [17], Due to the additional cost and the variety of salting procedures and quantities used, farmers legitimately enquire about which method is most effective.

This study was conducted on site with a view to implementing the findings in the prevention of FLD. To guarantee the comparability of the 2 groups, bales of hay from the same field were packed at the same time, under the same meteorological conditions, and stored in the same conditions. Furthermore, sampling was carried out when the proliferation of microorganisms in hay is highest (January-March) [16]. There were more than 100 samples. For fungi and actinomycetes, the amounts of micro-organisms were similar in our results ($2.8 \times 10^5$ cfu/g for salted hays and $2.3 \times 10^5$ cfu/g for unsalted hays) to those found in harvests in other years in our region ($7 \times 10^4$ cfu/g [15] and $3.6 \times 10^5$ cfu/g [16]). Nevertheless, the current study relies on a single harvest year and may not reflect the different climatic conditions over the years; some have been associated with the growth of particularly high concentrations of microorganisms involved in FLD, such as *S. rectivirgula*, *A. corymbifera* and *E. amstelodami*, as well as with an increase in FLD cases [16, 18, 19]. Hence, it is not excluded that salting may have an impact when the conditions are conducive to microbial growth.

The questionnaire allowed us to verify the salting methods and the amounts of salt used. Although only 2 salting procedures were used, the variability in hay-making techniques (bulk, round bales and cubic bales) and salting loads (15–120 kg/ha) induced a variability in procedure that may diminish the power of the experiment. However, the advantage of an on-site experiment is not negligible in that it reflects the way farmers actually work and takes into account the human factor in the way techniques are applied. We tested salting effects for different procedures and salting loads: none proved to be particularly effective. Some agricultural professionals have already noticed that salt is ineffective as a preservative (see: http://muextension.missouri.edu/explore/agguides/crops/g04575.htm), and that it probably needs to be applied in large amounts to be a preservative. However, larger amounts would entail increased costs, which would be dissuasive for farmers. Furthermore, high amounts can be physiologically harmful to animals.

In our study, hay samples were frozen at -18ºC overnight to kill mites. Indeed, hay contains a large quantity of mites, which scatter fungal spore on the Petri dish, and lead to an overestimation of cfu. We observed that this freezing causes a 10% decrease in microorganisms (personal data). It is known that adding salt lowers the freezing point, and although the impact of freezing on microbial viability is usually observed in liquid media, it is not excluded that freezing may have an influence on the results, and that some microorganisms survived freezing better in salted hays than in unsalted hays. With regard to the disadvantage of mites in the assessment of contamination in microorganisms, we consider it preferable to freeze samples overnight, rather than risk overestimating microorganisms in hays.

Our study showed that the amount of fungi and actinomycetes in hay was not significantly lower in salted hay than in unsalted hay. These results argue against a traditional practice in Doubs, which is reinforced by the misconception that palatable hay is healthy hay. Our results are important in that they can inform farmers and dispel the false sense of security induced by salting. Farmers need to be informed that salting seems to be ineffective in preventing farmer's lung disease.

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