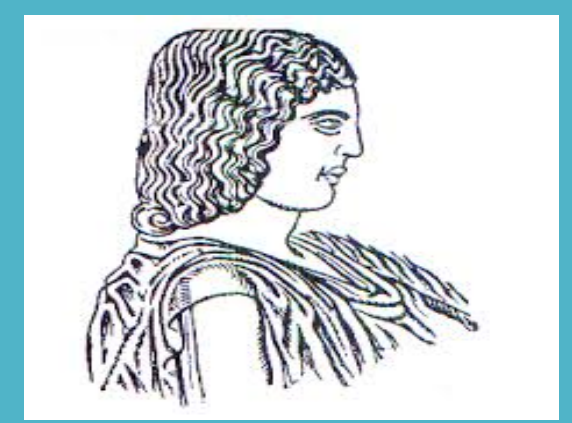




# Effect of the replacement of wheat straw by spent mushroom substrate on milk yield, composition, fatty acid profile, oxidation stability and udder health in dairy ewes during the final stage of lactation

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# What is Spent Mushroom Substrate (SMS)?

- A **by-product** of the mushroom growing process; the **exhausted residual lignocellulosic biomass** left after the harvest of mushrooms
- SMS **consisted of** straw, sawdust, wood chips and shavings, corn cobs, cottonseed hulls, livestock litter and manure, etc.
- SMS composition **varies** as an effect of the raw materials, the location, the mushroom genus and the cultivation method (~35% DM, 20% OM, 13% ash)



# How important is the environmental problem related with SMS?

- Production of mushrooms has increased worldwide by **65%** during the recent years (48.34 MTs in 2022 from 31.78 MTs in 2012)
- Approximately **5 kg of SMS** are generated per kg of fresh mushroom; disposal of SMS wastes is therefore a major problem
- In the past, discarding by **disposal, burying, or landfilling** and **incineration or burning** methods were used
- Nowadays **SMS re-utilization** is suggested in the context of sustainability, circular economy and protection of natural resources

# SMS composition

- Polysaccharides, i.e., cellulose and hemicellulose
- Proteins (as extra-cellular enzymes)
- Vitamins
- Trace elements, namely magnesium (Mg), zinc (Zn), calcium (Ca) and iron (Fe)
- However, SMS is abundant in lignin that impairs feed digestibility

# Can SMS be used in dairy ruminants' diets and why?

- The high cost of animal feed poses a significant challenge to livestock production.
- Utilization of agro-industrial by-products as alternative feed resources is a practical approach in low-input systems that improves sustainability of livestock production
- SMS has already been evaluated as an ingredient in cattle diets as raw, just dried and milled or after fermentation with bacterial inoculation with promising results

## Objective of the study

- **Scarce data** exist on the effects of using SMS as a dietary ingredient in lactating animals and especially small ruminants
- As a result, the aim of the present study was to investigate the effects of the replacement of wheat straw by spent mushroom substrate on milk production, composition, oxidative stability and udder health in dairy ewes during the final stage of lactation.

## Material and Methods (1) – Animals and Groups

- 30 Karagouniko dairy ewes → 2<sup>nd</sup> parity and same stage of lactation ~ 145 days after parturition)
- Assigned to 3 groups → Control (C) that was fed with a diet consisting of concentrates, alfalfa hay and wheat straw at a ratio of 1:0.6:0.4, whereas in the other two groups wheat straw was replaced by SMS at 50% or 100% (SMS1 and SMS2, respectively). The duration of the experiment was 4 weeks.
- Commercial SMS deriving from *Pleurotus ostreatus* industrial-scale cultivation (Manitus S.A., Athens, Greece)

## Material and Methods (2) – Animals and Groups

- Each ewe group was housed in an individual pen, which was divided into an outdoor and indoor area and had the same covered area (3 m<sup>2</sup>/ewe), similar orientation, and was equipped with 10 individual troughs for feeding
- At the beginning of the experiment, all animals were provided with concentrates, alfalfa hay and wheat straw at a ratio of 1:0.6:0.4 for a week, serving as an adaptation period

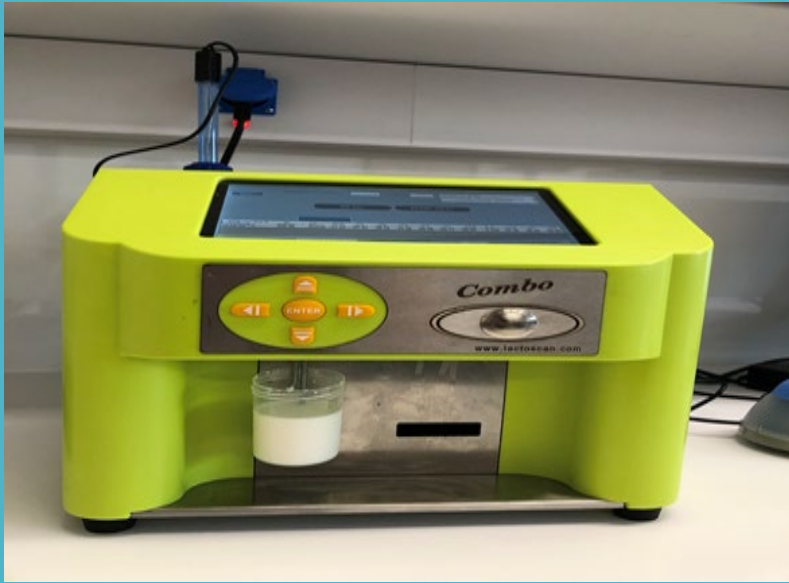
|                                    |                     |                    |                    |            |
|------------------------------------|---------------------|--------------------|--------------------|------------|
| <i>Ingredients (%)</i>             |                     |                    |                    |            |
| Corn                               | 23.4                |                    |                    |            |
| Wheat                              | 17.5                |                    |                    |            |
| Barley                             | 17.5                |                    |                    |            |
| Soybean Meal (44%)                 | 18.25               |                    |                    |            |
| Sunflower Meal (28%)               | 5.0                 |                    |                    |            |
| Wheat Bran                         | 15.0                |                    |                    |            |
| Sodium Chloride (NaCl)             | 1.0                 |                    |                    |            |
| Limestone                          | 1.85                |                    |                    |            |
| Monocalcium Phosphate              | 0.4                 |                    |                    |            |
| Vitamins and Trace elements Premix | 0.1                 |                    |                    |            |
| <i>Analysis</i>                    | <i>Concentrates</i> | <i>Alfalfa hay</i> | <i>Wheat straw</i> | <i>SMS</i> |
| Dry Matter (%)                     | 86.0                | 93.5               | 95.2               | 94.0       |
| Crude protein (%)                  | 17.0                | 10.2               | 3.4                | 4.7        |
| Crude Fiber (%)                    | 6.0                 | 34.2               | 51.7               | 26.7       |
| Ash (%)                            | 6.5                 | 7.4                | 7.2                | 18.4       |
| Fat (%)                            | 2.1                 | 2.3                | 1.0                | 1.4        |

## Material and Methods (3) – Assessed parameters during milking

- Milking of ewes was carried out twice daily, at 6:00 a.m. and 18:00 p.m., in a 12-stall milking parlor (Westfalia, Germany)
- Milk yield was determined on day 1 prior to, and on days 7, 14, 21 and 28 after, SMS addition
- Individual milk samples were also collected for the assessment of lactose, protein, fat, total solids-not-fat content, pH and somatic cell count (Lactoscan COMBO Milk Cell Analyser)

## Material and Methods (4) – Assessed parameters during milking

- Milk oxidative stability was determined by measuring malondialdehyde concentration (ng/mL)
- Milk fatty acids composition was analysed using gas chromatography Shimadzu GC-17 with AOC-20s Auto Sampler (day 28)
- Milk samples were also collected (day 28) for isolation of milk somatic cells and classification to polymorphonuclear granulocytes (P), monocytes/macrophages (M) and lymphocytes (L)



# Statistical analysis

- A repeated measures analysis of variance was applied to data for milk yield, protein, fat, lactose, total solids-non-fat, pH, somatic cell count, and MDA values with the MIXED procedure of SAS software (SAS/STAT, 2011)
- Data for immune cell profile and fatty acid profile were analyzed with the dietary treatment as fixed effect.
- The linear dose responses to dietary SMS were tested with orthogonal polynomials with the CONTRAST procedure.
- Classification of milk samples according to the dietary treatment with SMS was evaluated by a discriminant analysis of the fatty acid profile
- Differences were tested at 0.05 significance level by Bonferroni test and results are presented as LS Means  $\pm$  SEM

## Results (1)

- Mean daily feed intake was not different among the experimental groups (1.76 vs. 1.78 vs.  $1.79 \pm 0.05$  kg for CON, SMS1 and SMS2 group, respectively).
- A similar trend was also observed for daily DM intake (1.59 vs. 1.60 vs.  $1.60 \pm 0.05$  kg), CP intake (0.216 vs. 0.220 vs.  $0.223 \pm 0.006$  kg) and ME (15.14 vs. 14.99 vs.  $14.70 \pm 0.42$  MJ) for CON, SMS1 and SMS2 group, respectively ( $p > 0.05$ ).

# Results (2)

| Parameter          | Sampling Day | CON  | SMS1 | SMS2 | SEM  | p-Value | P-Linear |
|--------------------|--------------|------|------|------|------|---------|----------|
| Milk yield (mL/kg) | 0            | 620  | 625  | 660  | 69   | NS      | NS       |
|                    | 7            | 840  | 875  | 875  | 105  | NS      | NS       |
|                    | 14           | 825  | 795  | 815  | 102  | NS      | NS       |
|                    | 21           | 795  | 870  | 920  | 98   | NS      | NS       |
|                    | 28           | 835  | 825  | 845  | 104  | NS      | NS       |
|                    | p-value      | NS   | NS   | NS   |      |         |          |
| Fat (%)            | 0            | 5.49 | 5.37 | 5.39 | 0.38 | NS      | NS       |
|                    | 7            | 5.08 | 5.36 | 5.43 | 0.35 | NS      | NS       |
|                    | 14           | 6.29 | 6.18 | 6    | 0.25 | NS      | NS       |
|                    | 21           | 5.96 | 5.9  | 5.47 | 0.37 | NS      | NS       |
|                    | 28           | 5.7  | 5.83 | 5.75 | 0.23 | NS      | NS       |
|                    | p-value      | NS   | NS   | NS   |      |         |          |
| Protein (%)        | 0            | 4.7  | 4.92 | 4.82 | 0.09 | NS      | NS       |
|                    | 7            | 4.64 | 4.62 | 4.49 | 0.06 | NS      | NS       |
|                    | 14           | 4.67 | 4.6  | 4.57 | 0.11 | NS      | NS       |
|                    | 21           | 4.74 | 4.71 | 4.69 | 0.06 | NS      | NS       |
|                    | 28           | 5.04 | 4.93 | 4.76 | 0.2  | NS      | NS       |
|                    | p-value      | NS   | NS   | NS   |      |         |          |

# Results (3)

| Parameter                | Sampling Day | CON    | SMS1   | SMS2   | SEM  | p-Value | P-Linear |
|--------------------------|--------------|--------|--------|--------|------|---------|----------|
| Lactose (%)              | 0            | 4.45   | 4.66   | 4.55   | 0.08 | NS      | NS       |
|                          | 7            | 4.39   | 4.37   | 4.25   | 0.06 | NS      | NS       |
|                          | 14           | 4.41   | 4.35   | 4.32   | 0.1  | NS      | NS       |
|                          | 21           | 4.48   | 4.46   | 4.44   | 0.06 | NS      | NS       |
|                          | 28           | 4.76   | 4.66   | 4.51   | 0.19 | NS      | NS       |
|                          | p-value      | NS     | NS     | NS     |      |         |          |
| Total solids-not-fat (%) | 0            | 10.04  | 10.37  | 10.14  | 0.16 | NS      | NS       |
|                          | 7            | 9.76   | 9.73   | 9.46   | 0.13 | NS      | NS       |
|                          | 14           | 9.82   | 9.68   | 9.62   | 0.22 | NS      | NS       |
|                          | 21           | 9.97   | 9.92   | 9.88   | 0.13 | NS      | NS       |
|                          | 28           | 10.6   | 10.37  | 10.04  | 0.42 | NS      | NS       |
|                          | p-value      | NS     | NS     | NS     |      |         |          |
| pH                       | 0            | 6.67 A | 6.64 A | 6.68 A | 0.04 | NS      | NS       |
|                          | 7            | 6.66 A | 6.68 A | 6.72 A | 0.03 | NS      | NS       |
|                          | 14           | 6.6 A  | 6.57 A | 6.65 A | 0.06 | NS      | NS       |
|                          | 21           | 6.62 A | 6.49 A | 6.59 A | 0.04 | NS      | NS       |
|                          | 28           | 7.05 B | 6.94 B | 7.06 B | 0.04 | NS      | NS       |
|                          | p-value      | <0.001 | <0.001 | <0.001 |      |         |          |

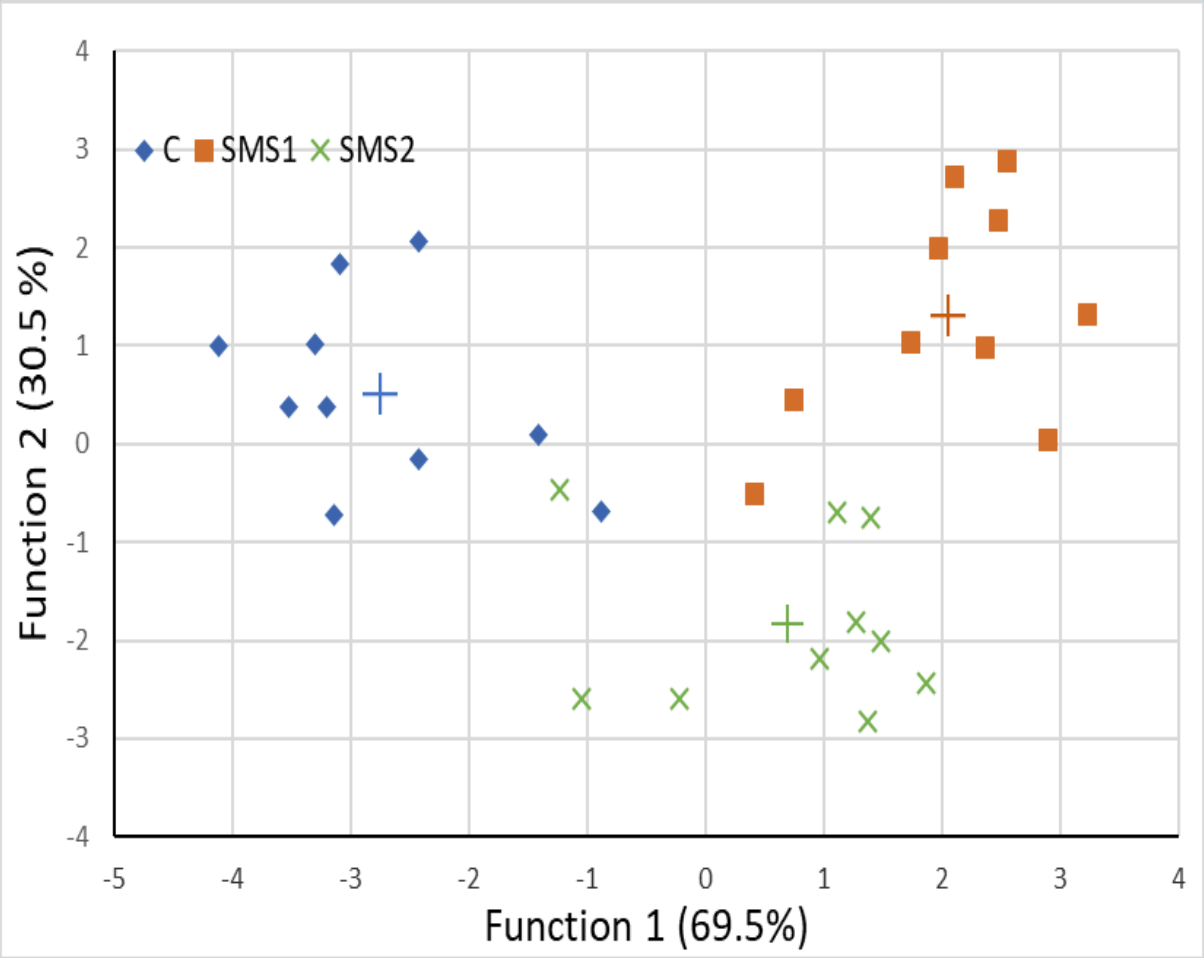
# Results (4)

| Parameter   | Sampling Day | CON    | SMS1     | SMS2   | SEM  | p-Value | P-Linear |
|-------------|--------------|--------|----------|--------|------|---------|----------|
| Log SCC     | 0            | 5.01   | 5.01     | 4.9    | 0.16 | NS      | NS       |
|             | 7            | 4.93   | 4.82     | 5.21   | 0.16 | NS      | NS       |
|             | 14           | 4.65   | 5.01     | 5.05   | 0.14 | NS      | NS       |
|             | 21           | 4.54   | 4.82     | 5      | 0.16 | NS      | NS       |
|             | 28           | 5.11   | 5.18     | 5.15   | 0.17 | NS      | NS       |
|             | p-value      | NS     | NS       | NS     |      |         |          |
| MDA (ng/mL) | 0            | 3.48   | 3.81 A   | 3.32   | 0.25 | NS      | NS       |
|             | 7            | 3.59 a | 2.32 bB  | 2.62 b | 0.33 | <0.05   | <0.05    |
|             | 14           | 3.75 a | 2.99 bAB | 2.79 b | 0.4  | <0.05   | NS       |
|             | 21           | 3.87 a | 2.96 bAB | 2.53 b | 0.25 | <0.01   | <0.001   |
|             | 28           | 3.68 a | 3.24 bAB | 2.88 b | 0.2  | <0.05   | <0.01    |
|             | p-value      | NS     | <0.05    | NS     |      |         |          |

# Results (5)

| Fatty Acid,<br>g/100 g Fat | Treatment |      |      | SEM  | p-Value | P-Linear |
|----------------------------|-----------|------|------|------|---------|----------|
|                            | CON       | SMS1 | SMS2 |      |         |          |
| SFA                        | 71.3      | 72.9 | 72.0 | 1.00 | 0.558   | 0.642    |
| MUFA                       | 22.5      | 21.2 | 21.8 | 0.88 | 0.556   | 0.544    |
| PUFA                       | 3.87      | 3.64 | 4.05 | 0.16 | 0.196   | 0.411    |

9 out of 27 fatty acids contributed to the discrimination of milk samples (C11:0, C12:0, C15:1, C16:1n7, C17:1, C18:2n6, CLA, C18:3n3 and C20:4n6)



# Results (6)

|                                      | Treatment |         |         | SEM  | p-Value | P-Linear |
|--------------------------------------|-----------|---------|---------|------|---------|----------|
|                                      | CON       | SMS1    | SMS2    |      |         |          |
| Lymphocyte (L) (%)                   | 11.22     | 10.52   | 12.90   | 2.42 | NS      | NS       |
| Macrophage (M) (%)                   | 1.02      | 1.30    | 1.58    | 0.19 | NS      | <0.05    |
| Polymorphonuclear leucocytes (P) (%) | 45.94 a   | 42.73 a | 22.82 b | 6.52 | <0.05   | <0.05    |
| L/(M + P)                            | 0.41      | 0.37    | 0.78    | 0.17 | NS      | NS       |

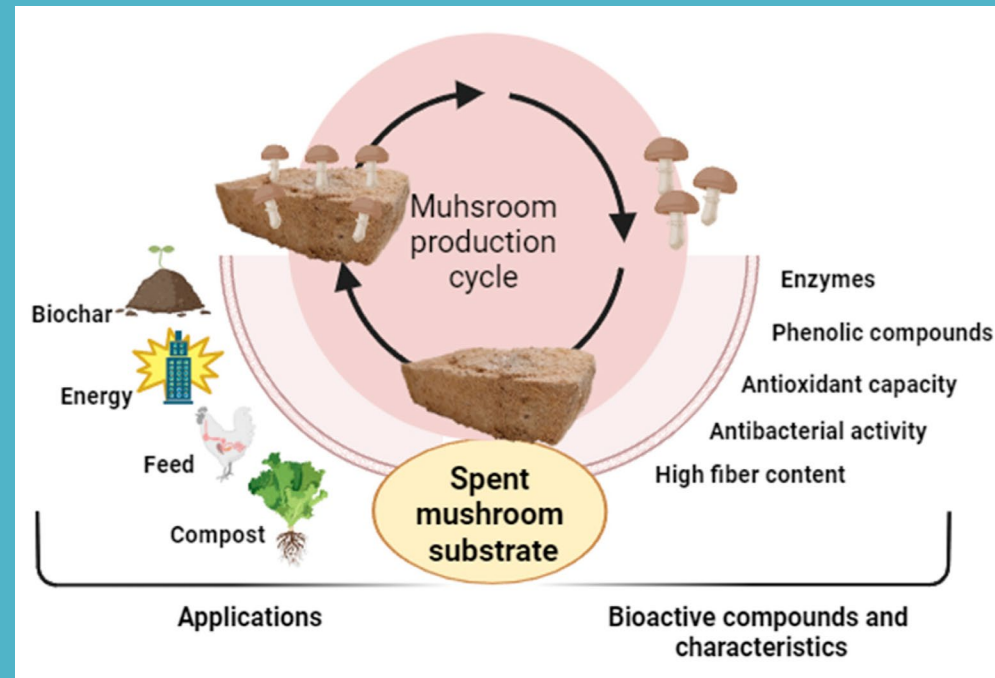
# Discussion

- No effect on feed intake was observed as a result of SMS inclusion at 10–20% of the diet
- No effects of wheat straw hay replacement by SMS on milk yield, composition, fatty acid profile and pH were shown.
- On the other hand, milk oxidative stability was ameliorated, possibly due to SMS antioxidant properties attributed to its high phenolic content
- Finally, the count of polymorphonuclear leukocytes in milk decreased in the SMS2 group (index of a healthier mammary gland) and the macrophage proportion linearly increased (low percentages in all treatment groups, typical for ewe milk, indicating a minimal contribution of this cell type to phagocytosis in the udder)

# Conclusion

- SMS appears as a potential unconventional feedstuff in the diets of dairy ewes in the context of sustainability, circular economy and protection of natural resources.
- Replacement of wheat straw by SMS improves milk oxidative stability, without negatively affecting milk yield, composition and the health status of ewes during their final stage of lactation.
- Further experimentation is warranted

# *Thank you for your attention*



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