

# Regrouping in Dairy Ewes—Effects on Productive Performance and Specific Behavioral Traits

**Table S1.** Composition and analysis of dairy ewes' diet.

| <i>Components (g/kg)</i>          |      |                    |
|-----------------------------------|------|--------------------|
| Corn                              | 465  |                    |
| Wheat                             | 120  |                    |
| Soybean Meal (44%)                | 210  |                    |
| Sunflower Meal                    | 50   |                    |
| Alfalfa Meal                      | 30   |                    |
| Wheat Bran                        | 40   |                    |
| Plant Oil                         | 25   |                    |
| Molasses                          | 15   |                    |
| Sodium Chloride (NaCl)            | 9    |                    |
| Calcium Carbonate                 | 18   |                    |
| Monocalcium Phosphate             | 14   |                    |
| Vitamins & Trace elements Premix* | 4    |                    |
| <i>Calculated Analysis</i>        |      |                    |
| <i>Concentrates</i>               |      | <i>Alfalfa hay</i> |
| Dry Matter – DM (%)               | 88.0 | 93.5               |
| Crude protein – CP (%)            | 17.0 | 10.2               |
| Crude Fiber (%)                   | 5.4  | 34.2               |
| Ash (%)                           | 8.0  | 7.4                |
| Fat (%)                           | 5.0  | 2.3                |
| Calcium (%)                       | 1.0  | -                  |
| Phosphorus (%)                    | 0.7  | -                  |
| Sodium (%)                        | 0.4  | -                  |

\*Premix contained per kg: 150 mg Mg, 35 mg Mn, 50 mg Fe, 60 mg Zn, 0.8 mg Se, 0.75 mg Co, 1.25 mg I, 60 mg Se, 200 mg Mo, 15 kIU vitamin A, 2 kIU vitamin D3, 25 mg vitamin E (kIU: 1000 International Units).

**Table S2.** Effects of ewe breed on milk yield, composition, oxidative stability, flow rate and kick responses.

| Breed           | Examined Parameter                        | Day before and after Regrouping <sup>1</sup> |                    |                    |                    |                    |                    |                     |                     |      |
|-----------------|---|--|--------------------|--------------------|--------------------|--------------------|--------------------|---------------------|---------------------|------|
|                 |   | -1   | 1                  | 2                  | 3                  | 4                  | 5                  | 13                  | 20                  | SEM  |
| Chios           | Daily milk yield (mL)                     | 1369 <sup>a</sup>                            | 1272 <sup>b</sup>  | 1409 <sup>a</sup>  | 1319 <sup>ab</sup> | 1384 <sup>a</sup>  | 1362 <sup>a</sup>  | 1309 <sup>ab</sup>  | 1209 <sup>b</sup>   | 101  |
|                 | Milk fat (%)                              | 4.35 <sup>a</sup>                            | 4.40 <sup>a</sup>  | 4.52 <sup>a</sup>  | 3.89 <sup>b</sup>  | 4.33 <sup>a</sup>  | 4.23 <sup>a</sup>  | 4.11 <sup>ab</sup>  | 4.15 <sup>ab</sup>  | 0.16 |
|                 | Milk protein (%)                          | 5.06   | 5.21               | 5.19               | 5.21               | 5.22               | 5.19               | 5.07                | 5.09                | 0.10 |
|                 | Milk lactose (%)                          | 4.61   | 4.56               | 4.62               | 4.67               | 4.63               | 4.61               | 4.65                | 4.69                | 0.06 |
|                 | Milk total solids-not-fat (%)             | 10.22  | 10.32              | 10.36              | 10.43              | 10.51              | 10.34              | 10.26               | 10.22               | 0.11 |
|                 | Milk malondialdehyde (MDA) levels (ng/mL) | 8.97 <sup>a</sup>                            | 11.86 <sup>b</sup> | 8.76 <sup>a</sup>  | 9.60 <sup>a</sup>  | 10.93 <sup>b</sup> | 10.03 <sup>a</sup> | 9.95 <sup>a</sup>   | 10.57 <sup>ab</sup> | 0.49 |
|                 | Milk flow rate (mL/s)                     | 6.00   | 6.10               | 5.92               | 5.50               | 5.80               | 6.21               | 6.22                | 5.86                | 0.91 |
|                 | Kick responses <sup>2</sup>               | 0 (2)  | 2 (2)              | 0 (1)              | 1 (1)              | 0 (1.5)            | 0 (0)              | 0 (1)               | 0 (3)               |      |
| Karagouniko     | Daily milk yield (mL)                     | 1207 <sup>a</sup>                            | 1082 <sup>b</sup>  | 1186 <sup>a</sup>  | 1229 <sup>a</sup>  | 1239 <sup>a</sup>  | 1217 <sup>a</sup>  | 1025 <sup>b</sup>   | 989 <sup>b</sup>    | 108  |
|                 | Milk fat (%)                              | 5.30 <sup>a</sup>                            | 5.25 <sup>a</sup>  | 5.09 <sup>ab</sup> | 5.03 <sup>b</sup>  | 5.06 <sup>b</sup>  | 5.03 <sup>b</sup>  | 5.11 <sup>ab</sup>  | 4.99 <sup>a</sup>   | 0.17 |
|                 | Milk protein (%)                          | 5.21   | 5.25               | 5.21               | 5.32               | 5.33               | 5.32               | 5.43                | 5.21                | 0.10 |
|                 | Milk lactose (%)                          | 4.78   | 4.68               | 4.73               | 4.64               | 4.69               | 4.69               | 4.66                | 4.69                | 0.06 |
|                 | Milk total solids-not-fat (%)             | 10.54  | 10.49              | 10.49              | 10.52              | 10.58              | 10.57              | 10.65               | 10.45               | 0.11 |
|                 | Milk malondialdehyde (MDA) levels (ng/mL) | 7.85 <sup>a</sup>                            | 10.75 <sup>b</sup> | 8.73 <sup>a</sup>  | 10.53 <sup>b</sup> | 9.38 <sup>a</sup>  | 9.45 <sup>a</sup>  | 10.14 <sup>ab</sup> | 9.72 <sup>ab</sup>  | 0.52 |
|                 | Milk flow rate (mL/s)                     | 10.86  | 9.09               | 10.32              | 9.69               | 9.02               | 9.86               | 8.86                | 9.36                | 0.98 |
|                 | Kick responses <sup>2</sup>               | 0 (0)  | 0 (1)              | 0 (1)              | 0 (1)              | 0 (1)              | 0 (0)              | 0 (0)               | 0 (0)               |      |
| <i>p</i> -value | Breed                                     | Day * Breed                                  |                    |                    |                    |                    |                    |                     |                     |      |
|                 | Daily milk yield (mL)                     | 0.176  | 0.157              |                    |                    |                    |                    |                     |                     |      |
|                 | Milk fat (%)                              | 0.001  | 0.127              |                    |                    |                    |                    |                     |                     |      |
|                 | Milk protein (%)                          | 0.224  | 0.057              |                    |                    |                    |                    |                     |                     |      |
|                 | Milk lactose (%)                          | 0.513  | 0.063              |                    |                    |                    |                    |                     |                     |      |
|                 | Milk total solids-not-fat (%)             | 0.129  | 0.140              |                    |                    |                    |                    |                     |                     |      |
|                 | Milk malondialdehyde (MDA) levels (ng/mL) | 0.132  | 0.173              |                    |                    |                    |                    |                     |                     |      |
|                 | Milk flow rate (mL/s)                     | 0.001  | 0.563              |                    |                    |                    |                    |                     |                     |      |
|                 | Kick responses                            | 0.445  | 0.749              |                    |                    |                    |                    |                     |                     |      |

<sup>1</sup> Day -1: 1 day before and Days 1, 2, 3, 4, 5, 13 and 20: 1st, 2nd, 3rd, 4th, 5th, 13th and 20th day after regrouping<sup>a,b</sup> Means within a row with different superscripts are significantly different (*p* < 0.05)<sup>2</sup> Presented as medians and interquartile range in parenthesis.

**Table S3.** Effects of ewe breed on the average, minimum and maximum heart rate (beats/min), the number of escape attempts and vocalizations per min, and the flight distance (m) during the isolation—flight distance test.

| Breed           | Examined Parameter                   | Day after Regrouping <sup>1</sup> |                      |                       |                        | SEM  |
|-----------------|--------------------------------------|-----------------------------------|----------------------|-----------------------|------------------------|------|
|                 |                                      | 1                                 | 6                    | 13                    | 20                     |      |
| Chios           | Average heart rate                   | 129 <sup>a</sup>                  | 123 <sup>a</sup>     | 104 <sup>b</sup>      | 105 <sup>b</sup>       | 4.38 |
|                 | Maximum heart rate                   | 186 <sup>a</sup>                  | 160 <sup>a</sup>     | 128 <sup>b</sup>      | 132 <sup>b</sup>       | 7.95 |
|                 | Minimum heart rate                   | 102 <sup>a</sup>                  | 98 <sup>a</sup>      | 87 <sup>b</sup>       | 87 <sup>b</sup>        | 3.21 |
|                 | Number of vocalizations <sup>2</sup> | 10 (5) <sup>a</sup>               | 6.5 (1) <sup>b</sup> | 5.5 (5) <sup>bc</sup> | 4 (3) <sup>c</sup>     |      |
|                 | Flight distance (m) <sup>2</sup>     | 2 (1.5) <sup>a</sup>              | 1 (1.5) <sup>b</sup> | 0.5 (1) <sup>b</sup>  | 0.5 (0.8) <sup>b</sup> |      |
| Karagouniko     | Average heart rate                   | 131 <sup>a</sup>                  | 126 <sup>a</sup>     | 120 <sup>ab</sup>     | 118 <sup>b</sup>       | 4.69 |
|                 | Maximum heart rate                   | 174 <sup>a</sup>                  | 172 <sup>a</sup>     | 155 <sup>b</sup>      | 139 <sup>b</sup>       | 8.50 |
|                 | Minimum heart rate                   | 101 <sup>a</sup>                  | 99 <sup>a</sup>      | 93 <sup>b</sup>       | 93 <sup>b</sup>        | 3.43 |
|                 | Number of vocalizations <sup>2</sup> | 3 (6)                             | 2 (5.5)              | 3.5 (6)               | 3.5 (4.5)              |      |
|                 | Flight distance (m) <sup>2</sup>     | 3 (3) <sup>a</sup>                | 1.5 (2) <sup>b</sup> | 1.5 (1) <sup>b</sup>  | 1 (1) <sup>c</sup>     |      |
|                 |                                      | Breed                             | Day * Breed          |                       |                        |      |
| <i>p</i> -value | Average heart rate                   | 0.084                             | 0.144                |                       |                        |      |
|                 | Maximum heart rate                   | 0.284                             | 0.078                |                       |                        |      |
|                 | Minimum heart rate                   | 0.196                             | 0.144                |                       |                        |      |
|                 | Number of vocalizations              | 0.001                             | 0.001                |                       |                        |      |
|                 | Flight distance (m)                  | 0.001                             | 0.429                |                       |                        |      |

<sup>1</sup> Day 1, 6, 13 and 20: 1st, 6th, 13th and 20th day after regrouping <sup>a,b,c</sup> Means within a row with different superscripts are significantly different (*p* < 0.05). <sup>2</sup> Presented as medians and interquartile range in parenthesis.