Feed Additives

✓Feed additives hold considerable potential to reduce the environmental impact of the livestock sector

✓Feed additives carry both environmental burdens from their production and environmental benefits from their usage, environmental assessments have often failed to address this complexity.

✓Both have to be considered to understand the environmental foot print of feed additives

FAO, Environmental performance of feed additives in livestock supply chains, Guidelines for assessment, 2020

Feed Additives

- ✓ Among the numerous effects of additives, particularly interesting is the one related to the improvement of animal health, which involves a reduction in the use of drugs to treat pathologies and better production efficiency.

- ✓ Among the various additives that can be used for this purpose, vitamin E, a micronutrient capable of influencing the oxidative state and the immune response, is certainly one of the most interesting.

- ✓ The use od additive is regulated by the REGULATION (EC) No 1831/2003:

Vitamin E is categorized as nutritional additive

Vitamin E

- ✓ Plasma vitamin E levels fall significantly around parturition in the dairy cow so that it is difficult to maintain levels, in this period, at those considered adequate for health.

- ✓ It has been suggested that this is one of the mechanisms for the decrease in peripartum immune system efficiency.

- ✓ Decreased DMI in the peripartal period and increased vitamin E losses with colostrum are not sufficient to explain this decrease.

- ✓ Changes in vitamin E partitioning may be impaired in this period. The liver plays a central role in the release of a-tocopherol into the circulation and consequently its transfer to peripheral tissues.

Vitamin E in dairy cows

A. Baldi*

Livestock Production Science 98 (2005) 117–122

Vitamin E

✓ Vitamin E intake is generally considered adequate when a-tocopherol plasma levels are above:

3–3.5 μg/ml (6.97-8,12 μmol/L)

Vitamin E in dairy cows

A. Baldi*

Livestock Production Science 98 (2005) 117–122

Changes of blood fatty acids, β-hydroxybutyrate and α-tocopherol in the periparturient period in dairy cows

Table 1. Daily milk yield and milk composition from the 4 participating farms[1]

Trait	Farm A	Farm B	Farm C	Farm D
Milk yield (L/d)	45.65 ± 0.87	46.47 ± 0.88	38.20 ± 1.12	39.40 ± 1.14
Fat (%)	3.59 ± 0.07	3.14 ± 0.09	4.15 ± 0.02	4.16 ± 0.02
Protein (%)	2.91 ± 0.03	2.99 ± 0.04	3.45 ± 0.03	3.43 ± 0.03
Log SCC/mL	1.60 ± 0.07	1.49 ± 0.08	1.96 ± 0.04	1.86 ± 0.06

[1]Farms A and B were the Italian farms and farms C and D were the Greek farms. All values are LSM ± SEM.

Four farms were involved in the trial, A and B from Italy, C and D from Greece

Milk yield was approximatevely 20% higher in Italian farms than in Greek farms

Short communication: **Associations between blood fatty acids, β-hydroxybutyrate, and α-tocopherol in the periparturient period in dairy cows: An observational study**

Journal of Dairy Science Vol. 99 No. 10, 2016

A. Pilotto,* G. Savoini,* A. Baldi,* G. Invernizzi,* C. De Vecchi,† G. Theodorou,‡ P. Koutsouli,‡ and I. Politis‡

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Changes of blood fatty acids, β-hydroxybutyrate and α-tocopherol in the periparturient period in dairy cows

Table 2. Changes in levels of blood fatty acids, BHB, α-tocopherol (α-T), total cholesterol (TC), and the ratio of α-T to total cholesterol during the periparturient period in dairy cows[1]

Time of sampling	Fatty acids (mmol/L)	BHB (mmol/L)	α-T (μmol/L)	TC (mmol/L)	α-T (μmol/L):TC (μmol/L) ($\times 10^3$)
Dry-off	0.155[a] ± 0.017	0.394[a] ± 0.024	8.900[a] ± 0.206	3.898[a] ± 0.099	2.422[a] ± 0.061
Calving	0.511[b] ± 0.017	0.512[b] ± 0.024	4.372[b] ± 0.206	2.471[b] ± 0.099	1.863[b] ± 0.062
30 d postpartum	0.255[c] ± 0.017	0.620[c] ± 0.024	9.062[a] ± 0.212	3.988[a] ± 0.099	2.361[a] ± 0.063

- ✓ Fatty acid concentrations were low at dry-off, reached maximum value at calving, and then declined at 30 d postpartum, 50% less than at calving.
- ✓ BHB (β-hydroxybutyrate) concentrations were low at dry-off, increased by 27% at calving, and continued to increase by another 20% at 30 d postpartum.
- ✓ α-T (α-tocopherol) concentrations were lowest at calving, and no differences in α-tocopherol concentrations were detected at dry off or 30 d post partum. The same trend was observed for TC (Total Cholesterol) and α-T : TC

- ✓ Significant negative correlations (strong at dry-off and weak at 30 d postpartum) between BHB and α-tocopherol after adjustment with cholesterol were detected, that could suggest an effect of vitamin E on the metabolic function of the liver

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Short communication: **Associations between blood fatty acids, β-hydroxybutyrate, and α-tocopherol in the periparturient period in dairy cows: An observational study**

A. Pilotto,* G. Savoini,* A. Baldi,* G. Invernizzi,* C. De Vecchi,† G. Theodorou,‡ P. Koutsouli,‡ and I. Politis‡[1]

Journal of Dairy Science Vol. 99 No. 10, 2016

Changes of blood fatty acids, β-hydroxybutyrate and α-tocopherol in the periparturient period in dairy cows

Table 3. Spearman's rho correlations between the levels of blood fatty acids, BHB, α-tocopherol (α-T), and the ratio of α-T to total cholesterol (TC) during the periparturient period in dairy cows[1]

Time	Item		Fatty acids	BHB	α-T	α-T:TC
Dry-off	Fatty acids	Rho	1	0.114	−0.169	−0.002
		P-value	—	NS	NS	NS
	BHB	Rho		1	−0.370	−0.352
		P-value		—	***	***
	α-T	Rho			1	0.348
		P-value			—	***
	α-T:TC	Rho				1
		P-value				—
Calving	Fatty acids	Rho	1	0.116	−0.084	0.053
		P-value	—	NS	NS	NS
	BHB	Rho		1	−0.010	0.165
		P-value		—	NS	NS
	α-T	Rho			1	0.207
		P-value			—	*
	α-T:TC	Rho				1
		P-value				—
30 d postpartum	Fatty acids	Rho	1	−0.030	−0.300	0.028
		P-value	—	NS	***	NS
	BHB	Rho		1	−0.104	−0.188
		P-value		—	NS	*
	α-T	Rho			1	0.388
		P-value			—	***
	α-T:TC	Rho				1
		P-value				—

- ✓ Overall, a weak correlation was found between fatty acids and BHB throughout the periparturient period, indicating that the two markers are not correlatedduring the periparturient period
- ✓ Significant negative correlations (strong at dry-off and weak at 30 d postpartum) between BHB and α-tocopherol after adjustment with cholesterol were detected, that could suggest an effect of vitamin E on the metabolic function of the liver

A. Pilotto,* G. Savoini,* A. Baldi,* G. Invernizzi,* C. De Vecchi,† G. Theodorou,‡ P. Koutsouli,‡ and I. Politis‡[1]

Changes of blood ROS, SAC and Osi in the periparturient period in dairy cows

Table 1. Changes in levels of reactive oxidative substances (ROS, CarrUnits), serum antioxidant capacity (SAC, µmol HClO/mL) and oxidative stress index (OSi, ROS/SAC) during the periparturient period in dairy cows from four farms[1].

	Farms (F)				Sampling time (T)			Effects		
	1 ($n = 30$)	2 ($n = 29$)	3 ($n = 36$)	4 ($n = 36$)	Dry-off	Calving	30 days postpartum	*F*	*T*	FxT
ROS	$69.578^{a} \pm 1.829$	$80.179^{b} \pm 1.860$	$58.349^{c} \pm 1.674$	$56.973^{c} \pm 1.673$	$60.924^{A} \pm 1.141$	$73.871^{B} \pm 1.223$	$64.015^{A} \pm 1.275$	***	***	ns
SAC	$453.556^{a} \pm 10.889$	$458.460^{a} \pm 11.075$	$408.717^{b} \pm 9.958$	$415.375^{bc} \pm 9.956$	$64.015^{A} \pm 1.275$	$380.412^{B} \pm 6.847$	$461.863^{A} \pm 6.555$	**	***	**
Osi	$0.163^{a} \pm 0.006$	$0.183^{a} \pm 0.006$	$0.148^{b} \pm 0.005$	$0.143^{b} \pm 0.005$	$0.134^{A} \pm 0.003$	$0.200^{B} \pm 0.004$	$0.143^{A} \pm 0.004$	***	***	***

- ✓ Dairy cows experience oxidative stress around parturition and calving is associated with the highest values of two measures of oxidative stress (ROS and Osi) and the lowest value of serum antioxidant capacity (SAC) This implies that they may be useful biomarkers for oxidative stress

- ✓ Values of oxidative biomarkers in farms 1 and 2 are, on average, higher during the whole experimental period compared farms 3 and 4 . This could be due to a major effect of the production level on the general health status of the animals; indeed, milk was 20% higher in farms 1 and 2 compared to farms 3 and 4

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Oxidative indices as metabolic stress predictors in periparturient dairy cows
Guido Invernizzi[a], Panagiota Koutsouli[b], Giovanni Savoini[a], Elena Mariani[a], Raffaella Rebucci[a], Antonella Baldi[a] and Ioannis Politis[b]
ITALIAN JOURNAL OF ANIMAL SCIENCE
2019, VOL. 18, NO. 1, 1356–1360

Correlations between blood ROS, SAC, Osi and α-tocopherol in the periparturient period in dairy cows

Table 2. Spearman's rho correlations between reactive oxygen substances, serum antioxidant capacity, oxidative stress index and blood α-tocopherol in dairy cows ($n = 131$)[a].

Item		α-T[b] Dry-off	α-T[b] Calving	α-T[b] 30 days postpartum
ROS[c]	Rho	−0.500	−0.282	−0.671
	p-Value	***	**	***
SAC[d]	Rho	0.123	0.100	0.178
	p-Value	NS	NS	NS
OSi[e]	Rho	−0.552	−0.267	−0.634
	p-Value	***	**	***

[a]Dairy cows from four herds, two of them in Italy and two in Greece. [b]α-tocopherol. [c]Reactive oxidative substances. [d]Serum antioxidant capacity. [e]Oxidative stress index. **Correlation is significant at $p < .01$; ***$p < .001$ (2-tailed); NS: nonsignificant differences.

- ✓ α-tocopherol (a-T) is the main antioxidant vitamin in dairy cows (Baldi et al. 2000; Politis 2012).
- ✓ ROS and OSi were negatively correlated with a-T at all three sampling points
- ✓ The lack of expected positive correlation between SAC and a-T can be attributed to the inability of OXY adsorbent test to correlate with the single antioxidant measure

Oxidative indices as metabolic stress predictors in periparturient dairy cows

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Correlations between the three markers of oxidative status (ROS, SAC, OSi) and the two markers of negative energy balance (FFA and BHB)

- ✓ Reactive oxygen species (ROS) were positively correlated with BHB at all three sampling points, but only at dry off with FFA
- ✓ Oxidative stress index (Osi) was positively correlated with BHB at dry-off and 30 days post partum, and with FFA at dry off and calving
- ✓ Serum antioxidant capacity was negatively correlated with FFA at dry-off and 30 days postpartum , but wasnot correlated with BHB at any of the three sampling points

- These findings suggest that a positive correlation may exists between oxidative and metabolic markers, especially for fatty acids
- Thanks to the correlation with FFA and BHB, ROS could be the most cost-effective oxidative stress marker to be used on large datasets, also for the prevention of metabolic disorders

Table 3. Spearman's rho correlations between the levels of blood free fatty acids, β-hydroxybutyrate, reactive oxidative substances, serum antioxidant capacity and oxidative stress index in dairy cows ($n = 131$)[a].

Time of sampling	Item		ROS[b]	SAC[c]	OSi[d]
Dry-off	FFA[e]	Rho	0.233	−0.197	0.327
		p-Value	**	*	***
	BHB[f]	Rho	0.274	−0.004	0.261
		p-Value	**	NS	**
Calving	FFA	Rho	0.121	−0.138	0.215
		p-Value	NS	NS	*
	BHB	Rho	0.184	0.169	0.040
		p-Value	*	NS	NS
30 days postpartum	FFA	Rho	0.037	−0.400	0.162
		p-Value	NS	***	NS
	BHB	Rho	0.272	−0.023	0.254
		p-Value	**	NS	**

[a]Dairy cows from four herds, two of them in Italy and two in Greece. [b]Reactive oxidative substances. [c]Serum antioxidant capacity. [d]Oxidative stress index. [e]Free fatty acids. [f]β-hydroxybutyrate. *Correlation is significant at $p < .05$; **$p < .01$; ***$p < .001$ (2-tailed); NS: nonsignificant differences.

Oxidative indices as metabolic stress predictors in periparturient dairy cows

Guido Invernizzi[a], Panagiota Koutsouli[b], Giovanni Savoini[a], Elena Mariani[a], Raffaella Rebucci[a], Antonella Baldi[a] and Ioannis Politis[b]

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Effects of the health o the udder (healthy or mastitic) on blood ROS, SAC and Osi in the periparturient period in dairy cows

Healthy animals group A (n = 90)
Mastitic cows were group B (n = 20)

Table 1. Effect of the health of the udder (healthy or mastitic) on ROS (CarrU), SAC (μmol HClO/ml) and OSi (ROS/SAC) in the blood of dairy cows at dry-off, calving and 30d post-partum. Group A – healthy animals; Group B – mastitic animals. Values are presented as LSM ± SEM

Parameter	Group A	Group B
Dry-off		
ROS	$51.87^{a,1} \pm 0.85$	$54.85^{a,1} \pm 1.87$
SAC	$447.31^{a,1} \pm 2.52$	$431.64^{a,1} \pm 5.52$
OSi	$0.117^{a,1} \pm 0.002$	$0.127^{a,1} \pm 0.005$
Calving		
ROS	$65.89^{a,2} \pm 0.86$	$75.04^{b,2} \pm 1.87$
SAC	$347.38^{a,2} \pm 2.54$	$312.38^{b,2} \pm 5.52$
OSi	$0.191^{a,2} \pm 0.002$	$0.240^{b,2} \pm 0.005$
30 d post-partum		
ROS	$53.46^{a,1} \pm 0.86$	$57.07^{a,1} \pm 1.87$
SAC	$451.42^{a,1} \pm 2.54$	$440.36^{a,1} \pm 5.52$
OSi	$0.118^{a,1} \pm 0.002$	$0.130^{a,1} \pm 0.005$

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J Vet Res 64, 2020
DOI:10.2478/jvetres-2020-0053

SHORT COMMUNICATION

Association of oxidative stress biomarkers and clinical mastitis incidence in dairy cows during the periparturient period

George P. Laliotis[1], Panagiota Koutsouli[1], Kyriaki Sotirakoglou[2], Giovanni Savoini[3], Ioannis Politis[1]

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- ✓ No differences in ROS, SAC and Osi between the two groups at both dry-off and 30 d post-partum.
- ✓ At calving time, ROS and OSi levels were higher by 13.9% and 25.7%, while SAC levels were lower by 10% in mastitic cows than in healthy cows

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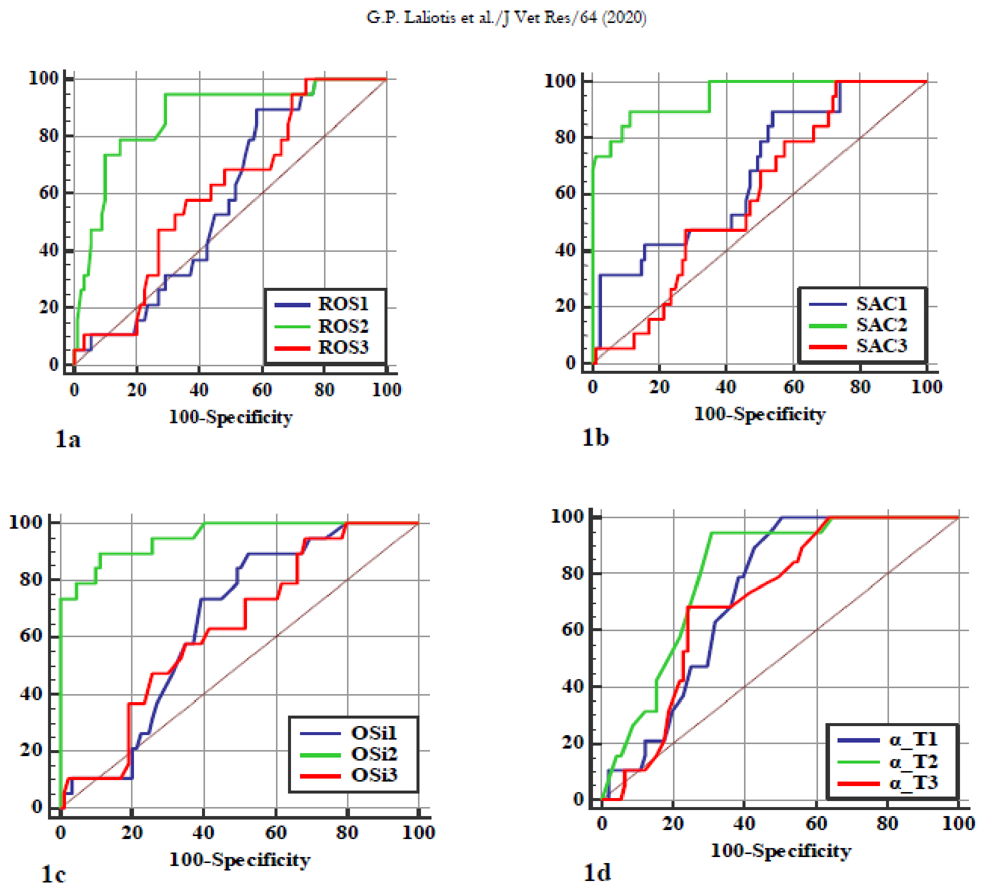
Blood ROS, SAC, OSi and α-tocopherol can be used to predict mastitis incidence in dairy cows



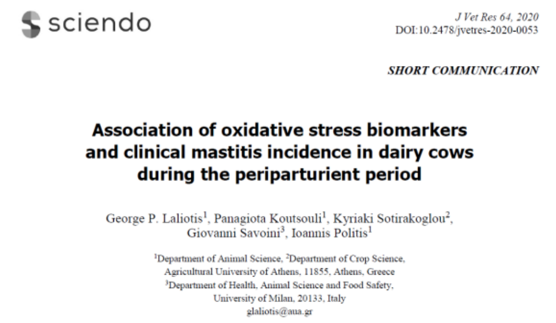
ig. 1. ROC curve analysis in detecting mastitis incidents based on the determined concentrations of the ROS (1a); SAC (1b); OSi (1c); ıd α-T levels (1d). Numbers (1, 2, 3) after the name of each biomarker correspond to the examined time points (1 – dry-off; 2 – calving; – 30 d post-partum)

ROS : reactive oxigen species
SAC : serum antioxidant capacity
OSi : oxidative stress index, ROS/SAC
α_T : α tocopherol (vitamin E)

1 : dry off
2 : calving
3 : 30 d post partum

- ✓ ROS and OSi values were higher and SAC and α_T values lower at calving for cows that developed mastitis in subsequent lactation”

- ✓ The accuracy of prediction using ROC curve analysis is classified into one of four categories (10): a) poor (0.5 < AUC < 0.7); b) acceptable (0.7 ≤ AUC ≤ 0.8); c) excellent (0.8 ≤ AUC ≤ 0.9), and d) perfect (AUC ≥ 0.9).
- ✓ Based on these criteria, SAC and OSi at calving exhibited perfect predictive ability and ROS and α-T showed excellent ability in detecting mastitis incidence



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Conclusion

- ✓ Vitamin E is an important micronutrient for dairy cows because is the main antioxidant vitamin
- ✓ Blood α-tocopherol concentration was negatively correlated with markers of oxidative status (ROS and Osi)
- ✓ A proper inclusion in the diet as additive, especially in the periparturient period, is essential to try to keep correct blood level
- ✓ Blood α-tocopherol concentration showed excellent ability in detecting mastitis incidence