

# Using plant extracts to help mitigate heat stress in pre-weaned calves

Faced with climate change, heat stress is one of the major challenges for livestock. Indeed, the heat felt by animals leads to many physiological (rectal temperature, respiratory rate) and behavioural changes (rumination time, sleeping time, feed intake) leading to a decrease of zootechnical performance and constitutes an additional risk factor for morbidity.

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In reared calves, several studies have shown that in a situation of heat stress, exposed animals had lower growth performance (average daily gain, weaning weight) and lower feed efficiency than calves reared in thermoneutral conditions: this is partly explained by a reduced dry matter intake and a greater susceptibility to disease.

The period before weaning is a sensitive and a critical period for the career of the heifer. The consequences of heat stress can therefore have significant economic repercussions for the breeders.

**Table 1. Effect of Heat Stress Control supplementation in milk replacer on growth, feed efficiency and health status of calves (trial in Italy).**

	Trial 1 (2019)			Trial 2 (2020)		
	Control	Experimental	p-value	Control	Experimental	p-value
Number of calves (n)	20	20	–	20	20	–
Weight at three days (kg)	39.4	38.7	0.67	38.7	39.0	0.76
Weight at 63 days (kg)	69.1	72.0	0.05	67.0	75.6	<0.01
Weight gain 3-63 days (kg)	29.7	33.3	0.05	28.3	36.6	<0.01
Average daily gain (ADG) (g/j)	490	570	0.05	470	610	<0.01
Feed efficiency (dry matter intake/weight gain)	1.9	1.6	0.13	2.52	1.98	<0.01
Morbidity (%)	11	9	0.65	11	11	1
Relapses (n)	7	1	0.01	17	9	0.01
Number of treatments (n)	21	11	<0.01	30	20	<0.01

Temp °C	Humidity (%)																	
	0	5	10	15	20	25	30	40	45	50	55	60	65	70	75	80	85	90
21	63	64	64	64	65	65	65	66	66	67	67	67	68	68	68	69	69	69
22	64	64	65	65	66	66	66	67	68	68	69	69	70	69	70	70	70	71
23	65	65	66	66	67	67	67	68	69	69	70	70	71	71	71	72	72	73
24	66	66	67	67	68	68	69	70	70	71	71	72	72	73	73	74	74	74
25	67	67	68	68	69	69	70	71	71	72	72	73	73	74	74	75	75	76
26	67	68	69	69	70	70	71	72	73	73	74	74	75	75	76	77	77	78
27	68	69	69	70	71	71	72	73	74	75	76	76	76	77	77	78	78	79
28	69	70	70	71	72	72	73	74	75	76	76	77	78	78	79	80	80	81
29	70	71	71	72	73	73	74	76	76	77	78	78	79	80	81	81	82	83
30	71	71	72	73	74	74	75	77	78	78	79	80	81	81	82	83	84	84
31	71	72	73	74	75	76	76	78	79	80	80	81	82	83	84	85	85	86
32	72	73	74	75	76	77	77	79	80	81	82	83	84	84	85	86	87	88
33	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
34	74	75	76	77	78	79	80	82	83	84	84	85	86	87	88	89	90	91
35	75	76	77	78	79	80	81	83	84	85	86	87	88	89	90	91	92	93
36	75	77	78	79	80	81	82	84	85	86	87	88	89	90	91	93	94	95
37	76	77	79	80	81	82	83	85	86	87	89	90	91	92	93	94	95	96
38	77	78	79	81	82	83	84	86	88	89	90	91	92	93	95	96	97	98
39	78	79	80	82	83	84	85	88	89	90	91	92	93	95	96	98	99	100
40	79	80	81	82	84	85	86	89	90	91	93	94	95	96	98	99	100	101
41	80	81	82	83	85	86	87	90	91	93	94	95	97	98	99	101	102	103

**Fig. 1. Temperature Humidity Index (THI) table for pre-weaned calves.**

Different strategies can be used to attenuate the effects of heat stress. Adapting the environment of the animal is one of the essential levers

for reducing the heat it feels. For example, it is necessary to promote water consumption to compensate losses caused by breathing and sweating. It is also important to adapt the barns to provide areas of shade for the calves and promote good ventilation. Another possibility is to install equipment such as fans or sprayers.

To prevent the effects of heat stress, one of the other interesting levers is the nutrition approach. Today, many feed supplements have been developed for lactating cows, but no solutions exist for calves.

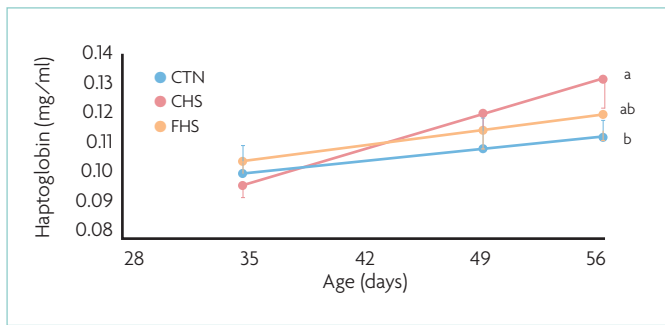
### Critical THI for pre-weaned calves

The reference indicator used to measure the heat felt by animals is the Temperature Humidity Index (THI) (NRC, 1971;  $THI = (1.8 \times \text{Temperature } (^{\circ}\text{C}) + 32) - (0.55 - 0.0055 \times \text{Relative Humidity } (\%)) \times (1.8 \times \text{Temperature } (^{\circ}\text{C}) - 26)$ ). While for lactating cows the THI limit beyond which they are under heat stress is

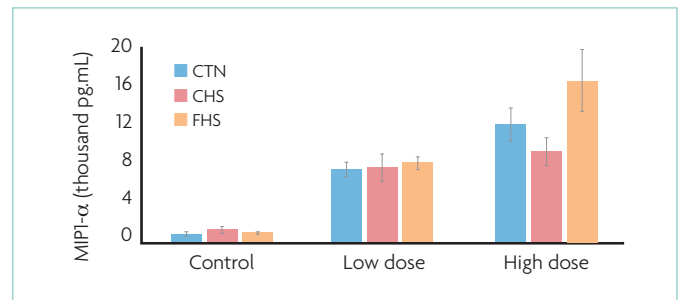
well known, this has been little studied for calves. Bonilait conducted two consecutive trials in Italy (2019, 2020) from June to September including 80 female calves. Based on 2,221 measures of the thermo-physiological parameters (respiratory rate and rectal temperature), a critical THI limit for calves before weaning could be determined.

When the THI is higher than 80 on average during the day, the calf is in a situation of pronounced heat stress (significant acceleration of the respiratory rate). The state of heat stress is confirmed by a rectal temperature significantly higher than normal indicating that the regulatory mechanisms are overwhelmed. For example, from a THI of 77 to 83, we observe 10 more breaths per minute and an increase in rectal temperature of 0.2°C. The results are consistent with a study carried out by Kovács et al. (2019): based on the respiratory rate, they determined that the THI limit for the calves before weaning was 82.

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**Fig. 2. Blood haptoglobin in dairy calves fed with control milk replacer in thermoneutral conditions (CTN), control milk replacer in heat stress conditions (CHS), and supplemented milk replacer in heat stress conditions (FHS)**



**Fig. 3. Macrophage inflammatory protein 1-α response to ex-vivo challenge of blood immune cells collected from dairy calves fed with control milk replacer in thermoneutral conditions (CTN), control milk replacer in heat stress conditions (CHS), and supplemented milk replacer in heat stress conditions (FHS).**

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### Benefits of Heat Stress Control solution

These two trials conducted in Italy also aimed to demonstrate the effectiveness of a nutritional solution implemented by Bonilait: Heat Stress Control. It is a combination of antioxidants and plant extracts that help to reduce the effects of heat stress in rearing calves from birth to weaning integrated directly into the milk replacer. Oxidative stress biomarkers were analysed by a laboratory using a specific method from blood samples taken from the calves.

In a heat stress situation, the antioxidant capacity of calves supplemented with Heat Stress Control is significantly improved by 6% and the production of free radicals tends to decrease by 8%.

This leads to improve health status and zootechnical performances of calves under heat stress conditions.

This work has shown that Heat Stress Control permit to:

- Improve starter consumption (+1.4 kg/calf from birth to weaning) and therefore feed efficiency (see table 1).
- Increase the average daily gain (ADG) of calves (Trial 1: 570g/j versus 490g/j; Trial 2: 610g/j versus 470g/j).
- Reduce significantly the number of relapses (Trial 1: 6 less relapses; Trial 2: 8 less relapses) and therefore the

number of veterinary treatments (Trial 1: 11 treatments versus 21 for control calves; Trial 2: 20 treatments versus 30 for control calves) due to respiratory and digestive troubles.

### Metabolism and immunity under heat stress conditions

To dissipate heat and regulate their body temperature, animals expend additional energy which can lead to changes in their metabolism. In addition, we know that the production of free radicals increases when the animal is subject to attacks caused by its environment.

Several studies have determined that under heat stress conditions, the blood concentration of antioxidants in stressed animals is lower and the production of free radicals increases: this is oxidative stress.

One of the repercussions of oxidative stress is the reduction of the body's immune defences and this leads to an increase in susceptibility to disease.

In order to better understand the mechanisms involved in heat stress at the physiological level, Bonilait conducted another study in partnership with the University of Barcelona (2021). Thus, 32 bull calves were involved in the study at 12 days and three groups were formed:

- Group 1 (CTN): Calves fed with non-supplemented milk replacer under

thermoneutral conditions (THI: 64 during the day and 60 at night).

- Group 2 (CHS): Calves fed with non-supplemented milk replacer under heat stress conditions (THI: 83 during the day and 77 at night).

- Group 3 (FHS): Calves fed with milk replacer supplemented with Heat Stress Control under heat stress conditions (THI: 83 during the day and 77 at night).

#### ● Metabolism:

Blood samples were taken from the animals every week. The results show that calves subjected to heat stress have lower glucose levels and higher non esterified fatty acids (NEFA) levels. This is explained by the fact that glucose is used as an energy source to dissipate heat feel by stressed animals.

As a result, calves under heat stress mobilise their fatty acid reserves and release NEFAs for ATP production.

The level of urea, usually known to reflect the amount of protein ingested, is higher for non-supplemented calves raised under heat stress conditions (CHS). This is explained by an increase in body protein breakdown which are the source of blood urea in heat stress situation.

For the supplemented animals (FHS), the level of urea is not significantly different from the animals of the CTN group, suggesting a similar protein efficiency with calves raised under thermoneutral conditions.

Finally, the liver function was evaluated with the blood levels of two enzymes: alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The trial shows that the blood level of AST increases in heat stress situations and the release of this enzyme into the blood reflects damage to the liver. In this way, the solution tends to improve liver function in heat stress situations (table 2).

#### ● Chronic inflammation:

Blood levels of haptoglobin were higher in animals submit to heat stress at the end of the trial period: this reflects the state of chronic systemic pro-inflammation.

For stressed animals with Heat

Stress Control supplementation, the haptoglobin level was between unsupplemented stressed animals and unstressed animals. Supplementation can reduce chronic pro-inflammatory status under heat stress conditions (Fig. 2).

#### ● Immunity:

To measure what is happening at the immune level, immune cells (from blood samples) were taken for an ex vivo challenge with lipopolysaccharide (LPS).

Blood samples were stimulated with different doses of LPS: control (0µg of LPS/mL), low (0.01µg of LPS/mL) or high (5µg of LPS/mL). The low dose corresponding to a physiological challenge and the high dose to a potential immune response. Upon contact with pathogens, the release of cytokines and chemokines increases.

In this trial, the response of 15 cytokines were evaluated. Overall, cytokine production is lower when calves are under heat stress.

For supplemented animals, the production of cytokines approaches that of calves reared under thermoneutral conditions.

Macrophages inflammatory protein 1-α (MIP 1-α) promote neutrophil migration during inflammation and contribute to alveolar macrophage activation.

When the LPS challenge is high, the number of macrophages is lower in heat stress situations. For supplemented animals, the number of macrophages is higher (Fig. 3).

### Conclusion

The studies carried out have demonstrated that in a situation of heat stress, nutrition is an efficient and easy to implement solution that can be complementary to environmental strategies. The physiological mechanisms involved during exposure to heat stress are complex. The Heat Stress Control solution has an effect at the metabolic level, and therefore helps to improve feed efficiency, at the immune level at a decisive stage of life for the animal. ■

**Table 2. Blood metabolites levels in dairy calves fed with control milk replacer in thermoneutral conditions (CTN), control milk replacer in heat stress conditions (CHS), and supplemented milk replacer in heat stress conditions (FHS).**

Item	CTN	CHS	FHS	p-value
Glucose (mg/dL)	96.7 <sup>a</sup>	85.5 <sup>b</sup>	84.7 <sup>b</sup>	0.035
NEFA (mmol/L)	0.096 <sup>b</sup>	0.121 <sup>a</sup>	0.128 <sup>a</sup>	0.047
Total protein (g/dL)	6.04	5.99	5.95	0.515
Albumin (g/dL)	3.23	3.25	3.26	0.691
Urea N (mg/dL)	13.3 <sup>b</sup>	16.5 <sup>a</sup>	14.8 <sup>ab</sup>	0.044
Creatinine (mg/dL)	0.98 <sup>b</sup>	1.10 <sup>a</sup>	1.07 <sup>a</sup>	0.036
ALT (U/L)	11.7	11.7	11.1	0.093
AST (U/L)	60.2	66.2	57.0	0.086