

Effect of isoquinoline alkaloids and betaine supplementation on haematological properties and betaine distribution of heat stress pigs

H. H. Le^{A,D}, J. B. Furness^B, V. Artuso-Ponte^C, J. J. Cottrell^A and F. R. Dunshea^A

^A Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Australia;

^B Department of Anatomy and Neuroscience, The University of Melbourne, Australia;

^C Phytobiotics Futterzusatzstoffe GmbH, Germany

^D Email: huull1@student.unimelb.edu.au

Heat stress (HS) compromises efficient production of pigs, in part by increasing gastrointestinal oxidative stress (Collin *et al.*, 2001), inflammation (Ganesan *et al.*, 2017) and altering haematological properties (Mendoza *et al.*, 2017). Management of HS is a topic of increasing concern due to the effects of climate change. Nutritional formulation is viewed as a cost-effective and flexible strategy (Cottrell *et al.*, 2015). Previous studies have shown that isoquinoline alkaloids (IQA) extracted from *Macleaya cordata* may ameliorate the effects of HS. Additionally, the organic osmolyte and methyl donor betaine (BET) can reduce oxidative stress, prevent tissue damage and has anti-inflammatory actions (Gabler *et al.*, 2013; Bingul *et al.*, 2016). Therefore, the aim of this experiment was to investigate the impacts of IQA and BET supplementation on haematological status and the distribution of betaine in grower pigs under HS conditions.

50 female Large White x Landrace grower pigs were fed either control (CON), IQA (+ 0.15 g/kg), BET (+ 1 g/kg) diets for 14 days under thermoneutral conditions (TN, 20 °C), then given a climate challenge of either TN (constant 20 °C) or cyclic HS (8 h/d 35 °C, 16h/d 28 °C/d) for 3 days. The respiration rate (RR), rectal temperature (RT) and skin temperature (ST) were observed during the environmental challenge. At the end of experiment, blood samples and tissues were collected for analysis of haematology, inflammation markers and betaine distribution. All data was statistically analyzed by ANOVA using unbalanced design with Genstat software¹⁸ (VSN international, Hemel Hempstead, England). Avoiding skew, urinary concentration of betaine was transformed before analyzing.

The HS protocol increased indices of HS, including respiration rate and rectal temperature, with improvements observed with both BET and IQA treatments. Heat stress reduced haematocrit (P<0.001), haemoglobin (P<0.001) and increased erythrocyte settling rate (P=0.001) (Table 1), a marker of systemic inflammation. However, no effects of either diet were observed. In addition, HS reduced thyroid hormones concentrations (P<0.001) with no influence of diet observed. The distribution of betaine was quantified, and independently of diet, HS increased betaine concentrations in the liver (P=0.07) and blood (P=0.08), possibly indicating osmotic stress. The BET diet increased betaine concentration in liver (P=0.007), kidney (P<0.001), jejunum (P<0.001), ileum (P<0.001), plasma (P<0.001) and urine (P=0.002) samples (Table 1).

Temperature Diet	TN			HS			SED	P-value		
	CON	IQA	BET	CON	IQA	BET		T	D	T*D
<i>Haematological status</i>										
Haematocrit (%)	35.42	35.29	35.11	33.20	31.17	30.74	1.34	<0.001	0.31	0.45
Haemoglobin (g/dL)	11.82	11.95	11.93	11.30	10.64	10.45	0.44	<0.001	0.50	0.25
ESR (mm/h)	8.26	7.80	8.68	12.21	11.30	14.12	2.10	0.001	0.45	0.83
Free T ₃ (pg/mL)	0.71	0.58	0.68	0.45	0.50	0.48	0.07	<0.001	0.68	0.35
Free T ₄ (ng/dL)	1.70	1.32	1.58	1.01	0.92	0.93	0.14	<0.001	0.03	0.26
<i>Betaine concentration</i>										
Plasma (µM)	731	791	1271	949	831	1293	98.41	0.08	<0.001	0.28
Urine (log10)	3.24	3.20	3.43	3.34	3.18	3.84	0.20	0.24	0.02	0.34
Liver	0.94	1.14	1.54	1.38	1.22	1.56	0.18	0.07	0.007	0.20
Kidney	0.93	0.83	1.19	1.06	0.61	1.05	0.14	0.42	<0.001	0.21
Jejunum	0.34	0.36	0.45	0.40	0.33	0.52	0.12	0.15	<0.001	0.18
Ileum	0.45	0.45	0.62	0.49	0.43	0.52	0.04	0.52	<0.001	0.09

Table 1. Haematological status and betaine distribution in growing pig fed control (CON), isoquinoline alkaloid (IQA) or betaine (BET) under thermoneutral (TN) or heat stress (HS) condition.

In conclusion, both IQA and BET treatments ameliorated symptoms of HS in the grower pig. Furthermore, the wide distribution of BET indicates that it may serve multiple roles when protecting against HS.

References

- Bingul I, Basaran-Kucukgergin C, Aydin AF, Coban J, Dogan-Ekici I, Dogru-Abbasoglu and Uysal M (2016) *Environmental Toxicology and Pharmacology*. **45** 170-178.
- Collin A, Lebreton Y, Fillaut M, Vincent A, Thomas F and Herpin P (2001) *Experimental Physiology*. **86** 83-91.
- Cottrell JJ, Liu F, Hung AT, DiGiacomo K, Chauhan SS, Leury BJ, Furness JB, Celi P and Dunshea FR (2015) *Animal Production Science*. **55** 1391-1402.
- Gabler N, Frouel S, Awati A, Owusu-Asiedu A, Amerah A, Patridge G, Dunshea F (2013) *Australasian Pig Science Association (APSA), Melbourne, Australia*. 85.
- Ganesan S, Volodina O, Pearce SC, Gabler NK, Baumgard LH, Rhoads RP and Selsby JT (2017). *Physiological reports*. **5** e13397.
- Mendoza SM, Boyd RD, Ferket PR and van Heugten E (2017) *Journal of Animal Science*. **95** 5040-5053.

Funding by Phytobiotics Futterzusatzstoffe GmbH is gratefully acknowledged.