Activity of plant wastes on acute phase proteins in cows

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ABSTRACT: The effect of two patented protected plants wastes (PW), named in the paper as PE and CO, has been evaluated in dairy heifers under ACTH challenge. The experimental protocol used for each compound involved 3 groups of 5 heifers, 18 days of adaptation to the experimental diets and 5 days (22-26) of ACTH treatment (0.5 mg of Synachten – Novartis - twice a day). In addition to the basal diet, 1 kg of a mixture of dehy beet pulp and PW in the ratio of 1.0:0.0 (EXP-0), 0.1:0.9 (EXP-1) and 0.3:0.7 (EXP-2) was fed in the morning meal to the experimental groups. Blood was sampled before (days 19 and 22) and during (days 24 and 26) ACTH treatment and analysed for cortisol, glucose, ceruloplasmin and haptoglobin. Acute phase proteins increased in the EXP-1 group for PE ($P<0.01$) and in EXP-2 group for CO ($P<0.05$), while the higher concentration of CO reduced haptoglobin concentration ($P=0.01$). These results suggest that the efficacy of PW integration is dose dependent and the optimal dose seems to be different between the 2 selected compounds.

Key words: Acute phase proteins, Plant wastes, Dairy heifers.

INTRODUCTION – The emerging interest for nutraceutics in alternative care medicine has been widely discussed and post-genomic researches have indicated that nutritional intervention can be associated with gene expressions, which are responsible for a variety of biological functions (Stefanon *et al.*, 2006). There are different classes of known compounds in plants having proven nutraceutical properties, and most of them are already available on the market for human and pet animal use. The effect of bioactive compounds of plant origin in the control of oxidative stress is also of interest for farm animals. Preventing or retarding the onset of diseases has become a more attractive and cost effective strategy in the animal production systems. The beneficial role of nutraceutics in enhancing the endogenous defences, through the regulation of gene expressions and the enhancement of key enzymes of animal antioxidant systems, have also been underlined (Sgorlon *et al.*, 2005).
This research focuses on the investigation of immune modulatory and antioxidant activities of new functional additives in animal feed, produced with organic residual from food and plant-based additives industries. In this paper, the effect of two wastes on cortisol response and acute-phase protein levels was tested in heifers, using a model of ACTH-induced stress.

MATERIAL AND METHODS – The experimental protocol was used to test two patented protected plant wastes (PW), named in the paper as PE and CO. For each compound tested, 15 Friesian heifers, homogeneous for age and body conditions, were randomly assigned to three groups of 5 animals each (EXP-0, EXP-1 and EXP-2) and fed twice a day a basal diet (2 kg/day of concentrate and 6 kg/head/day of forage) formulated to cover maintenance requirements. In addition to the basal diet, 1 kg of a mixture of dehydrated beet pulp and PW in the ratio of 1.0:0.0 (EXP-0), 0.1:0.9 (EXP-1) and 0.3:0.7 (EXP-2) was fed in the morning meal. The adaptation period lasted 18 days. At day 22, all cows were injected twice a day with 0.5 mg of ACTH (Synachten, Novartis, Varese) for 5 days. At days 19 and 22 (before ACTH administration) and at days 24 and 26 (during ACTH treatment), blood samples were collected from jugular vein at morning just prior to feed distribution. Blood samples were centrifuged for 15 min at 3000 rpm and plasma stored at –20°C for analysis. Cortisol was measured employing an immunoenzymatic kit (Cayman Chemical, USA) and glucose, ceruloplasmin and haptoglobin were determined by an automatic analyser (ILab 600, Instrumentation Laboratory) according to Bertoni et al. (1998). For each PW, biochemical data obtained from blood samples (4 for each cow) were analyzed with a factorial model, with fixed effects for diet supplementation (3 levels) and ACTH (before and during treatment) (SPSS, 1997).

RESULTS AND CONCLUSIONS – As expected, cortisol and glucose levels dramatically increased after ACTH treatment (P<0.001; Tables 1 and 2). Surprisingly, cortisol was also significantly higher in EXP-1 for PE (P<0.01) and in EXP-1 and EXP-2 for CO groups (P<0.05), in comparison to the EXP-0 group. At the best of our knowledge, there is only one literature report indicating a positive cortisol response to plant polyphenols (Rakimov *et al.*, 1986). Furthermore, more recent papers (Kim *et al.*, 2004; Park *et al.*, 2001) have clearly reported a pro-inflammatory stimulation of cyclooxygenase-2 (COX-2) in cells after treatment with green tea polyphenols, that activate adrenal response and cortisol secretion (Vakharia and Hinson, 2005). Moreover the addition of a consistent quantity of lignified and fibrous food, such as PE and in particular CO, could have caused the rise of cortisol levels (Odensten et al., 2007). PW were selected for their content in polyphenols and flavonoids and phytochemical analysis are in progress to investigate if other classes of compounds (as essential oils, alkaloids, glycosides, saponins) are contained in these wastes.

From Tables 1 and 2, is evident that PE and CO had an effect on the acute phase protein response. A linear relationship between mean cortisol concentrations and mean haptoglobin or ceruloplasmin levels can be drawn, particularly after the ACTH challenge. This positive relationship between cortisol and acute-phase proteins (Ting *et al.*, 2004), further confirms a stimulatory effect of PE and CO on the hypothalamic-pituitary-adrenal (HPA) axis.

Table 1. Effects of plant waste "PE" administration and ACTH treatment on blood parameters of EXP-0 (control), EXP-1 (high dose) and EXP-2 (low dose) groups.

	Cortisol (nmol/L)	Glucose (mmol/L)	Haptoglobin (g/L)	Ceruloplasmin (mmol/L)
Pre-ACTH				
EXP-0	8.93	4.68	0.07	2.75
EXP-1	16.75	4.80	0.34	3.04
EXP-2	13.05	4.60	0.05	2.45
Post-ACTH				
EXP-0	300.40	7.15	0.12	3.17
EXP-1	345.10	6.78	0.41	3.55
EXP-2	290.40	6.84	0.07	2.80
Main effects and interactions (P < 1)				
Group (G)	0.005	NS	NS	0.000
ACTH (T)	0.000	0.000	NS	0.010
G x T	NS	NS	0.050	NS
MSE	4.994	0.085	0.042	0.063

Considering the effect of PW on the response of acute phase proteins, a significant increase of haptoglobin and ceruloplasmin was observed for the highest dose of PE, whereas the highest dose of CO decreased haptoglobin levels (P=0.01). This pattern suggess that the biological response of animals was different in the 2 experiments. Furthermore, the lowest dose of PE showed a positive effect on the reduction of ceruloplasmin synthesis (P=0.000) if compared with EXP-0 values, while the same dose in CO trial increased ceruloplasmin production (P=0.05). From these results, we can state that a positive effect of PW on acute phase protein synthesis can be obtained employing the highest dose of CO, whereas PE seems to be more effective in the control of acute phase response at the lowest dose. Blood biochemical and biomolecular analysis are in progress to better understand the adaptation mechanisms that are involved in restoring animal homeostasis.

Table 2. Effects of plant waste "CO" administration and ACTH treatment on blood parameters of EXP-0 (control), EXP-1 (high dose) and EXP-2 (low dose) groups.

	Cortisol (nmol/L)	Glucose (mmol/L)	Haptoglobin (g/L)	Ceruloplasmin (mmol/L)
Pre-ACTH				
EXP-0	7.49	4.49	0.13	2.45
EXP-1	11.07	4.51	0.15	2.75
EXP-2	11.14	4.51	0.10	2.54
Post-ACTH				
EXP-0	306.00	6.95	0.13	2.85
EXP-1	333.10	7.18	0.10	3.16
EXP-2	333.60	7.30	0.29	3.12
Main effects and interactions (P < 1)				
Group (G)	0.049	NS	NS	0.050
ACTH (T)	0.000	0.000	NS	0.000
G x T	NS	NS	0.010	NS
MSE	4.829	0.960	0.016	0.049

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