

THE ASSOCIATION OF ALOE AND β -CAROTENE
SUPPLEMENTATION IMPROVES OXIDATIVE STRESS
AND INFLAMMATORY STATE IN PREGNANT BUFFALO COWS

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ABSTRACT

The complex polysaccharides of Aloe have been reported to improve immunity and oxidative status either *in vitro* or *in vivo* studies. Further, β -carotene has been shown to influence immune function and health in the cow. In this study, the effects of VigoorsanTM, a commercial supplement containing *Aloe arborescence* and β -carotene, on biochemical profile and oxidative/inflammatory status, were evaluated in pregnant buffalo cows. VigoorsanTM was supplied (50 g/day/head) to pluriparae buffalo cows for 30 days before delivery. A significant increase of BAP and OXY and a decrease of d-ROMs ($P<0.01$) were detected in blood from cows supplemented with VigoorsanTM. Further, a decrease of TNF- α , IL1- β and MCP-1 and an increase of IL-10 were showed ($P<0.01$). Blood chemistry profiles showed no adverse effects on health status. Results showed that VigoorsanTM supplementation improves oxidative and inflammatory status with no negative effects on metabolism in water buffalo cow.

Keywords: buffaloes, *Bubalus bubalis*, *Aloe arborescence*, β -carotene, oxidative status,

inflammation

INTRODUCTION

Transition is a highly demanding period that can be affected by many factors that could compromise the health, productivity and reproductive performance of dairy cows. Nutritional strategies can be used to prevent metabolic diseases in the early days post-calving, and to improve milk production. A wide variety of additional feed additives exist that may also have potential for use over a few weeks pre-partum. The genus Aloe plant, whose four species namely, *Aloe barbadensis* Miller, *Aloe ferox*, *Aloe arborescens* and *Aloe perryi* baker have been traditionally applied for the medicinal practice over thousands of years in many cultures of the world. Recent studies suggested it could play an interesting role in improving health status and, as a consequence, performance in ruminants.

Several studies showed a lot of beneficial properties of *Aloe* spp. (Boudreau and Beland, 2006). Aloe gel possesses wound healing (Hegggers, 1993), anti-inflammatory (Vazquez, 1996), to treat

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oncology pain (Looney, 2010), antiviral (Saoo, 1996), spermicidal (Fahim, 1996) and gastro-protective (Danhof, 1991), cicatrising (Davis *et al.*, 1994) properties. Immune-stimulating (Infascelli *et al.*, 2010), anti-proliferative (Di Luccia *et al.*, 2013) and cholesterol-lowering (Tizard *et al.*, 1989) activities have also been shown.

Several researchers have demonstrated that the mucilaginous polysaccharides contained in the clear pulp of Aloe leaf are the major ingredient responsible for the healing. Many of Aloe effects may derive from its activity on intestinal function, thus influencing absorption and availability of key substances (Calabrò *et al.*, 2013). Indeed, the complex polysaccharides of Aloe have been reported as potent B cell stimulators either *in vitro* (Leung *et al.*, 2004) and *in vivo* (Liu *et al.*, 2006) studies.

Moreover, several studies have shown the importance of β -carotene, the natural precursor for vitamin A (retinol) in ruminants, on reproduction, immune function and health in the cow and calf (Michal *et al.*, 1994; Kume and Toharmat, 2001). Dietary β -carotene has been showed to elevate *peri-partum* concentrations of blood β -carotene, thus enhancing host defence mechanisms by potentiating lymphocyte and phagocyte functions, and decreasing the incidence of certain reproductive disorders (Michal *et al.*, 1994). The majority of raw materials used to feed dairy cows are very poor sources of β -carotene and plasma concentrations of β -carotene have been shown to decrease in dairy cows during the *pre-partum* period, therefore, a supplementation of this precursor is recommended (Kawashima *et al.*, 2009). In a recent study, a dietary supplement of β -carotene given in late-gestation was able to increase β -carotene concentrations in cows blood and in colostrum but was unable to increase colostral IgG (Kaewlamun *et al.*, 2011).

This research aimed to explore the influence of supplying the diet of pregnant buffalo cows with Vigoorsan™, a feeding supplies containing *Aloe arborescens* and β -carotene, on the biochemical profile, oxidative stress and inflammatory state.

MATERIALS AND METHODS

Twenty Italian Mediterranean pluriparae buffalo cows were divided into two groups (homogeneous for parity, BCS and milk yield in the previous lactations).

Health status of animals was monitored daily based on clinical signs of disease by trained individuals and on a weekly basis by a veterinary practitioner. Starting at 60 days prior to the expected date of calving (evaluated by ultrasonography) cows were monitored daily and fed a diet (12.5 kg dry matter/head/die: crude proteins 12% DM; 6.0 MJ/kg DM) constituted by oat straw, corn silage and a commercial concentrate. For 30 days before calving, group B received a supplementation of 50g/day/head of Vigoorsan™, while group A was used as control.

Before and after 30 days of Vigorsaan™ supplementation, blood samples were obtained from the coccygeal vein at 07:00 am before feeding. All blood samples were collected into 10 ml vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and allowed to clot and kept at 4°C until separation of serum. Clotted blood was centrifuged at 1600 x g at 4°C for 20 minutes. The separated serum samples were stored at -80°C until analysis to avoid loss of bioactivity and contamination and were thawed on ice for approximately 2 h before use.

Blood chemistry analyses were performed by an automatic biochemical analyser AMS

Autolab (Analyzer Medical System, Rome, Italy) using reagents from Spinreact (Girona, Spain) to determine: blood urea nitrogen (BUN), creatinine (CREA), glucose (GLU), aspartate aminotransferase (AST), cholesterol (CHO) and triglycerides (TRI); reagents from Catachem (Bridgeport-Connecticut-USA) to determine β -hydroxybutyric acid (B-HBA), reagents from Randox (Ireland) for non esterified fatty acids (NEFA), and from Diacron International s.r.l. (Grosseto, Italy) to assess reactive oxygen metabolites (d-ROMs), biological antioxidant potential (BAP), OXY-adsorbent (OXY) tests. Serum levels of interleukin-1 β (IL-1 β), interleukin-10 (IL-10), tumour necrosis factor (TNF- α), and monocyte chemo-attractant protein-1 (MCP-1) were measured using commercially available ELISA kits (Genorise Scientific, INC).

One-way ANOVA was used to detect statistical differences between groups using the statistical model (GLM procedure, SAS, 2000):

$$y_{ij} = \mu + \text{Diet}_i + \varepsilon_{ij}$$

Where: y is the experimental data, μ the general mean, Diet ($i = A, B$), ε the error term.

RESULTS AND DISCUSSION

Blood chemistry parameters (Table 1)

were in the normal range for the physiologic state of animals and no significant difference was detected between groups, thus showing animals were in good health and no adverse effect occurred after VigoorsanTM supplementation.

Results concerning the oxidative status after 30 days of treatment strongly suggest the beneficial effects of VigoorsanTM in terms of cellular health (Table 2). The d-ROMs were lower in group B showing a general decrease of ROS production.

A high level of ROS due to an increased production of oxidant species and/or a decreased efficacy of antioxidant system can lead to oxidative stress, an emerging health risk factor involved in many diseases, including inflammatory, infectious and degenerative disorders, both in humans and in animals (Halliwell and Cross, 1994; Bildik *et al.*, 2004; Kiral *et al.*, 2005; Roncoroni *et al.*, 2014; Vajdovich *et al.*, 2005). The d-ROMs test provides a measure of the whole oxidant capacity of plasma and its decrease is considered a measure of cellular health. The lower d-ROMs level in the VigoorsanTM group is probably due to an improvement of the biological antioxidant potential, as confirmed by the increase of BAP and OXY levels. Such a biological antioxidant potential is attributed to the major component of plasma barrier to oxidation (vitamin C, vitamin E, uric acid, bilirubin and so on) (Benzie and Strain, 1996; Dohi *et al.*, 2005; Hetey *et al.*, 2007).

Table 1. Blood chemistry parameters in control (Group A) and supplemented with 50 mg/kg/die of VigoorsanTM for 30 days (Group B) cows.

Group	GOT	TRI	GLU	CHOL	NEFA	BHBA	BUN	CREA
	<i>U/L</i>	<i>mg/dl</i>	<i>mg/dl</i>	<i>mg/dl</i>	<i>μmol/l</i>	<i>mg/dl</i>	<i>mg/dl</i>	<i>mg/dl</i>
A	127	25.6	75.5	34.5	69.9	5.1	15.8	0.9
B	119	37.7	71.8	34.3	59.9	5.8	17.7	0.8
MSE	6.1	3.2	5.4	2.4	9.1	0.4	1-0	0.04

The beneficial effects of Vigoorsan™ are also confirmed by the decrease of IL-1 β , TNF- α and MCP-1 and by the increase of IL-10. Interleukin-1 β is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis (Auron *et al.*, 1984). TNF- α is produced primarily by macrophages, but also by a broad variety of cell types including lymphoid cells, mast cells, endothelial cells, cardiac myocytes, adipose tissue, fibroblasts, and neuronal tissue (Aggarwal *et al.*, 2003). Cellular response to TNF- α results in activation of pathways that favour both cell survival and apoptosis depending on the cell type and biological context. TNF- α plays a key regulatory role in inflammation and host defence against bacterial infection and causes many of the clinical problems associated with autoimmune disorders (Lin *et al.*, 2007). Monocyte chemoattractant protein-1 (MCP-1) is a representative of the CC chemokine group, and its main known function is related to guiding monocytes to leave the circulation and become tissue macrophages, the first step in the initiation of inflammation. Several studies show that alterations in plasma MCP-1 concentrations in metabolic disease states, the presence of circulating chemokine reservoirs and recent novel mechanisms

of action are associated with metabolic disturbances and suggest that MCP-1 might have systemic role in metabolic regulation (Rull *et al.*, 2010).

Interestingly, TNF- α , and IL-1 β concentrations were significantly decreased in Vigoorsan™ group compared to control, indicating a possible anti-inflammatory role of Vigoorsan™. This hypothesis has been further supported by results related to IL-10. This anti-inflammatory cytokine was found to increase by nearly two-fold in the Vigoorsan™ group compared to control. IL-10 is an anti-inflammatory cytokine that is produced by T cells, NK cells, mast-cells and macrophages (Pestka *et al.*, 2004; Akuffo *et al.*, 1999; Grimbaldston *et al.*, 2007). It is capable of inhibiting synthesis of pro-inflammatory cytokines like TNF- α . In addition, IL-10 is an important negative regulator of the immune response, which allows for maintenance of pregnancy (Pestka *et al.*, 2004).

Our data provide evidence that dietary supplementation with Vigoorsan™ decreases inflammation and oxidative stress. Such beneficial effects could have important consequences on animal performance, so far, it could be involved in the improvement of immunity, as suggested by Infascelli *et al.* (2010). In any event, the possible benefits in terms of animal welfare and animal

Table 2. Oxidative status and inflammatory state parameters in control (Group A) and supplemented with 50 mg/kg/die of Vigoorsan™ for 30 days (Group B) cows.

Group	TNF-α	IL-1β	IL10	MCP1	d-Roms	BAP	OXY
	<i>pg/ml</i>	<i>png/ml</i>	<i>pg/ml</i>	<i>pg/ml</i>	<i>U CARR</i>	<i>μmoli/L</i>	<i>mmoli/L</i>
A	441.2 A	363.4 A	53.20 B	142.5 A	57.50 A	1854 B	298 B
B	376.4 B	287.6 B	96.11 A	79.11 B	47.22 B	2948 A	347 A
MSE	10.1	11.4	5.8	8.7	1.6	268	17.3

A, B: P<0.01

production merits further studies, in particular concerning the intracellular transduction pathways involved for the beneficial effects of Vigorsan™.

Recent studies have demonstrated that *Aloe arborescens* has immunostimulating activity in animal trials (Infascelli *et al.*, 2010) and it was also found to have beneficial phytotherapeutic and anti-tumoral properties (Lissoni *et al.*, 2009). It is known that the anti-tumoral properties of Aloe depend not only on its immuno-modulatory effect, but also on a direct inhibition of cancer cell proliferation (Bedini *et al.*, 2009). Di Luccia *et al.* (2013) analysed the composition of an *Aloe arborescens* leaf extract by gas chromatography-mass spectrometry analysis and found it is rich in Aloe-emodin, a hydroxylanthraquinone with known anti-tumoral activity and in several compounds with anti-oxidant properties. Accordingly, they showed that the Aloe extract has antiproliferative effects on several human transformed cell lines and exhibits pro-differentiative effects on both primary and immortalized human keratinocyte. Proteomic analysis of whole cell extracts revealed the presence of proteins with a strong anti-proliferative and antimicrobial activity specifically induced in human keratinocytes by Aloe treatment supporting its application as a therapeutic agent.

In conclusion, this study shows that the supplementation of *Aloe arborescens* and β -carotene in the diet of buffalo cows ameliorates the inflammatory state and oxidative stress. Also, results suggest an immunostimulating activity that may be of great importance for ruminants during transition. Further studies will be addressed to the identification of these components and the molecular mechanisms underlying their effects.

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