Journal of Animal and Veterinary Advances 10 (9): 1127-1132, 2011
ISSN: 1680-5593

Effects of Ginger (*Zingiber officinale*) and European Stoneseed (*Lithospermum officinale*) Extracts on Performance, Meat Quality and Fatty Acid Composition of Finishing Bulls

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Abstract: About 4 farms breeding Blonde d'Aquitaine (farm A), Piemontese (farms B and D) and Limousin (farm C) bulls were used in a 3 year research project to study the effects of supplementation of a phytotherapic compound (containing ginger and European stoneseed) on performance, meat quality and fatty acid composition of finishing bulls. In each farm, 20 bulls were divided in two balanced groups: treatment (TRT, n = 10) and control (CTR, n = 10) group. The two groups were placed in two separate pens where concentrate and straw were offered *ad libitum*. During the last 60 days before slaughtering (finishing period), the phytotherapic compound (50 g/head/day) was added into the diet of the TRT group. Animals were weighted every 2 weeks in order to calculate the Average Daily Gain (ADG) of the trial. At the end of the experimental period, bulls were slaughtered and muscle conformation, fat covering, pH and temperature were measured on carcasses. On beef samples were analyzed color, drip losses, cooking losses, meat cooking shrinkage and shear force. Moreover, fatty acid profile and sensory traits of meat were also investigate on samples collected in farm A and D. The TRT groups of farms A and B showed higher ($p<0.05$) ADG compare to the respective CTR group. Very few significant differences of physical, chemical and sensory characteristics emerged on beef samples. Globally, the supplementation with the phytotherapic compound did not negatively affect meat quality. The obtained results thus suggest that the considered plant extracts might enhance bulls' performance but further investigations should help to clarify the dose-response relationship.

Key words: Plant extracts, growth promoters, bull performance, beef quality, fatty acids, sensory characteristics

INTRODUCTION

The relationship between the ruminant animal and its resident ruminal microbial population is clearly symbiotic and allows ruminants to utilize fibrous plants and material via a microbial fermentation. However, the ruminal fermentation is intrinsically inefficient. An aliquot of dietary carbon and energy may be converted to methane and heat, two end-products that are unusable by the animal. Similarly, the dietary protein can be degraded to ammonia and lost in the urine.

Making nutrients unavailable to the ruminal microorganism by allowing them to by-pass the ruminal fermentation represents, among the others, one of the strategies that have been used to improve ruminal feed efficiency. The addition of antimicrobial compounds in the diet to alter the ruminal microbial ecosystem has been investigated as well. Since the 70's, antibiotics (e.g., ionophores) were originally used as preventive measures to control diseases such as coccidiosis and bacterial enteritis in poultry. Subsequently, they were widely used in ruminants as methods to decrease the incidence of subclinical infections and improve productivity. In fact, antibiotic growth promoters have extensively been employed to enhance animal health and productivity in livestock systems of many countries of the world. Monensin, one of the most commonly used ionophores, has been proved as a methane inhibitor and propionate enhancer for cattle. It also reduces dietary protein deamination and decreases the lactic acid production. Several studies highlighted that the increment of energy availability and nitrogen retention improves the efficiency of feed utilization of ruminants and thus improves animal productivity and production performance.

The 50 years practice of antibiotic use for non-therapeutic purposes led to an increasing public and scientific concern on their negative effects. This emergence mainly arises from the development of antibiotic resistance in several human pathogenic bacteria (Barton, 2000). Moreover, the use of antibiotics

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as production enhancers has been criticized for the risk of residues into animal derived food products and into the environment. For all these reasons, the European Union introduced in 2006 a general ban of the use of antibiotics as animal growth promoters (European Union, 2003). As a consequence of this situation, consumers increasingly demand for even more natural and safe products in the food chain, forcing producers to look for alternative feed additives. Herbs and plant extracts represent common responses to these demands. Several substances deriving from vegetables can offer some of the benefits that antibiotics provide. Natural plant extracts have been in use since the beginning of recorded history. Although, little is known about their mechanisms on human and animal metabolisms, several researches revealed beneficial effects of the inclusion of plant extracts as feed micro-ingredients (Benchaar *et al*., 2008; Jouavi and Morgavi, 2007).

The vast source of different molecules with intrinsic bioactivities showed antioxidant, antiseptic and immunomodulator actions on animal physiology. In particular, some compounds extracted from herbs and spices showed positive effects on the digestive system. In particular, they have proven to act as appetite and digestion stimulants, gastric stimulants, antidiarrhoeics, antiseptics, tonics and carminative (Richard, 1992; Charalambous, 1994). Among numerous herbs used as natural medicine, ginger (*Zingiber officinale*) is a rhizome that is widely used as herbal remedy for some common ailments. It contains zingiberol, gingerol and shagoals which are active constituents with antipyretic, antiemetic, analgesic, anti-inflammatory (Evans and Trease, 1979), antioxidant and anti-stress activities (Lakshmi and Sudhakar, 2010). The European stoneseed (*Lithospermum officinale*) belongs to the Borraginaceae, a wide family of medical plant. *L. officinale* contains several bioactive substances, including phenolic compounds (e.g., lithospermic acid) and it is traditionally used for the treatment of fever, gouty diseases and intestinal pain (Krenn *et al*., 1994).

The aim of this study was to evaluate the effect of the supplementation of a phytotherapic compound containing ginger (*Zingiber officinale*) and European stoneseed (*Lithospermum officinale*) extracts on performance, meat quality and fatty acid composition of finishing bulls. This phytotherapic compound is a rich source of glucosides, organic acids and flavonoids (such as rutin and quercitin) and it is supposed to encourage feed intake and thus improving feed efficiency.

MATERIALS AND METHODS

During a 3 years research program, the same experimental trial was repeated four times in four different beef breeding farms located in Piedmont region (N-W, Italy). The beef bulls used in the trials belonged to tree breeds: Blonde d'Aquitaine (farm A), hypertrophied Piemontese (farms B and D) and Limousin (farm C).

In each farm, 20 subjects were selected from all animals available at that time in the farm and randomly divided into two groups (CTR: control group; TRT: treatment group) in order to be balanced for age and body weight. During the trial the two groups were housed in two pens with straw and received the same amount of the same diet (based on concentrate and hay) with some differences in concentrate composition between the farms. The diets were formulated to correctly meet the requirements for late maturing beef cattle. Water was available all the time. During the last 60 days before slaughtering (finishing period), the phytotherapic compound (50 g/head/day) was added into the diet of the TRT group. Animals were individually weighted every 2 weeks in order to calculate the Average Daily Gain (ADG) of the trial.

At the end of the experimental period, all animals were stunned by a captive bolt and were slaughtered in commercial abattoirs according to standard procedures. The carcasses were weighted, split into two parts and stored at 2°C in a chilling room. Carcasses muscle conformation and fat covering were determined according to the European SEUROP classification scheme (European Union, 2006).

About 45 min after slaughter the pH was measured (pH_0) on the right half-carcass at the 13th thoracic vertebra level by a Crison pHmeter with an Ingold spear electrode and automatic temperature compensator. About 24 h post-mortem the carcasses were weighted again and the pH was measured (pH_{24}) again as described above. About 7 days after slaughter the portion of longissimus thoracis et lumborum between the 9th thoracic and 1st lumbar vertebra was taken from the right half-carcass of each animal.

The meat samples were kept at 3±1°C for 7 days after slaughter when the following analyses were performed:

- Meat color using a Minolta Chromameter Reflectance II CR200/08 (CIE L*, a*, b*) (Boccard *et al*., 1981)
- Drip losses, on a steak weighing about 80 g and 1.5 cm thick and kept for 48 h in a plastic container with a double bottom (Lundstorm and Malmfors, 1985)
- Cooking losses and meat cooking shrinkage, on a meat round sample (diameter: 5.5 cm; thickness: 1.0 cm) cooked in a electric oven to an internal temperature of 70°C (Barbera and Tassone, 2006)
- Shear force (kg) on cylindrical cores 2.54 cm in diameter, taken parallel to muscle fibres and obtained from the steaks used to determine cooking losses

The shear force was measured by an Instron 1011 equipped with a Warner-Bratzler shear device and calibrated at a speed of 100 mm min^{-1}. Moreover, fatty acid profile and sensory characteristics of meat were also investigated on samples collected in farms A and D. For the analysis of fatty acid composition, lipids were extracted from samples of muscle according to Folch *et al.* (1957) and Fatty Acid Methyl Esters (FAME) were prepared by alcoholysis in an essential non-alcoholic solution (Chistopherson and Glass, 1969) and analyzed by gas chromatograph (SHIMADZU-GC 17A) using a HP88 capillary column (100 m×0.25 mm ID, 0.2 μm film thickness; J and W Scientific). The column temperature was held at 60°C for 1 min and then raised at 20°C min^{-1} to a final temperature of 190°C where it remained for 40 min. Temperature of the injector and flame-ionization detector were maintained at 250 and 280°C, respectively. The injection volume was 0.1 μL and nitrogen constant linear flow rate was at 40 mL min^{-1}.

Sensory analysis was carried out by assessors selected and trained for beef evaluation, according to guidelines of American Meat Science Association. An 8-point structured scale was adopted with 1 and 8 representing the minimum (the worst) and maximum (the best) scores, respectively. Sensory characteristics were determined for the appearance of the raw meat and eating qualities of the cooked meat. Eating qualities included: tenderness (ease of sinking; friability; residue after chewing), initial and sustained juiciness and overall acceptability. The steaks were cooked into an electric oven, preheated at 165°C to an internal temperature of 70°C. Parametric and non-parametric variables were statistically analyzed with Student's t-test and Mann-Whitney U-test, respectively. Significance was declared at $p<0.05$. All statistical analysis was performed using SAS (2006) procedures.

RESULTS AND DISCUSSION

Data reported in the following figures and tables compare the TRT and CTR groups in the farms in which trials took place. They aim at highlighting the effects of the inclusion of the phytotherapic compound in the diets of TRT groups. It is worth mentioning here that the proposed results do not intend to compare the fou farms involved in the research. In fact, even if they share common characteristics (region of location, farm management, etc.), they represent different experimental realities (breeds, feed ingredients, etc.) analyzed during a 3 year research project.

Figure 1 shows the ADG of animals during the trials. Regardless of the treatment, the results obtained in the trials are similar to those that usually occurred in the four farms. Concerning the effect of the phytotherapic compound, a significant difference emerges in the ADG between TRT and CTR groups both in farm A and B. There was no difference in live weight at the beginning of the experimental fattening period but due to a higher ADG, TRT bulls showed a heavier average final live

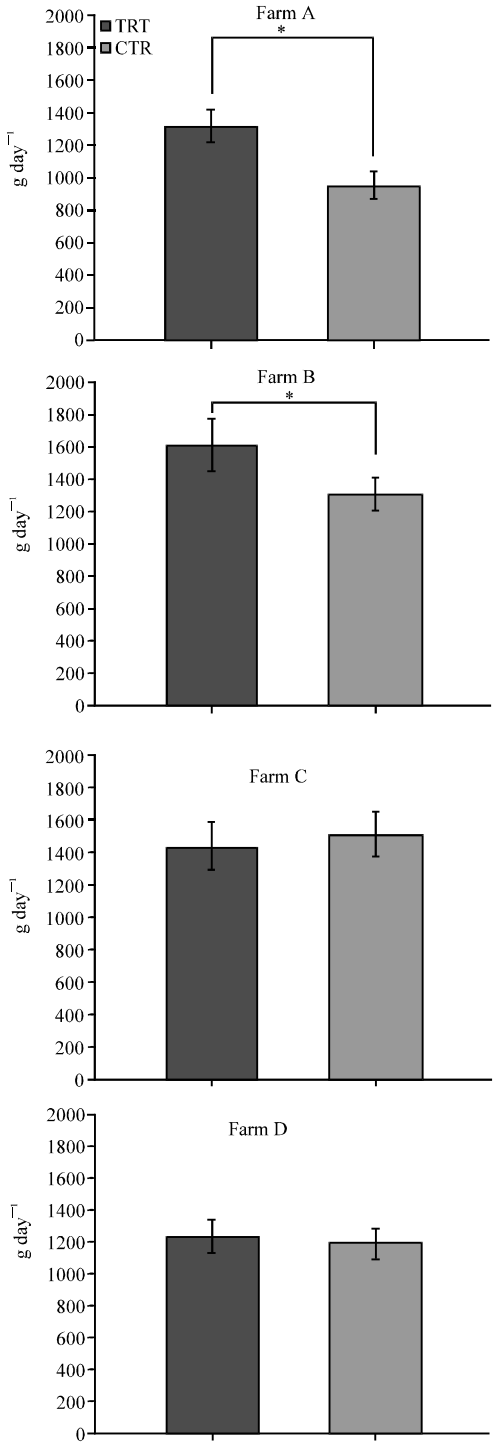

Fig. 1: Average daily gain (g day^{-1}) of bulls during the trials (*: $p<0.05$)

Table 1: Average color parameters of beef samples

	Farm A			Farm B			Farm C			Farm D		
Parameters	TRT	CTR	P[§]	TRT	CTR	P	TRT	CTR	P	TRT	CTR	P
L*	46.6	44.8	*	32.2	31.1	NS	35.6	35.7	NS	33.6	32.8	NS
a*	23.1	23.6	NS	22.2	21.5	NS	22.3	21.0	NS	19.5	20.0	NS
b*	7.5	7.5	NS	6.3	6.0	NS	6.6	6.2	NS	5.8	5.9	NS
Chroma	23.6	24.5	NS	23.1	22.3	NS	23.2	22.0	NS	20.3	20.9	NS
Hue	0.3	0.3	NS	0.3	0.3	NS	0.3	0.3	NS	0.3	0.3	NS

[§]Significance referred to comparison between TRT and CTR group in the same farm;NS: Not Significant;*: p<0.05. L*: lightness; a*: redness; b*: yellowness

Table 2: Average physical parameters of beef samples

	Farm A			Farm B			Farm C			Farm D		
Parameters	TRT	CTR	P[§]	TRT	CTR	P	TRT	CTR	P	TRT	CTR	P
DL (%)	3.9	4.1	NS	3.9	3.1	*	4.8	4.1	NS	4.1	4.2	NS
MCS (%)	18.9	19.8	NS	18.1	20.2	NS	16.3	16.5	NS	14.9	14.1	NS
CL_{MCS} (%)	25.7	25.4	NS	25.2	21.8	*	20.9	22.4	NS	17.8	16.9	NS
WB (kg)	6.0	6.4	NS	8.1	7.9	NS	8.0	8.1	NS	6.9	6.6	NS

[§]Significance referred to comparison between TRT and CTR group in the same farm;NS: Not Significant;*: p<0.05. DL: Drip Losses; MCS: Meat Cooking Shrinkage; CL_{MCS}: Cooking Losses based on MCS method; WB: Warner-Bratzler shear force

weight than the CTR ones (farm A: 647 vs. 632 kg; farm B: 692 vs. 645 kg). The same difference between ADG of TRT and CTR groups did not occur in the farms C and D. Very few studies have been published on effects of plant extracts on beef cattle performance. Supplementation with a commercial mixture containing thymol, eugenol, vanillin and limonene was not able to affect dry matter intake and ADG of beef cattle (Benchaar *et al*., 2006). Devant *et al*., (2007) supplemented Holstein bulls with a blend of plant extracts containing cynarin, ginseng and fenugreek. Rumen fermentations were significantly affected by plant extract supplementation while only a numerical improvement in ADG was observed.

The bulls were slaughtered when they reached the optimal finishing status set by the breeder and the beef expert of the abattoirs. Bulls of TRT and CTR groups are similar for SEUROP and fatness scores. At the slaughterhouse, SEUROP and fatness scores of carcasses are in close agreement with data reported by Alberti *et al*. (2008). Similarly, pH_0, pH_{24} and their relative temperature recorded on the carcasses are not different and indicate a correct process of carcass cooling and meat acidification. The correctness of these processes is widely recognized as a positive influence on several meat parameters such as tenderness (Lawrie, 1998).

The addition of the phytotherapic compound in the diets does not have any effect on the lightness, redness and yellowness of the meat samples (Table 1). Only in the farm A, the TRT group shows greater meat lightness (L*) compared to the CTR group. However, this difference was not confirmed in the other three farms. As reported in some studies, the inclusion of plant extracts in the diet may have effects on meat color stability to oxidation (Rochfort *et al*., 2008). High intake of tocopherols, flavonols (e.g., quercitin) and polyphenolics correlate with a greater stability of meat color (Demeyer *et al*., 2004). Table 2 shows physical parameters measured on beef samples. Drip and cooking losses, cooking shrinkage and shear force are not globally influenced by the use of the phytotherapic compound in the diets.

Only in the farm B drip and cooking losses show significant differences. From the very few studies available in the literature, the diet seems not affecting the drip loss (Lawrie, 1998). Age and stress are indeed pre-slaughter factors that may influence water losses of carcasses and meat. To the best of the knowledge, no studies on meat drip losses take into account the effect of adding plant extracts into diet.

Results on fatty acid profile are shown in Table 3. TRT beef samples of farm B are richer in alphalinolenic acid (C18:3 n3). However, this difference is not supported by other significances: Polyunsaturated Fatty Acids (PUFA) for example are similar in the two groups. Nutritional studies highlight that not only macronutrients (protein, fat, carbohydrate) but also plant bioactives may affect carcass and meat composition. Sillence (2004) show that essential fatty acids such as Conjugated Linoleic Acid (CLA) can improve the fat:lean ratio of carcasses in some circumstances.

Bas and Morand-Fehr (2000) suggest that the amount and composition of lipids in the diets of ruminants may have an influence on the fatty acid composition of fat and muscle tissues. In fact such improvements in the fatty acid profile of meat were obtained by feeding ruminants on pasture. In these conditions, fresh grass that it is a rich source of PUFA, represented the main or in some cases, the only it can be expected that the small amount (50 g $days^{-1}$ for 60 days) of the phytotherapic compound in the diet will be unable to determine a significant variation in the fatty acid composition of meat.

Table 3: Fatty acid composition (% of total fatty acids) of M. longissimus thoracis et lumborum

	Farm A			Farm D		
Fatty acid	TRT	CTR	P[§]	TRT	CTR	P
C14:0	2.34	2.32	NS	2.12	2.80	NS
C14:1	0.46	0.51	NS	0.42	0.37	NS
C15:0	0.52	0.61	NS	0.40	0.42	NS
C15:1	1.09	1.14	NS	0.18	0.25	NS
C16:0	27.05	27.92	NS	28.10	27.58	NS
C16:1	2.71	2.83	NS	2.90	3.42	NS
C18:0	22.58	22.75	NS	17.27	19.00	NS
C18:1	32.85	33.27	NS	38.69	35.79	NS
C18:2 n6	9.49	7.77	NS	9.24	9.89	NS
C20:1	0.10	0.10	NS	0.09	0.08	NS
C18:3 n3	0.46	0.45	NS	0.30	0.18	*
CLA c9t11	0.38	0.33	NS	0.31	0.21	NS
∑SFA	52.48	53.60	NS	47.88	49.81	NS
∑MUFA	37.20	37.85	NS	42.26	39.92	NS
∑PUFA	10.32	8.55	NS	9.86	10.29	NS
PUFA/SFA	0.20	0.16	NS	0.21	0.21	NS

[§]Significance referred to comparison between TRT and CTR group in the same farm; NS: Not Significant; *: $p<0.05$. SFA: Saturated Fatty Acid; MUFA: Monounsaturated Fatty Acid; PUFA: Polyunsaturated Fatty Acid

Table 4: Average sensory characteristics of beef samples

	Farm A			Farm D		
Characteristics	TRT	CTR	P[§]	TRT	CTR	P
Appearance	5.96	6.15	NS	5.21	5.59	NS
Ease of sinking	5.70	5.75	NS	6.21	5.94	NS
Friability	5.19	5.35	NS	5.66	5.77	NS
Residue	5.25	5.30	NS	3.75	3.19	NS
Initial juiciness	5.40	5.47	NS	5.62	5.94	NS
Sustained juiciness	4.97	4.99	NS	5.18	5.42	NS
Overall acceptance	5.42	5.42	NS	5.03	5.34	NS

[§]Significance referred to comparison between TRT and CTR group in the same farm; NS: Not Significant; *: $p<0.05$. All parameters are expressed following an 8-point structured scale (1 = minimum score; 8 = maximum score)

Many of the above mentioned detected parameters are strongly correlated with sensory traits of meat samples. It is therefore not surprising that no significant differences occurred in TRT groups compared to the respective CTR groups (Table 4). Although, tenderness evaluation by the taste panel did not always correspond with the instrumental shear force (Raes *et al*., 2003), the presented results recorded with the Warner-Bratzler shear device confirm the lack of difference in sensorial traits related to tenderness (e.g., ease of sinking). Similarly, comparable fat amounts and its acid profiles detected in beef samples support the substantial equivalence recorded by panelists between TRT and CTR groups. Finally, overall acceptance values suggest that the pythoterapic supplementation did not negatively affect sensory characteristics of beef samples.

CONCLUSION

The risk of development of antibiotic resistance in humans, strongly associated with the wide use of antibiotics in livestock as growth promoters, led to their ban in the European Union and contributed to increase worldwide the interest of plant extracts in animal nutrition. However, most studies to date have been *in vitro* experiments and only few papers referred to *in vivo* studies often with contrasting results.

Compared to antibiotics, plant extracts have multiple and subtler modes of actions that still need to be understood and explored. Moreover, ingredients of diets and physiological status of animals may influence their efficacy as feed additives. This can explain the significant effect of the phytotherapic compound on bulls' growth performance observed in two of the four farms examined. Increased amounts of supplemented plant extracts in well-designed experiments should help to clarify the dose-response relationship. Finally, the obtained results suggest that plant extracts might enhance performance without negatively affecting beef quality whilst at the same time, they highlight the need for further researches to validate plant bioactivity.

ACKNOWLEDGEMENTS

This research was made possible by the financial contribution of Government of Piedmont Region-Directorate of Agricultural Development, under the project Impiego di prodotti fitoterapici nell'alimentazione dei bovini da latte e da carne.

The researchers thank Claudio Malavasi of Biotrade (Modena, Italy) and Simone Mellano of Piedmontese Association of Beef Producers for their precious cooperation and technical support during the trials.

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