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Contact: tel.: +421 915 984 669
e-mail: folia.veterinaria@uvlf.sk

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INFLUENCE OF *SALVIA OFFICINALIS* ESSENTIAL OIL ON DIGESTION PARAMETERS AND INTESTINAL MICROFLORA OF BROILER CHICKENS

Marcin, A.[1], Levkut, M.[1], Revajová, V.[1], Šoltysová, B.[2], Naď, P.[1]

[1]University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
[2]NAFC Nitra, Agroecology Research Institute, Špitálska 1273, 071 01 Michalovce
The Slovak Republic

andrej.marcin@uvlf.sk

ABSTRACT

This study was aimed at the comparison of the effects of dietary applications of sage essential oil (*Salvia officinalis* L.) on some digestive enzyme activities in the chyme of the jejunum, digestive characteristics, and selected bacterial microflora in the caecum. Seventy, one-day-old broiler chickens (Ross 308) were allocated into two equal groups for 42 days. The feed mixture of the experimental group was supplemented with the essential oil at the level of 2.306 g.kg^{-1}. This supplementation was absent in the control feed mixture. The main volatile compounds were analysed: Eucalyptol 85, alpha-thujon 148, beta-thujon 72, camphor 149 and borneol 37 g.kg^{-1}. The digestive enzyme activities in the chyme of the jejunum increased as follows: amylolytic on days 16 ($P < 0.01$) and 29 ($P < 0.001$) as well as cellulolytic on days 16 ($P < 0.05$), 29 ($P < 0.001$), and 42 ($P < 0.01$). The proteolytic activity decreased on day 16 ($P < 0.01$). The intake of the additive, increased the digestibility of crude fibre ($P < 0.01$) on days 16, 29 and 42. The apparent assimilable mass coefficient of crude protein, corrected for protein catabolism, was increased in the experimental group on days 29 ($P < 0.05$) and 42 ($P < 0.01$). The counts of *Escherichia coli* in the caecum decreased ($P < 0.05$) on days 29 and 42. The supplementation of chickens with the sage essential oil increased the crude fibre digestibility, the amylolytic and cellulolytic activities in the chyme of the jejunum, and decreased the counts of *E. coli* in the *caecum*.

Key words: camphor; chickens; digestibility; essential oil; sage; thujone

INTRODUCTION

The search for new substances has been intensified since the ban of antibiotic performance enhancers in 2006 [26]. Therefore, an important group of effective and low-cost feed additives is no longer available.

One possible class of alternatives are plant extracts which are sources of different bioactive molecules with an effect on animal physiology and metabolism. Many of these compounds have been used in the form of whole plant ex-

tracts for food or medicinal applications for human beings [35]. Phytoadditives, which are herbs or herbal derivates, have attracted attention in animal nutrition because of their potential role as alternatives to antibiotic growth promoters. Their efficacy in the nutrition of chickens depends on: composition, inclusion level of phytogenic preparations into feed, bird genetics, and composition of the diets [25].

These types of additives are containing various chemical compounds such as: saponins, essential oils, tannins, and flavonoids. These phytochemicals are isolated from medicinal, aromatic and spicy plants and possess potential values for the manipulation of poultry digestion and productivity.

Essential oils are very complex mixtures of volatile, lipophilic compounds originating from plants. Due to their lipophility, they possess good intestinal and percutaneous absorption properties. After oral intake, they stimulate the secretion of digestive enzymes and increase gastric and intestinal motility [36]. Essential oils are able of enhancing the production of digestive secretions, stimulating blood circulation, exerting antioxidant properties, reducing levels of pathogenic bacteria and may enhance the immune status [3].

The value of many research studies is quite limited. This is because the use of blends of phytogenic feed additives in their studies obscures the process of evaluating the individual effects of the separate essential oils utilized.

Whereas, numerous studies demonstrated the effects of sage extracts on the health status and blood chemistry of poultry *in vivo* [7, 11]; there is a supposition that phytogenic compounds are able to enhance the enzyme activities and nutrient absorption in the digestive apparatus of poultry [37]. The effects of sage essential oil on the digestive characteristics, the enzyme activities in the small intestine, and selected intestinal microbial population are not entirely evident. Therefore, the objective of this study was to compare the influence of dietary intake of the sage essential oil on some digestive enzyme activities in the chyme of the jejunum, digestive characteristics and selected bacterial microflora in the caecum of broiler chickens.

MATERIALS AND METHODS

Chickens and diets

Seventy, one-day-old broiler chickens of hybrid Ross 308, were delivered from a commercial hatchery. They were divided at random into 2 groups of 35 animals (control/sage). The chickens were housed in two floor pens located in one hall of a chicken fattening farm (Michalovce, Slovak Republic) with constant access to feed and water. Both pens were identical with regard to the same direction and the same area (0.12 m^2 per broiler chicken).

Both groups were fed with mash diets (domica Ltd., Slovak Republic) for 42 days (Table 1). The methionine was used as the first limiting amino acid. The diets were prepared and formulated without antibiotics and growth promoters. The anticoccidial agents were added into the starter and grower feed mixtures of both groups.

The essential oil was isolated from the tops of sage (*Salvia officinalis* L., family *Lamiaceae*) by steam distillation of the plant biomass in Calendula Inc. (Nová Ľubovňa, Slovak Republic). The percentages of the main active compounds were analysed by gas chromatography (GC) using Hewlet-Packard 5890 Series II (injection input split splitless, capillary column HP-5, detector FIF, automatic injection HP 7673) with nitrogen as the carrier gas [10]. The herbal extract was added to diets of the experimental group with the resulting concentration of essential oils at the level of 2.306 $g.kg^{-1}$. The dosage of the sage essential oil, for the addition to the feed mixture, was selected according to previous results of chemical, microbiological and palatability tests [22]. The herbal ingredient was absent in the diets of the control group. The body weights of chickens were assessed once a week. The feed was weighed to evaluate the feed consumption.

Feed analysis

The samples of diets were analysed (Table 1) according to the official methods of the Association of Official Analytical Chemists [6]. The analyses were conducted for the determination of: dry mater (DM), crude protein (CP), crude fat (CF), ash, starch, and total carbohydrates. CF was analysed by the common method [34].

The ingredient composition of the experimental diets ($g.kg^{-1}$ diet) (starter/grower/finisher) was as follows: wheat 150.0/160.0/150.0; maize 453.5/482.5/495.5; soybean meal 300.0/221.0/213.0; soya 30.0/59.0/57.0; sunflower meal 20.0/20.0/20.0; rape-seed oil 5.0/17.0/28.0; calcium carbonate 13.0/14.5/14.0; sodium chloride 4.0/4.0/4.0; calcium hydrophosphate 13.5/10.5/10.5; L-Lysine 4.0/5.0/2.5; DL — Methionine 2.0/1.5/0.5; and Vitamin-mineral premix 5.0/5.0/ 5.0. Anticoccidial agent in diets: starter — Robenidin, grower — Narasin, and finisher — absent.

Table 1. Chemical composition of the experimental diets (g.kg^{-1} diet)

Ingredients	Experimental diets		
	Starter	Grower	Finisher
Dry mater	881.90	890.27	898.04
Crude protein	248.20	223.10	214.40
Crude fat	26.15	38.62	46.30
Crude fibre	44.10	35.20	31.30
Crude ash	66.00	57.90	55.30
Starch	340.34	398.02	416.55
Total carbohydrates	42.92	59.85	52.20
Calcium	9.68	9.41	9.19
Phosphorus	7.59	7.38	7.21
Sodium	1.82	1.80	1.41
Methionine	3.92	4.56	4.23
Lysine	10.05	13.39	12.05
Cystine	2.98	2.86	2.60
Metabolizable energy*	11.78 MJ	12.42 MJ	12.71 MJ

* — Calculation based on Kirchgesner and Roth [18]

Mineral content (mg.kg^{-1} diet): Mg 100.0, Mn 80.0, Zn 60.0, Fe 60.0, Cu 5.0, Co 0.2, J 1.0, and Se 0.15. Vitamin content (mg.kg^{-1} diet): retinol (A) 2.4, cholecalciferol (D3) 30.0, tocopherol (E) 20.0, menadione (K3) 4.0, thiamine (B1) 6.0, riboflavin (B2) 3.0, pyridoxine (B6) 5.0, cobalamin (B12) 0.02, folic acid (B9) 1.0, D-biotin (B7) 0.05, calcium D-panthothenate (B5) 10.0, and niacin (B3) 25.0.

The amino acid analyses of the experimental diet were performed by high performance liquid chromatography with AAA 400 amino acid analyser (INGOS, Czech Republic). This analyser is designed for the determination of amino acids on an ion exchanger column with a post-column derivatisation by means of ninhydrin. Lysine was determined after hydrolysis for 24 h at 110 °C with 6 mol.l^{-1} HCl. Sulphur-containing amino acids, methionine and cystine were analysed after cold formic oxidation for 16 h before the acid hydrolysis. The mineral composition of the feed (Ca, Na) was determined by atomic absorption spectrophotometry (AAS) with Shimadzu AA 6200 after the feed sample ashing in a muffle furnace [33]. The quantitative determination of phosphorus was performed spectrophotometrically [5]. The insoluble portion of ash in HCl was determined in the feed mixture as the residue of ash, after dissolving ash in diluted hydrochloric acid by weighing [8].

Blood analysis

Ten broilers from each group were randomly selected and anaesthetized with intraperitoneal injections of xylazine 0.6 ml.kg^{-1} (Rometar 2 %, SPOFA, Czech Republic) and ketamine 0.7 ml.kg^{-1} body weight (Narkamon 5 %, SPOFA, Czech Republic) on 16, 29 and 42 days of age. The numbers of birds were 25, 15 and 5 in the groups on days 16, 29 and 42 after selection. Blood samples for the preparation of the sera were taken by intracardial puncture after laparotomy and applied into anticoagulant-free tubes. The separation of sera was performed by centrifugation and the samples

were kept at 20 °C until examination. The determination of the total protein concentration in the serum was performed by the Bradford method at a wavelength of 595 nm [2]. The contents of calcium, magnesium and potassium were determined by AAS [33]. The quantitative determination of phosphorus was performed spectrophotometrically [5].

Analysis of intestinal contents

The samples of chyme from the jejunum and the caecum were placed into sterile tubes for digestive enzyme analyses and microbiological assays immediately after necropsy. The preparation of samples for the quantification of digestion enzymes activities was performed as follows. One gram of fresh sample was diluted with 49 ml sterile TBS buffer (TRIS-hydroxymethyl aminomethane 10 mmol.l^{-1}, HCl 0.5 mol.l^{-1}, pH 7.0) and homogenised. The samples were subsequently taken for the measurement of nonspecific proteolytic activity [4] with the substrate azocasein (Merck Ltd., Germany). The cellulolytic and the amylolytic activity [21] were analysed with the substrates methylhydroxyethylcellulose (Merck Ltd., Germany) and starch (Fisher Slovakia Ltd.), respectively. The quantification of the protein concentration was performed by the Bradford method [2].

For the microbiological examination, the caecal digesta were diluted 10 fold (1 : 9 w/v) by blending them with the anaerobically sterilized TBS buffer. Thereafter, a 0.1 ml sample was diluted by 10-2–10-7 and spread onto sterilized selective nutrient media McConkey agar (Merck Ltd., Germany) for *Escherichia coli*; Slanetz-Bartley agar (Merck Ltd., Germany) for *Enterococcus* spp.; and Rogosa agar (Merck Ltd., Germany) for *Lactobacillus* spp. for the purpose of cultivation. The numbers of Colony Forming Units (CFU.g^{-1}) of wet caecal digesta of *E. coli* and *Enterococcus* spp., were optically enumerated after aerobic cultivation for 24 h, whereas *Lactobacillus* spp. after stationary anaerobical cultivation for 48 h at 37 °C.

Check of digestibility

The digestibility was checked on 16, 29 and 42 days of age. The excreta was sampled directly from the cloaca into sterile glass containers on the designated day. The quantifications of crude fibre, ash and portion of insoluble ash in HCl were performed in excreta according to methods of Daněk et al. [8].

The digestibility coefficient (dc) was calculated according to the formula:

$$100 - \frac{\text{sample of excreta/ash insoluble in HCl in excreta}}{\text{sample of feed mixture/ash insoluble in HCl in feed mixture}}$$

The digestibility was determined by calculating the analysed content of nutrients in feed and excreta concerning the content of the insoluble portion of ash. The digestibility measurement of crude protein of broiler chickens with the correction for uric acid nitrogen was performed as follows: the contents of crude protein were measured in the excreta by Kjeldahl method [19] with the instrument Foss 2300 Kjeltec analyser (Foss Tecator, Sweden).

Because a part of the crude protein in the excreta originates from uric acid, the faecal crude protein should be corrected for uric acid nitrogen. At first, the correction for the endogenous losses resulting from protein catabolism was performed by determining the crude protein balance (Nb) of the animal as follows:

$$Nb = QiNi - QeNe$$

where Ni represents the crude protein content (g.kg^{-1}) of the feed, and Ne the crude protein content (g.kg^{-1}) of the excreta, Qi and Qe are the rates of feed intake and excretal output (g.kg^{-1}.day^{-1}). Subsequently, the crude protein losses to the moles of uric acid were converted to calculate the mass losses associated with uric acid excretion [29]. The mass correction for uric acid nitrogen is 3 g.g^{-1} N [17]. Given these values, the equation for the apparent assimilable mass coefficient of crude protein corrected for protein catabolism (AMCN) was as follows:

$$AMCN = 1 - ([Qe + 3(Nb)] / Qi) \qquad [14, 16]$$

Statistical analysis

The data are expressed as means ± standard deviation (SD) of single values (SAS, Version 8.2; SAS Institute Inc., 1999, Cary, NC USA). Means of the results from the treatments were compared by one-way analysis of variance. Treatment means were statistically compared by Tukey-Kramer multiple comparison test. Significance was declared at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

RESULTS

The percentage ranges of the active compounds of the sage essential oil are reported in Table 2. Digestive enzyme activities (amylolytic, cellulolytic, proteolytic) were assessed in the chyme of the jejunum (Table 3). The intake of the sage essential oil added into the feed mixture caused, on the one hand, enhancement of amylolytic activity on days 16 ($P < 0.01$) and 29 ($P < 0.001$), and on the other hand, a decrease on day 42 ($P < 0.05$). The cellulolytic activity was increased on days 16 ($P < 0.05$), 29 ($P < 0.001$) and 42 ($P < 0.01$). The decrease of the proteolytic activity was observed in the experimental group on day 16 ($P < 0.01$). The digestibility data of crude fibre and ash, as well as, the apparent assimilable mass coefficient of crude protein corrected for protein catabolism are summarized in Table 4.

The comparison of digestibility coefficients demonstrated a better utilisation of the experimental feed mixture, as far as the digestibility of crude fibre is concerned. The values of this parameter were increased on days 16 ($P < 0.01$), 29 ($P < 0.01$) and 42 ($P < 0.01$). The AMCN value increased in the experimental group on days 29 ($P < 0.05$) and 42 ($P < 0.01$). The changes of total protein and macroelements (phosphorus, potassium, calcium, magnesium) in the serum are shown in Table 5. The level of serum total protein was significantly higher ($P < 0.05$) in the experimental group on day 29. The microbiological counts in the caecal chyme of broiler chickens are summarized in Table 6. The total counts of *E. coli* were significantly lower ($P < 0.05$) in the experimental group on days 29 and 42. The basic growth data and the average feed intake of broiler chickens on days 16, 29 and 42 are summarized in Table 7. There was observed a significantly higher body weight ($P < 0.05$) in the experimental group in comparison to control on day 42.

Table 2. Gas chromatography analysis of active volatile compounds of the sage essential oil

Plant source	Compound	Content [g.kg^{-1}]
Sage*	Eucalyptol	85 ± 1
	Alpha-thujon	148 ± 1
	Beta-thujon	72 ± 1
	Camphor	149 ± 1
	Borneol	37 ± 1

* — the analysed density of sage essential oil was 0.915 ± 0.001 g.cm^{-3}

DISCUSSION

As far as the chemical composition of the sage essential oil is concerned, it is divided into ketones (camphore, α,β-thujone), terpenes (limonene, α,β-pinene) and alcohols (borneol, linalool) [12]. The sage essential oil tested in our study contained the following active com-

Table 3. Digestive enzyme activities in the chyme of the jejunum of broiler chickens
(n = 16; mean ± SD)

Age [day]	Group	Amylolytic (glucose) [mol.l^{-1}.min^{-1}]	Cellulolytic activity (glucose) [mol.l^{-1}.min^{-1}]	Proteolytic activity (azocasein) [g.ml^{-1}.min^{-1}]
16	Control	0.17^{a} ± 0.011	0.11^{a} ± 0.025	0.77^{a} ± 0.063
	Sage	0.26^{c} ± 0.038	0.13^{b} ± 0.023	0.48^{c} ± 0.062
29	Control	0.10^{a} ± 0.013	0.07^{a} ± 0.015	0.41^{a} ± 0.048
	Sage	0.15^{d} ± 0.032	0.13^{d} ± 0.038	0.41^{a} ± 0.035
42	Control	0.14^{a} ± 0.017	0.16^{a} ± 0.026	0.60^{a} ± 0.066
	Sage	0.12^{b} ± 0.015	0.23^{c} ± 0.018	0.55^{a} ± 0.070

Means with different superscript letters differed significantly:
^{a,b} — $P < 0.05$; ^{a,c} — $P < 0.01$; ^{a,d} — $P < 0.001$

Table 4. Apparent assimilable mass coefficient of crude protein and digestibility of crude fibre and ash
(n = 8; mean ± SD)

Age [day[	Group	AMCN	Crude fibre (dc)	Ash (dc)
16	Control	$0.25^{a} \pm 0.023$	$28.48^{a} \pm 0.712$	$39.04^{a} \pm 2.467$
	Sage	$0.22^{a} \pm 0.017$	$43.36^{c} \pm 0.997$	$44.64^{a} \pm 3.162$
29	Control	$0.28^{a} \pm 0.019$	$33.99^{a} \pm 0.510$	$55.80^{a} \pm 3.471$
	Sage	$0.35^{b} \pm 0.016$	$43.51^{c} \pm 1.650$	$52.94^{a} \pm 1.853$
42	Control	$0.25^{a} \pm 0.018$	$33.6^{a} \pm 0.874$	$46.18^{a} \pm 3.048$
	Sage	$0.44^{c} \pm 0.065$	$46.07^{c} \pm 0.848$	$49.84^{a} \pm 3.489$

AMCN — apparent assimilable mass coefficient of crude protein corrected for protein catabolism; dc — digestibility coefficient
means with different superscript letters differ significantly: ^{a,b} —P < 0.05; ^{a,c} — P < 0.01; ^{a,d} — P < 0.001

Table 5. Biochemical parameters in the serum of broiler chickens (
n = 8, mean ± SD)

Age [day]	Group	Total protein [g.l^{-1}]	P [mg.dl^{-1}]	K [mg.dl^{-1}]	Ca [mg.dl^{-1}]	Mg [mg.dl^{-1}]
16	Control	$154.85^{a} \pm 4.94$	n.d.	n.d.	n.d.	n.d.
	Sage	$153.99^{a} \pm 2.80$	n.d.	n.d.	n.d.	n.d.
29	Control	$115.08^{a} \pm 5.65$	$15.41^{a} \pm 2.98$	$19.07^{a} \pm 1.4$	$11.70^{a} \pm 3.33$	$3.09^{a} \pm 0.44$
	Sage	$120.64^{b} \pm 5.19$	$13.70^{a} \pm 1.96$	$20.27^{a} \pm 2.85$	$11.28^{a} \pm 2.39$	$3.03^{a} \pm 0.45$
42	Control	$176.88^{a} \pm 12.18$	$14.54^{a} \pm 2.74$	$20.32^{a} \pm 2.72$	$9.03^{a} \pm 2.52$	$2.68^{a} \pm 0.48$
	Sage	$181.14^{a} \pm 7.16$	$12.91^{a} \pm 1.83$	$20.27^{a} \pm 2.56$	$12.01^{a} \pm 3.51$	$3.11^{a} \pm 0.68$

n.d. — not determined; means with different superscript letters differ significantly: ^{a,b} — P < 0.05

pounds: eucalyptol (1,3,3-trimethyl-2-oxabicyclo[2,2,2] octane), α-thujone (1S,4R,5R)-4-methyl-1-(propan-2-yl) bicyclo[3.1.0] hexan-3-one, β-thujone (1S,4S,5R)-4-methyl-1-propan-2-ylbicyclo[3.1.0]hexan-3-one, camphor (1,7,7-trimethylbicyclo[2.2.1]heptan-2-one) and borneol (endo-1,7,7-timethyl-bicyclo[2.2.1]heptan-2-ol). In addition to these compounds, the essential oil, if an extract equivalent to 14.9 % of dry sage is used, contains: 6.9 % rosmarinic acid (55 % recovery), 10.6 % carnosic compounds (75 % recovery) and 7.3 % essential oil (42 % recovery) [9]. According to the cluster analysis [20], the leaf age and origin of the plants, has a significant impact on the composition of the essential oils. Additionally, Zheljazkov et al. [38] evaluated the effect of distillation times on sage essential oil yields and its composition. They concluded that the duration of the extraction process with the steam distillation has the potential to change the chemical composition of the produced essential oil.

Our study demonstrated that the dietary addition of essential oil from sage can be substantial for avian and herbivores in various aspects.

The significant increase of amylolytic and cellulolytic activity, as well as the decrease of proteolytic activity were observed in the jejunum in some sampling periods.

According to Rideau et al. [27] it is proposed that the modification of the chyme and transit rates during the day

Table 6. Microbial counts in the caecal chyme of broiler chickens
(n = 6; log CFU.g^{-1} wet digesta)

Age [day]	Group	*Lactobacillus* spp.	*Enterococcus* spp.	*E. coli*
16	Control	8.0[a]	5.60[a]	7.04[a]
	Sage	8.18[a]	6.36[a]	6.85[a]
29	Control	8.32[a]	6.20[a]	7.15[a]
	Sage	8.64[a]	6.60[a]	6.11[b]
42	Control	8.48[a]	6.89[a]	7.30[a]
	Sage	8.94[a]	7.08[a]	6.69[b]

Means with different superscript letters differ significantly [a, b] — $P < 0.05$; CFU — colony forming units

Table 7. Basic growth data and feed intake of broiler chickens in the experiment
(mean ± SD)

Group	IBW [g]	BW [g]			ADFI [g.day^{-1}]		
		day 16 (n = 35)	**day 29** (n = 25)	**day 42** (n = 15)	**day 1—16** (n = 35)	**day 17—29** (n = 25)	**day 30—42** (n = 15)
Control	38.86[a] ± 6.71	257.11[a] ± 53.66	773.29[a] ± 237.62	1746.67[a] ± 340.52	29.39[a] ± 6.33	80.45[a] ± 28.67	160.63[a] ± 41.58
Sage	40.57[a] ± 4.75	257.92[a] ± 53.35	886.38[a] ± 259.29	1947.06[b] ± 274.14	28.69[a] ± 6.42	89.52[a] ± 29.33	203.30[a] ± 26.87

Means with different superscript letters differ significantly [a, b] — $P < 0.05$; CFU — colony forming units; IBW — initial body weight; BW — body weight; ADFI — average daily feed intake

may affect the pancreatic enzyme fate and distribution in the small intestine as well as they observed that intestinal contents and enzyme activities were higher in egg-forming than in non-egg-forming laying hens. Basmacioglu et al. [1] determined the effect of dietary supplementation of oregano essential oil on the digestive enzymes and nutrient digestibility of broilers fed wheat-soybean meal based diets. The dietary supplementation of essential oil at two levels (250 and 500 mg.kg^{-1}) with or without enzyme, significantly increased the activity in the digestive system and improved crude protein digestibility.

Positive effects of the sage essential oil on the digestibility of the crude fibre in the sampling periods were observed, which is a positive relation with regard to the increased values of cellulolytic activity. Gugliemo and Karasov [14] calculated that the utilization of feed in birds is influenced by the mixing of urinary wastes with the undigested feed passing through the cloaca. Similarly, continual loss of mass and energy from the gastrointestinal tract in the form of epithelial cells, microbes and digestive secretions was observed. In cases where the endogenous losses of mass have not been quantified, the apparent assimilable mass coefficient (AMC) can be calculated as follows:

$$AMC = (Qi - Qe) / Qi = 1 - Qe / Qi$$

Hernández et al. [15] demonstrated that plant extract supplementation of diets for broiler chickens for 42 days improved apparently the whole-tract and ileal digestibility of the nutrients. The improvement of apparent faecal digestibility of DM and the ether extract digestibility were significant after dietary intake of 5,000 ppm essential oil extract from sage, thyme and rosemary in starter feed but no effect was detected for CP digestibility. Whereas, the extracts caused an improvement of the apparent faecal digestibility of DM and CP of the finisher diet.

The positive increase of total protein level in serum after intake of the sage essential oil was observed on day 29 in the experimental broiler chickens.

On the contrary, Traesel et al. [31], evaluated serum proteins with electrophoresis and plasma lipid peroxidation in broilers fed with diets supplemented with antibiotics or the blend of essential oils from oregano (*Origanum vulgare* L.), sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.) and pepper (*Capsicum frutescens* L.) crude extracts. The total globulins and betaglobulin fraction significantly decreased in the experimental groups after supplementation of the diet with 150 mg.kg^{-1} essential oils after 42 days of the experiment. However, the values of: albumin fractions, globulins (α-1, α-2, γ-glob), and the albumin/globulin ratio did not change significantly.

Therefore, the decrease in total globulins was due to a decrease in the betaglobulins. The decreased concentrations of betaglobulins or gamma globulins in the absence of hypoalbuminemia usually result from the decreased concentration of immunoglobulins which is the result of the effect of the blend of essential oils.

In addition to the dietary dosage of essential oils, the reason of the lower dosage of the blend of plant extracts with the carvacrol as the major component, into the diet, was the utilization of the microencapsulation technique, due to the volatility of essential oils [31]. Whereas, the sage essential oil was applied unprotected into feed mixture after dilution in the plant oil in the experiment.

The sage essential oil does not influenced the levels of macroelements in the sera of experimental animals after dietary intake. According to Čapkovičová et al. [7], the addition of sage extract (0.05 and 0.1 %) to the diet, led to a decreased concentration of plasma calcium (Ca reference values 2.09—2.52 mmol.l^{-1}); whereas, the levels of plasma phosphorus and magnesium did not differ significantly between treatments (phosphorus, magnesium reference values 1.60—2.10 and 0.75—1.3 mmol.l^{-1}, resp.). Similarly, Faixová et al. [11] observed that feeding chickens with diets supplemented with sage essential oil had a reducing effect on plasma calcium levels and demonstrated a reducing effect of borneol on plasma level of the element.

The *in vivo* experiment demonstrated a significant decrease of the *E. coli* population in the caecal chyme of broiler chickens after the intake of the sage essential oil as a result of our observations. Gut mucus and produced mucin could have influence on antibacterial protection of the gastrointestinal apparatus of broiler chickens against *E. coli*.

According to the published experimental results dealing with the antimicrobial activity of essential oils, the sage essential oil is typical with the lower activity in comparison with the similar extracts from oregano, thyme, clove and cinnamon. This declaration is in compliance with the results of Santurio et al. [28] who evaluated the antimicrobial activities of essential oils from *Origanum vulgare* (oregano), *Thymus vulgaris* (thyme), *Lippia graveolens* (Mexican oregano), *Cinnamomum zeylanicum* (cinnamon), *Zingiber officinale* (ginger), *Salvia officinalis* (sage), *Rosmarinus officinalis* (rosemary) and *Ocimum basilicum* (basil) against *Escherichia coli* strains isolated from poultry (n = 43) and cattle faeces (n = 36). They observed the highest antimicrobial activity of the essential oils from oregano, Mexican oregano, thymus and cinnamon. Further, Šarić et al. [30] determined the chemical composition and antimicrobial properties of *Salvia officinalis* with the main components of essential oil: 28.64 % of camphor, 21.90 % of 1,8 cineole and 19.92 % of α-thujone (16.92 %). The essential oil showed antimicrobial activity against all bacteria tested in the MIC range of 0.16—5.00 mg.ml^{-1} and MBC range of 0.63—5.00 mg.ml^{-1}. Similarly, Mekinić et al. [23] tested the contents of phenolics and antibacterial activity of five *Lamiaceae* plant extracts (sage, thyme, lemon balm, peppermint and oregano) against major foodborne pathogens such as *Campylobacter coli*, *Escherichia coli*, *Salmonella Infantis*, *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus*. They determined the highest content of total phenolics and non-flavonoids was in the sage extract, and it showed the best antibacterial activity, especially against Gram-positive bacteria and *E. coli*. However, the data presented by Golestani et al. [13] indicated that the potential antibacterial activity of the essential oil remedies (*Thymus vulgaris*, *Allium cepa, Allium sativum, Eucalyptus globulus, Salvia officinalis*, *Dianthus caryophyllus, Mentha spicata* and *Men-*

tha piperita) against *Escherichia coli* O157 : H7 by the disk diffusion method, could be suitable against colibacillosis, although this claim has to be confirmed in experimental and clinical trials.

The sage essential oil is able to influence the body weight, but not the average daily feed intake. Similar results were obtained by Traesel et al. [32] who evaluated the performance data in broilers fed diets supplemented with antibiotics or essential oils from oregano, sage, rosemary, and pepper crude extract. The dose of essential oils at the level of 100 mg.kg^{-1} is suitable for a final body weight and a weight gain similar to those which were observed in broilers supplemented with antibiotic growth promoters. Furthermore, Hernández et al. [15] did not observe any difference in feed intake or feed conversion after a dietary intake of 5,000 ppm essential oil extract from Labiatae plants (sage, thyme, rosemary) in broilers in the feeding period of 42 days. However, the growth of birds was faster in the controls.

CONCLUSIONS

The supplementation of diets for broiler chickens with the sage essential oil beneficially increased the amylolytic and cellulolytic activities in the chyme of the jejunum, the digestibility of crude fibre and caused a decrease of CFU of *E. coli* in the content of the caecum.

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DISTRIBUTION OF IODINE AND SELENIUM IN SELECTED FOOD COMMODITIES

Strapáč, I.[1], Baranová, M.[2]

[1]Department of Chemistry, Biochemistry and Biophysics, Institute of pharmaceutical chemistry
[2]Department of Food Hygiene and Technology, Institute of milk hygiene and technology
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
The Slovak Republic

imrich.strapac@uvlf.sk

ABSTRACT

The aim of this study was to investigate the distribution of the contents of iodine and selenium in selected food commodities. Fresh food commodities were mineralized and analysed for their iodine and selenium content by Inductively Coupled Plasma-Mass Spectrometry using the calibration curve as the method for determining the contents of the elements. The average fruit and vegetables concentrations of iodine were very low. The cow's milk, other dairy products, eggs, poultry, fresh water fish, beef, liver, and mushrooms are frequently regarded as the most important natural source of dietary iodine from common foods. The higher concentrations of selenium were recorded in the kidney, liver, pork, beef, poultry, fresh water fish, hen's eggs, cow's milk, other dairy products, wheat flour, fats, coffee, peppers, mushrooms and potatoes.

Key words: food commodities; Inductively Coupled Plasma-Mass Spectrometry; iodine; selenium

INTRODUCTION

The living cell is a colloidal suspension of the elements surrounded by a semipermeable membrane, which enables the exchange of mass and energy with the immediate environment, which is closely related to its auto reproduction. For the proper functioning of a living organism, it must have the so-called essential trace elements that are involved in the life cycle of the cells. Iodine and selenium are included in the group of essential trace elements. They participate in the management of important biochemical processes. Deficient or excess intake of iodine and selenium leads to disorders of the body.

Iodine belongs to the main group in the periodic table of the trace elements called halogens. In the valence sphere, iodine has seven electrons $(5s)^2(5p)^5$. It lacks one electron to obtain an octet. By receiving one electron, iodine forms a stable iodide anion and obtains the configuration of the nearest inert element, xenon. Grey to black crystals of iodine easily sublime as a purple vapour. Free iodine is not found in nature. Iodine is present in the Earth's crust in the amount of 1.10—4% [6]. It can be found in the form of

iodides, or iodates (Ca $(IO_3)_2$) in Chilean saltpetre and as periodates which are found in minerals and rocks which are released by weathering. Subsequently, rain water washes them into the rivers, seas and oceans. It accumulates in seaweeds, corals, fishes, crabs and many other animals and plants.

The major part of the essential iodine enters into the human body via food and food products. As per the World Health Organization [16] recommendation, the safe and adequate dietary intake of iodine for infants to adults ranges from 50 to 200 µg per day. Iodine, as one of the trace elements, is required for the production of thyroxine T4(3,5,3',5'-tetraiodothyronine) and triiodothyronine T3(3,5,3´-triiodothyronine) hormones for the proper growth and development of the human body. Iodine deficiency causes severe disorders like cretinism, mental retardation and its excessive intake can lead to thyroiditis [1].

Selenium belongs to the sixth main group of the periodic table of the elements commonly called chalcogens (yield-forming elements). In the valence sphere, it has six electrons$(4s)^2(4p)^4$. It lacks two electrons to obtain an octet. By receiving two electrons, selenium forms a stable anion Se^{2-}. In nature, selenium is present in the form of selenides accompanying sulphides.

Selenium is highly toxic for the human body, but in essential concentrations, it is very important for human life. The safe and adequate dietary intake of selenium for infants to adults ranges from 50 to 200 µg per day [16]. The optimum Daily Value (DV) for selenium intake is 1 $\mu g.kg^{-1}$ [13].

Selenium, primarily known for its antioxidant, anti-inflammatory and antiviral properties [8, 11], is an essential trace mineral for normal growth and development in livestock and humans. Selenium exerts its biological functions as a component of at least three groups of proteins: glutathione peroxidase that is responsible for the reduction of hydroperoxides in cells, plasma and gastrointestinal tract; the iodothyronine deiodinases, responsible for the peripheral deiodinates of thyroxin (T3) to 3,5,3' triiodothyronine (T2) and other metabolites; and thioredoxin reductases, which are involved in many cell functions, including the control of apoptosis and maintenance of the cellular redox state [12]. Selenium is present there as selenocysteine, selenomethionine and methylselenocysteine [3].

Selenium has an important role in human nutrition. The potential health benefits of dietary selenium includes improved immune response and thyroid function, as well as cancer chemoprevention. The importance of selenium to humans and livestock is described in detail in the study by Palmieri and Szarek [10].

The aim of this study was to investigate the distribution of iodine and selenium content in selected food commodities available in Slovakia.

MATERIALS AND METHODS

As experimental materials, we used food commodities which were delivered for analysis to the State Veterinary and Food Institute in Kosice. The samples included commonly consumed foods of vegetable and animal origin (Table 1 and 2). The reagents used were of the highest quality (Suprapur and p. a). Water was purified and deionised. Before the analysis, samples were mineralized with 65 % HNO_3 (Merck, Germany) in the microwave equipment MWS-2 BERGHOF (Germany). The weight of the samples were about 0.3 to 1.0 g of fresh weight (FW) or dried weight (DW) material. The final volume of the samples was 25 ml. Samples were analysed on an ICP-MS Agilent 7500C (USA) by accredited methods at the State Veterinary and Food Institute in Kosice. Iodine and selenium contents in $mg.kg^{-1}$ were obtained from the calibration curves.

RESULTS AND DISCUSSION

The concentration levels of iodine and selenium measured in dry (DW) or fresh (FW) matter of food commodities are summarized in Tables 1and 2 and in Fig. 1. The concentrations of iodine and selenium in the foods examined in this study were very low. This was probably caused by the low concentrations of these trace elements in the soil [6].

Higher values of iodine were found in milk and milk products and in hen's eggs (Table.1). The highest mean value of iodine was found in curds (0.382 $mg.kg^{-1}$). Lower mean values of iodine were found in cream cheese (0.278 $mg.kg^{-1}$), butter (0.150 $mg.kg^{-1}$), and fresh cow's milk (0.143 $mg.kg^{-1}$). The mean value of iodine in fresh cow's milk (0.143 $mg.kg^{-1}$) was lower in comparison to the published values [14] and ranged from 68.6 $\mu g.l^{-1}$ to 1000.6 $\mu g.l^{-1}$. Bhagat et al. [1] found 0.20 ± 0.01 $mg.kg^{-1}$ of iodine in skimmed cow's milk. The values of iodine and selenium in cow's milk mostly depend on the quality of feedstuffs and on the supplementa-

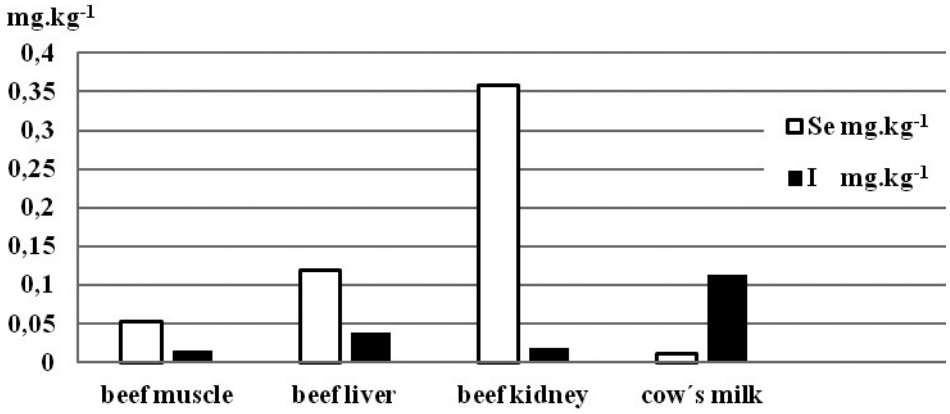

Fig. 1. Accumulation of selenium and iodine in beef muscle, liver, kidney and milk

tion of mineral additives which can increase the content of trace elements in milk [5]. Similarly, we were able to obtain eggs with higher values of selenium and iodine [15].

The iodine and selenium content of plant foods varied from species to species (Table 2). The mean iodine content in fruit (apple) and vegetables (onion, carrot, cabbage, tomato, potato) from domestic production was 0.001 mg.kg^{-1} FW. The mean selenium content in the commodities was also low and varied from 0.007 mg.kg^{-1} (cabbage) FW to 0.021 mg.kg^{-1} (potato) FW.

A higher mean value of iodine was detected in green pepper (*Capsicum annum*) (0.093 mg.kg^{-1} FW), and of selenium in oranges 0.258 mg.kg^{-1} FW). Haldimann et al. [4] determined the iodine content in fresh fruit in the range of 2 to 75 ng.g^{-1} DW and in samples of fresh vegetables in the range of 9 to 203 ng.g^{-1} DW.

In the samples of mushrooms (Table 2), the mean iodine content was between 0.007 mg.kg^{-1} (Shiitake – *Lentinula edodes*) and 0.027 mg.kg^{-1} (Oyster mushroom – *Pleurotus ostreatus*). Haldiman et al. [4] determined the iodine content in mushrooms to be in the range of 44 to 426 ng.g^{-1} DW.

The highest level of selenium in mushrooms was found in Parasol mushroom (*Macrolepiota procera*) (0.052 mg. kg^{-1} DW). The concentrations of selenium in the samples of mushrooms corresponded to the published values of selenium in edible mushrooms. Falandysz [3] found that the content of selenium in edible mushrooms was in a range from 5 µg.g^{-1} DW (*Lycoperdon, Lycoperdon* spp.) to 370 µg.g^{-1} DW (*Scutiger pes-caprae*, *Albatrellus pes-caprae*). A particularly rich source of selenium could be found in the selenium-enriched mushrooms that are cultivated on a substrate fortified with selenium (as inorganic salt or selenized yeast). The selenium-enriched Champignon mushroom (*Agaricus bisporus*) can contain up to 30 or 110 µgSe.g^{-1} DW [3].

Pork fat, butter and vegetable oil had almost the same values of selenium (Table 1); 0.074 mg.kg^{-1} FW on average. The mean iodine content in these fats, varied from 0.005 mg.kg^{-1} in vegetable oil, to 0.15 mg.kg^{-1} in butter.

The mean iodine content in skeletal muscles (Table 1) varied between 0.005 mg.kg^{-1} FW (wild boar, Sus scrofa) to 0.057 mg.kg^{-1} FW (chicken breast). The higher mean iodine (0.057 mg.kg^{-1}) and selenium (0.108 mg.kg^{-1}) values in chicken breasts and hen's eggs (0.341 mg I.kg^{-1} and 0.115 mg Se.kg^{-1}) were probably the result of iodine and selenium enriched mineral additives used to feed the chickens. Travnicek et al., [15] reported on the iodine content in consumer eggs. In the Czech Republic, 1 egg from a large flocks contained, on average, 31.2 µg iodine, while 1 egg from small flocks contained 10 µg.

The selenium content in the meat of farm animals was higher than in the meat of wild animals (Table 1). The selenium content in pork was 5.7 times higher than in the meat of wild boar (*Sus scrof*a) and the selenium content in beef was 3.1 times higher than in the meat of roe dear (*Capreolus capreolus*), and 6.9 times higher than in the meat of the red deer (*Cervus elaphus*).

Kursa et al. [7] evaluated the iodine and selenium contents in the skeletal muscles of red deer (*Cervus elaphus*), roe deer (*Capreolu scapreolus*) and wild boar (*Sus scrofa*) in the western and southern regions of the Czech Republic and in the Protected Landscape Area Šumava with a low content

Table 1. Mean content of selenium and iodine in food commodities of animal origin
(n = 3, with the exception of commodities marked with*)

Food commodities	FW/DW	Selenium [mg.kg^{-1}]	SD	Iodine [mg.kg^{-1}]	SD
Cow's milk	FW	0.027	0.0198	0.143	0.0776
Cream cheese	FW	0.108	0.0552	0.278	0.0566
Curds	FW	0.071	0,0176	0.382	0.3358
Butter	FW	0.072	0.0181	0.150	0.0694
Pork (muscle)	FW	0.160	0.0395	0.007	0.0050
Beef (muscle)	FW	0.096	0.0257	0.010	0.0009
Chicken breast	FW	0.108	0.0905	0.057	0.0001
Rabbit (muscle)	FW	0.049	–	0.006	–
Wild boar (muscle)	FW	0.028	0.0073	0.005	0.0013
Red deer (muscle)	FW	0.014	0.0037	0.021	0.0263
Roe deer (muscle)	FW	0.031	0.0160	0.008	0.0017
Freshwater fishes	FW	0.117	0.0347	0.049	0.0005
*Beef (liver)	FW	0.120	–	0.039	–
*Rabbit (liver)	FW	0.214	–	0.006	–
Wild boar (liver)	FW	0.063	0.0290	0.013	0.0001
*Red deer (liver)	FW	0.022	–	0.032	–
Roe deer (liver)	FW	0.075	0.0424	0.012	0.0005
*Hen (liver)	DW	0.168	–	0.018	–
*Beef kidney	FW	0.358	–	0.018	–
Pork (fat)	FW	0.080	0.0419	0.023	0.0111
Hen egg	FW	0.115	0.0880	0.341	0.2320

FWor DW — measured in dry (DW) or fresh (FW) matter; * —only one sample was examined

of iodine and selenium in the water and soil. In red deer muscles, the mean iodine content was 44.9 ± 15.2 μg.kg^{-1} FW, ranging from 6.9 to 82.0 μg.kg^{-1}. The lower concentration in roe deer meat (mean 39.3 ± 14.1 μg.kg^{-1}; range 18.3—84.4 μg.kg^{-1}) may have been due to the difference between biotopes and food. The mean iodine concentration in the *Musculus gracilis* of wild boars was 55.9 ± 27.0 μg.kg^{-1} FW. The mean values of selenium content in the meat of red deer, roe deer and wild boars were 16.2 ± 8.4, 36.9 ± 16.6 and 27.6 ± 19.8 μg.kg^{-1} FW, respectively.

Our results were within the range of the above results. The highest content of selenium was found in rabbit liver and a minimum content was determined in red deer liver. The content of selenium in beef liver was 5.5 times higher than the content of selenium in red deer liver and 1.6 times higher than the content of selenium in the roe deer liver (Table 1). The highest content of iodine was found in beef liver (0.039 mg.kg^{-1} FW). A similar mean value of iodine (0.032 mg.kg^{-1} FW) was found in the liver of red deer. The lowest mean values of iodine were determined in the liver of roe deer (0.012 mg.kg^{-1} FW) and wild boar (0.013 mg.kg^{-1} FW).

Table 1 shows also the mean values of selenium and iodine in the muscle tissue of freshwater fish. The content of

Table 2. Mean content of selenium and iodine in food commodities of vegetable origin
(n = 3, with the exception of commodities marked with*)

Food commodities	FW/DW	Selenium [mg.kg^{-1}]	SD	Iodine [mg.kg^{-1}]	SD
Onion	FW	0.019	0.0148	0.001	0.0007
Carrot	FW	0.009	0.0005	0.001	0.0009
Cabbage	FW	0.007	0.0025	0.001	0.0001
Tomato	FW	0.008	0.0037	0.001	0.0002
Green pepper	DW	0.039	0.0176	0.093	0.1232
Chilli pepper	DW	0.021	0.0067	0.016	0.0109
Potato	FW	0.021	0.0254	0.001	0.0004
Apple	FW	0.008	0.0012	0.001	0.0001
Orange	FW	0.258	0.4341	0.001	0.0006
Baby food	FW	0.007	0.0043	0.002	0.0023
Apple juice	FW	0.013	0.0100	0.001	0.0001
Champignon mushroom	DW	0.016	0.0057	0.016	0.0066
Oyster mushroom	DW	0.027	0.0137	0.027	0.0251
*Shiitake	DW	0.017	–	0.007	–
*Armillaria mellea	DW	0.018	–	0.013	–
*Parasol mushroom	DW	0.052	–	0.022	–
Beer	FW	0.017	0.0062	0.001	0.0001
Wine	FW	0.014	0.0156	0.002	0.0007
Coffee	DW	0.055	0,0042	0.001	0.0001
Rice	DW	0.018	0.0060	0.002	0.0005
Wheat flour	FW	0.104	0.0950	0.009	0.0007
Vegetable oil	FW	0.070	0.0476	0.005	0.0052

FW or DW — measured in dry (DW) or fresh (FW) matter; * — only one sample was examined

these elements in the muscle tissue depends on the species, age and weight of the fish. It is known, from the literature, that the content of iodine in the muscles of freshwater fish is 5 to 10 times lower than that in the muscles of saltwater fish [2, 4].

The comparison of the accumulation of selenium and iodine in beef, beef liver and kidney and in cow's milk evidently shows that most selenium accumulates in the kidney, less in the liver and in meat and the least in cow's milk (Fig. 1).

The maximum of iodine is accumulated in cow's milk and the minimum of iodine is accumulated in meat. Similar results were reported by Mehdi et al. [9] for bulls fed with selenium enriched feedstuffs. The maximum of selenium was accumulated in kidneys, less in liver and the least in muscle tissue. So the offal of animals fed with a diet supplemented with the essential trace minerals are a good source of these substances for human nutrition.

CONCLUSIONS

This study provided information on the distribution of iodine and selenium in food commodities that are part of the daily diet of people. The determination of iodine in

food has been a challenging analytical problem for a long time. The concentration of iodine in most foods is too low. Iodine deficiency can be controlled through the fortification of food and food products with added iodine. The basic source of iodine is iodized salt, but people with a salt-free diet must look for alternative sources of iodine. An alternative source of iodine could be domestic foods, such as: curds, chicken eggs, cream cheese, cow's milk, pepper, chicken breasts, freshwater fish, offal and mushrooms. Very low concentrations of iodine were detected in the samples of fruits, vegetables and beverages.

The highest value of selenium was accumulated in animal offal (kidneys and liver). Acceptable concentrations of selenium were found in pork, chicken breasts, freshwater fishes, milk and milk products, in wheat flour, fats, coffee, green pepper, mushrooms and potatoes.

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INTESTINAL MUCUS LAYER AND MUCINS (A REVIEW)

Tarabova, L., Makova, Z., Piesova, E., Szaboova, R., Faixova, Z.

Department of Pathological Physiology
University of Veterinary Medicine and Pharmacy, Kosice
The Slovak Republic

lucia.tarabova@uvlf.sk

ABSTRACT

The gastrointestinal tract, like the urinary, respiratory, reproductive tracts and the surface of the eye, has large surface areas which are in contact with the exterior environment. The mucosal tissues in the gastrointestinal tract are exposed to large number of exogenous, water or food born microbiota. Therefore, they serve as access routes for different types of bacteria, parasites, viruses, enzymes and toxins. In order to protect the mucosal tissues against pathogens and aggressive enzymes, which are necessary in digestive processes, they are covered by a resident microbial flora and also by a viscoelastic adherent mucous gel layer. The mucus layer acts as the first line of defense against threats and also as a positive environment for beneficial endogenous microbiota adapted for symbiotic living. The quantity and quality of mucus layers varies throughout the gastrointestinal tube and is often changed and disrupted during the occurrence disease. A disturbed mucus layer in the intestine can result in changes in the whole organism, such as: impaired immunity, loss of weight and weak food conversion, which is important, especially in food animals. That is why several researchers have focused on these changes, both in humans and other animals, to find out methods and countermeasures, which will facilitate the best protection for the mucus layer in the intestine. In this review, we describe the composition and function of the mucus layer and mucins in the intestine.

Key words: gastrointestinal tract; intestinal mucus layer; mucins

INTRODUCTION

The gastrointestinal tract is covered by a mucus layer, which is the product of secretory cells forming the inner layer of digestive tube (*tunica mucosa*), which is in contact with lumen. The mucus layer acts as a physical barrier, protecting the mucosal surface from dehydration and mechanical damage. It has an important role against destructive hydrolases, digestive enzymes, pathogenic bacteria, viruses and parasites and other chemical insults. By lubricating, it helps the passage of the digestive matter and creates an es-

sential and stable environment at the mucosal surface for the enteric microflora. The mucus layer has an important function with its selective permeability, which is adaptable and may be regulated in response to extracellular stimuli, such as nutrients, cytokines and bacteria. It may also affect the nutrient absorption by changing the permeability of small nutrients released by the activity of brush border digestive enzymes. The mucus layer protects the mucosal tissue against pathogens by inhibiting the binding sites for the bacterial adhesines, maintaining high concentrations of secreted IgA and lysozyme and acting as a free radical scavenger [8]. Normal intestinal mucosal epithelium has a tolerance to commensal microbiota because of its ability to distinguish them from pathogenic microorganisms by their molecular patterns, such as microbe associated molecular patterns and pathogenic-associated patterns, through pattern recognition receptors such as cell surface Toll-like receptors and cytoplasmic nucleotide-binding oligomerization domain like receptors [10].

MUCUS IN THE INTESTINE AND COLON

As mentioned in the introduction, mucus in the intestine and colon is produced by cells covering the *tunica mucosa*, which lies in contact with the lumen of the digestive tube and is built from four principal cells: enterocytes, enteroendocrine cells, Paneth cells and goblet cells. All these cells arise by mitosis from pluripotent stem cells located near the base of the crypts of Lieberkühn [12, 19]. The main producer of highly hydrated mucus cover in the gastrointestinal tract are goblet cells. These cells are cup-like shaped, containing segregated organelles and are in contact with the lumen. Although goblet cells are distributed throughout the entire length of the gastrointestinal tract, their contribution to the epithelial volume is not constant, so the thickness of the mucus layer is physiologically not the same in different parts of the intestine [25].

The mucus layer in the intestine and colon is comprised of water, ions, cytokines and molecules of the immune systems, such as: immunoglobulin A and anti-microbial peptides, defensins, protegrins, collectins, cathlecidins and histatins, which facilitate the clearance of pathogenic organisms [14, 27].

MUCINS

The viscosity of the mucus layer results from non–covalent interactions between large and highly hydrated glycoconjugates — mucins, which are also products of goblet cells synthetization. Mucus glycoproteins are polydisperse molecules, specific with their ability to carry out multiple tasks at the mucosal surface of the gastrointestinal tract and their antimicrobial activity. They consist of a protein backbone and a high proportion of O-linked carbohydrates. Commonly found monosaccharides in mucins are: N-acetylgalactosamine, N-acetylglucosamine, galactose, fructose and sialic acids. In small amounts, there can also be found N-linked mannose saccharide [26]. According to Corfield et al. [6], there are 13 mucin gene members, each with specific glycosylation and characteristic protein domains, divided into two basic groups: secreted, and membrane-associated. Linden et al. [16] divided mucins into three groups with 15 genes: secreted gel-forming mucins, cell-surface mucins, and secreted non-gel forming mucins. Cell-surface mucins are present on the apical membrane of all mucosal epithelial cells and provide a barrier to limit the access of large molecules and other cells to the cell surface. Secreted gel-forming mucins are major constituents of the mucus layer and provide its viscoelastic characteristics. They are the product of the cells in the epithelial surface or by glands in the *tela submucosa*. In the small intestine and colon can be found from secreted gel forming mucins, only MUC2 type, and from the cell-surface mucins: MUC1, MUC3A/B, MUC4, MUC12, MUC13, MUC15 and MUC17. Non-gel forming mucins are not present in the intestine or colon [13, 22]. Another division of mucin in the intestine is based on the affinity of mucin content to histological staining. Immature goblet cells of the intestinal neoplasms and foetal intestinal cells produce neutral, still incomplete mucin. Acid mucin is the product of mature, sulphomucin — containing Goblet cells. There can be found two types of acid mucin in the intestine: sulphomucins, and sialomucins. It is suggested, that acidic mucins are more resistant against microbiological degradation, than neutral mucins, because they appear to be less degradable by bacterial glycosidases and host proteases. It is known, that the synthesis of mucin and staining characteristics of mucins in goblet cells correlate with or lack of differentiation and maturity/immaturity of goblet cells through disease, especially in malignancy [1, 7, 24].

There are two mainly used histological methods for microscopic examination: formalin fixation followed by paraffin embedding and tissue freezing followed by embedding in cryo-protectant media. To detect neutral and acidic mucin in mucus, there is the use of Periodic Acid Schiff staining, and for the amount of only acid mucin in mucus layer, it is Alcian Blue staining [5]. As it is known, mucin can be secreted in three ways of secretion showed by surface mucous-secreting cells: single granule exocystis, apical expulsion or compound exocystis and cell exfoliation [9].

REGULATION OF MUCUS SECRETION

The regulation of mucus secretion is controlled by the neural, hormonal and paracrine system and also by the immune system. Under physiological conditions, without pathogens or other abusive elements, there is balance between the mucus secretion rate and its erosion through enzymatic digestion caused by luminal proteases and mechanical shear and removal by movement of luminal contents. This mechanism leads to relatively stable thicknesses of the adherent mucus layer [3]. If there is an influence of pathogens or other insults, there can be found alterations in goblet cells including their hyperplasia, increased mucin secretion, and changes in mucin glycosylation. In addition to other components of the host immune response, these changes lead to clearing of the infections [15]. Mucosal pathogens can penetrate not only the disrupted mucus layer, but they may also disregard the M cells, which are specifically designed to capture and present microbes to the underlying lymphoid tissue as a hole in the mucus barrier. The reason is because in the place of M cells, there is a lack of goblet cells, only a thin layer of mucus barrier and glycocalix, formed by glycoproteins and glycolipids. The average physiological thickness of the mucous layer is 700 μm. Destroyed thicknesses of the mucus layer is found in pathological states like: peptic ulcer disease, ulcerative colitis and infection by *Helicobacter pylori*, *Escherichia coli* and *Campylobacter jejuni* [11, 18, 21].

INTESTINAL ABSORPTION AND THE ROLE OF THE MUCUS LAYER

Knowledge about the role of the mucus layer is very important because of its influence on the whole organism, such as: protection against pathogens, good condition, nutrient absorption and connected food conversion, monitored and needed especially in farm animals, like chicken, pigs and cows kept for meal production. Intestinal absorption, means transport of substrates from the intestinal lumen through the mucus and epithelial layers into the blood and lymphatic system and its special function of the plasmatic membrane of enterocytes. Molecules can penetrate the lipid membrane by: simple passive diffusion, carrier – mediated diffusion, active transport or pinocytosis. To enlarge the surface on which absorption can occur, the intestinal lumen creates folds, villi and microvilli, which results in a 600 - fold increase of the surface area of the small intestine. Lipophilic substances pass through a lipid membrane normally faster than hydrophilic substances [4].

Polysaccharides cannot be absorbed in the intestine, only monosaccharides can, which are products of starch degradation or originated from dietary disaccharides. The specific enzymes of the brush border, which are necessary for saccharides absorption, are synthesized in maturing enterocytes and embedded in the apical membrane. Carbohydrates, which could not be resorbed in the small intestine because of malabsorption, poor digestion, intestinal hurry, or decreased intestinal surface lack of enzymes, will reach the colon and will be degraded by bacterial hydrolases. One role of the small intestine is the hydrolysis of food proteins to the molecules, which can be transported through the venous system. Protein assimilation occurs mainly in the distal jejunum and proximal ileum and has three phases: luminal phase, brush border membrane phase and cytoplasmatic phase [4]. The luminal phase consists of cleavage of polypeptides by pancreatic enzymes like trypsin, chymotrypsin, elastase and carboxypeptidase. The brush border membrane phase works on two mechanisms: brush border hydrolysis of oligopeptides and membrane translocation of small peptides. After intracellular intake, dipeptides in the cytoplasmatic phase are hydrolyzed to the amino acids and then reach the venous system. Fat is absorbed in the luminal and mucosal phase. During the luminal phase, fat from food are solubilized as micellar dispersions of monomeric lipids by the interaction of components of the upper gastrointestinal tract. In the mucosal phase, fatty acids, cholesterol and fat-soluble vitamins are transported through the jejunal mucosa into the blood [4].

Because the European Union implemented the ban on the use of antibiotics as antimicrobial growth promoters in

animal nutrition, various feed additives are researched for their efficiency to promote growth performance and health [2]. It has been determined, that the dietary administration of phytogenic feed additives, such as essential oils, oleoresins and flavonoids, affect broiler growth performance, nutrient utilization and caecal microflora composition [20). The effects of these probiotics has also been investigated. Probiotics such as *Lactobacillus planetarium* were reported to induce MUC2 and MUC3 and to inhibit the adherence of enteropathogenic *Escherichia coli*. Enhanced mucus layers and glycocalyx and occupied microbial binding sites by Lactobacillus sp. provided protection against invasion by the pathogens [17]. Several studies on newly hatched chicken have been done to find out, how nutrition can affect the mucin content. It has been determined, that food deprivation — starvation immediately after hatching caused delayed mucosa development and perturbed mucin dynamics. On the other hand, intra-anionic nutrient supply had a trophic effect on the chicken small intestine and enhanced goblet cell development [24]. The experiments mentioned above are only fragments from a number of other studies done, and are still in progress, on sheep, pigs, chicken and rats. All of these studies point to the necessity to continue more experiments focused on the protection of the mucus layer and its secretion and also on the influence of pathogens on mucus. The collection of results from different studies and comparison of them should lead to lower incidences of infectious diseases caused by enteropathogens, both in humans and other animals and to figure out the best countermeasures like probiotics and other nutritive additives against mechanical, chemical and biological insults. This should lead to increased health condition and taking on weight in meat animals and to the protection of gastrointestinal mucus layer in humans, who undergo long-time antibiotic treatment and are exposed to enteropathogens or parasites.

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THE EFFECT OF LUPIN (*LUPINUS ANGUSTIFOLIUS*) SUPPLEMENTATION ON ADAPTATION OF EWES AFTER SHORT TRANSPORT STRESS

Sopková, D., Andrejčáková, Z., Vlčková, R.

Department of Anatomy, Histology, and Physiology, Institute of Physiology
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
The Slovak Republic

drahomira.sopkova@uvlf.sk

ABSTRACT

Vehicle transportation represents acute stress to animals with release of catecholamines and glucocorticoids from the adrenal gland resulting in impaired metabolic state. Such changes in metabolism may be reduced by the application of suitable feed supplement. The aim of this study was to test the effects of lupin supplementation applied after 1-hour transportation. Ewes in the control group (n = 7) were fed on trefoil-grass silage and hay, while the diet of the experimental group (n = 7) was supplemented with lupin groats (*Lupinus angustifolius*, var. SONET; 500 g per head per day) for 8 days. In both groups, blood was collected on the day of transportation and on Days 6 and 11 thereafter. Total blood parameters were assayed using spectrophotometry and fractions of protein, cholesterol, and lactate dehydrogenase using agarose electrophoresis. Lupin increased the albumin: globulin (ALB : GLB) ratio and beta-hydroxybutyrate (BHB) concentration and reduced serum cholesterol and lactate, however it had no effect on body weight, body condition score (BCS), plasma glucose, serum protein, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) cholesterol, lactate dehydrogenase (LDH) or alkaline phosphatise (ALP). Lupin may therefore be used as suitable feed supplement for sheep at times of high nutrient requirement.

Key words: cholesterol; glucose; lactate dehydrogenase; lupin feeding; protein; sheep; stress

INTRODUCTION

Animals react to numerous challenges during their daily routines with a variety of responses, including physical and behavioural changes in their body [24]. Short-term transportation represents acute stress to animals [31, 32] with release of catecholamines and glucocorticoids from the adrenal gland resulting in hyperglycaemia [11], reduction of live weight [37], and impaired immune system [31]. The following metabolic changes have been observed in ruminants after transport and handling stress:

1) increased activity of blood enzymes (creatine phos-

phokinase — 34, 38; lactate dehydrogenase and aspartate aminotransferase — 34);

2) increased concentrations of blood lactate [36], ketones [15], and beta-hydroxybutyric acid [18, 83]; and

3) altered blood urea nitrogen concentration [13].

Many strategies have been used for helping animals to cope with transport and handling, such as use of high-energy diets [22]. Lupin grain is a legume plant used for livestock feeding of poultry, pigs and ruminants for its good source of protein [19], which is suggested as a good potential source of plant protein very comparable in its nutritional composition to that of animal protein [41]. Moreover, lupin contains low fat and starch, lacks antinutritional substances [21, 45], and is high in non-starch polysaccharides and oligosaccharides [43]. Lupin feeding does not affect body weight, whether consumed in the short (sheep; 44) or long term (humans; 8). Lupin consumption reduces appetite and energy intake in humans [8], lowers glycaemia in rats [17], chicken [45] and humans [7]; however in cyclic female sheep it increases levels of glucose and is thus associated with higher ovulation rate [3, 44]. Lupin feeding improves blood lipids in pigs [20], rats [40] and humans [7], resulting in lower blood concentrations of cholesterol.

To test whether such effects of lupin supplementation could be applied with the same effects to ewes exposed to short-term transportation (1 hour), this study set out to evaluate metabolic blood parameters such as serum protein and its fractions (albumin and globulins and albumin: globulin ratio), cholesterol and its fractions (high-density lipoprotein, low-density lipoprotein, and very low-density lipoprotein cholesterol; HDL-C, LDL-C, VLDL-C, respectively), lactate, beta-hydroxybutyrate (BHB), activity of lactic dehydrogenase (LDH) and its isoenzymes (LDH1-5), alkaline phosphatase (ALP), and plasma glucose.

MATERIAL AND METHODS

Animals

The experiment was conducted during the sheep anoestrous period (May to June). The ewes were transported by road from a nearby sheep farm. The transport lasted 1 hour at about 20 °C and 55 % relative humidity in the morning (9.00 to 10.00 a. m.). This short-term transport caused stress to the animals. The animals were transported at an average speed of 58 km/h, avoiding abrupt accelerations and decelerations. The experiment was carried out under standard conditions in the Experimental Station of the University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic. Fourteen Merino ewes of age 4—7 years were stabled in pens with the possibility of pasture. Water was provided to ewes ad libitum. All procedures were approved by the Ethical Committee of the State Veterinary and Food Administration of the Slovak Republic (Approval No. 2371/08-221).

Experimental Design

The ewes were divided into 2 groups: the diet of the control group (C; n = 7) consisted of trefoil-grass silage and hay, while the diet of the experimental group (L; n = 7) was supplemented with lupin groats (*Lupinus angustifolius*, var. SONET; 500 g per head per day). Sheep were fed with lupin once a day at about 5 a. m. A schematic representation of the experimental design is shown in Fig. 1. The ewes in the experimental group were fed with lupin for 8 days from Day 0 (day of transportation). The ewes were weighed on days 0 (day of transportation), 6 (fifth day of lupin supplementation), and 11 (2 days after the end of lupin feeding) and their body condition score was calculated [42].

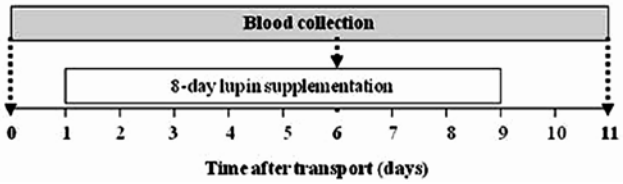

Figure 1. Schematic representation of the experimental design. Day 0 is the day of transport; Day 1 is the day of beginning of lupin supplementation which lasted 8 days (until Day 9). Blood was collected on Day 0, 6, and 11 after transport (dotted arrows)

Blood collection

Blood was collected routinely from the jugular vein of each animal with minimal disturbance to avoid excessive stress. Blood samples were collected without addition of anticoagulant on days 0 (immediately after transport), 6 and 11 of the experiment for metabolic profiles (protein, cholesterol, lactate dehydrogenase, alkaline phosphatase, beta-hydroxybutyrate, and lactate). Blood for glucose assessment was collected into centrifuge tubes with an addition of NaF. Samples were centrifuged for 10 min at 1500 × g after coagulation at room temperature (18 to 22 °C). Blood plasma for glucose was immediately assayed. Blood serum was stored at –20 °C until assayed.

Spectrophotometric assays

Assay in blood plasma

Analyses of glucose (GLUC) concentrations were performed on an ALIZÉ automatic biochemical analyzer (LISABIO, France) using commercial diagnostic kits (Randox, United Kingdom). Blood plasma was applied and replenished into substrate and absorbance was measured at 500 nm.

Assay in blood serum

In blood serum, the concentration of total cholesterol (TCH, λ 500 nm), total protein (TP, λ 540 nm), lactate (LACT, λ 500 nm), betahydroxybutyrate (BHB, λ 340 nm), activity of alkaline phosphatase (ALP, λ 405 nm) and activity of total lactate dehydrogenase (TLDH, λ 340 nm) were determined using commercial diagnostic kits (Randox, United Kingdom) with an ALIZÉ automatic biochemical analyzer (LISABIO, France).

Electrophoretic assay in blood serum

For electrophoretic study, 10 µl of serum was used for each separation. A HYDRASYS device (SEBIA, France) was used for the determination of protein and cholesterol fractions and activity of lactic dehydrogenase isoenzymes (LDH). The samples were separated using electrophoresis (HYDRAGEL 7 PROTEIN, HYDRAGEL 7 Lipo + Lp(a), HYDRAGEL 7 ISO-LDH, Ecomed, Žilina, Slovak Republic) on alkaline buffered (pH 9.2 for protein, pH 7.5 for cholesterol and pH 8.4 for LDH) agarose gels. The dried gels were prepared for visual examination and densitometry to obtain accurate relative quantification of individual zones. Then photographs of the gels were taken. Qualitative evaluations of the gels were done directly from the electrophoretograms, and the densitometric curves of the separations were created by means of EPSON PERFECTION V 700 PHOTO densitometer scanning at 570 nm.

Statistical Analyses

The experiment was conducted in two consecutive periods due to restricted capacity for animals to be stabled at the experimental station. The conditions of the experiment were kept the same in both periods. The results were calculated for both periods together. The groups of ewes fed and not fed with lupin consisted of 7 animals in each. Since there were no statistical differences between lupin-fed and control groups in terms of metabolic parameters, the dynamic (time-relative) changes in metabolic parameters were studied separately for the control and lupin-fed group. Variances between days after transportation in ewes fed and not fed with lupin were assessed with repeated measures ANOVA with Tukey's post test (GraphPad Prism 3.0 for Windows, GraphPad Software, San Diego California USA). All data are means with S.E.M. Differences from Day 0 are marked with superscript letters and declared to be significant at levels of $P < 0.05$, $P < 0.01$.

RESULTS

Live body weight and body condition score

Neither the body condition score nor body weight of ewes were affected by the lupin feeding after transportation (Table 1).

Metabolic profiles

Total protein and electrophoretic fractions of protein in ewes fed with lupin groats after short-term transport are

Table 1. Live body weight (BW) and body condition score (BCS) of ewes fed and not fed with lupin groats for 8 days after transport stress

Item	Day 0		Day 6		Day 11	
	Control	Lupin	Control	Lupin	Control	Lupin
BW [kg]	40.36 ± 2.07	43.43 ± 1.46	42.71 ± 1.96	45.29 ± 1.61	39.27 ± 2.32	43.26 ± 1.82
BCS	2.43 ± 0.19	2.82 ± 0.11	2.50 ± 0.16	2.82 ± 0.11	2.50 ± 0.16	2.82 ± 0.11

Table 2. Mean ± SEM concentrations of total protein (TP), albumin (ALB), alpha 1-globulin (alpha-1 GLB), alpha 2-globulin (alpha-2 GLB), beta globulin (beta GLB), gama globulin (gama GLB), and albumin: globulin ratio (ALB: GLB ratio) of ewes fed and not fed with lupin groats for 8 days after transport stress

Item [g.l^{-1}]	Day 0		Day 6		Day 11	
	Control	Lupin	Control	Lupin	Control	Lupin
TP	66.43 ± 7.10	72.42 ± 3.46	68.63 ± 3.62	72.13 ± 3.90	70.93 ± 3.83	69.50 ± 2.44
ALB	23.63 ± 2.64	33.18 ± 1.81	23.83 ± 3.55	35.27 ± 1.48	23.86 ± 4.03	34.73 ± 1.37
Alpha-1 GLB	5.78 ± 1.55	4.75 ± 0.37	7.22 ± 1.67	4.23 ± 0.44	6.86 ± 1.62	3.87 ± 0.15
Alpha-2 GLB	8.85 ± 1.71	8.40 ± 0.56	9.61 ± 1.36	7.86 ± 0.28	9.81 ± 1.55	7.18 ± 0.20
Beta GLB	12.00 ± 5.95	7.19 ± 1.70	9.94 ± 3.42	4.95 ± 1.16	10.07 ± 3.34	4.13 ± 0.61
Gama GLB	16.18 ± 1.92	18.90 ± 1.19	18.15 ± 4.58	19.83 ± 1.98	20.34 ± 5.11	19.69 ± 1.76
ALB: GLB ratio	0.66 ± 0.13	0.88 ± 0.10[a]	0.58 ± 0.12	1.00 ± 0.10[a]	0.56 ± 0.14	1.03 ± 0.10[a]

[a] — $P < 0.05$; values within rows, Day 6 and/or Day 11 compared to Day 0

Table 3. Mean ± SEM concentrations of total cholesterol (TCH), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), and very low-density lipoprotein (VLDL-C) cholesterol of ewes fed and not fed with lupin groats for 8 days after transport stress

Item [mmol.l^{-1}]	Day 0		Day 6		Day 11	
	Control	Lupin	Control	Lupin	Control	Lupin
TCH	1.74 ± 0.23	1.93 ± 0.24[a]	1.52 ± 0.13	1.71 ± 0.22[a]	1.05 ± 0.43	1.68 ± 0.21[a]
HDL	0.18 ± 0.03	0.19 ± 0.07	0.12 ± 0.03	0.21 ± 0.03	0.14 ± 0.03	0.29 ± 0.04
LDL	0.86 ± 0.10	0.96 ± 0.18	0.83 ± 0.12	0.78 ± 0.12	1.01 ± 0.20	0.67 ± 0.12
VLDL	0.79 ± 0.11	0.70 ± 0.10	0.57 ± 0.06	0.72 ± 0.10	0.68 ± 0.11	0.72 ± 0.09

[a] — $P< 0.01$; values within rows, Day 6 and/or Day 11 compared to Day 0

shown in Table 2. Lupin supplementation did not affect the concentration of total protein, albumin, and globulin fractions after short-term transport of ewes. However, the albumin: globulin (ALB : GLB) ratio was significantly increased ($P < 0.05$) during lupin supplementation and 2 days after lupin withdrawal (Day 11). In the control group, the protein parameters were not affected after transport stress.

Total cholesterol and its fractions in ewes fed and not fed with lupin groats immediately after transportation are shown in Table 3. Total cholesterol was significantly lowered ($P < 0.01$) by the lupin in ewes 6 days after transport, but its fractions were not significantly affected. Total cholesterol and its fractions in the control group were not affected after transport.

The activity of total lactate dehydrogenase (TLDH) in blood serum and LDH isoenzymes in blood plasma of ewes fed and not fed with lupin groats for 8 days were not affected after short transport (Table 4). The activity of alkaline phosphatase (ALP) in blood serum was not affected either in the control or in the lupin-fed group after short transport stress.

Serum concentrations of beta-hydroxybutyrate (BHB), lactate and plasma glucose of ewes fed and not fed with lupin groats for 8 days after transport stress are shown in Table 5. Serum BHB concentration in lupin-fed ewes significantly decreased 2 days after lupin withdrawal (Day 11). There was hyperglycaemia in both groups after transport. Plasma glucose concentrations significantly decreased after transport

Table 4. Mean ± SEM activity of total lactate dehydrogenase (TLDH), LDH isoenzymes (1—5), and alkaline phosphatase (ALP) of ewes fed and not fed with lupin groats for 8 days after transport stress

Item [μkat.l^{-1}]	Day 0		Day 6		Day 11	
	Control	Lupin	Control	Lupin	Control	Lupin
TLDH	14.97 ± 1.54	17.62 ± 1.21	15.22 ± 0.95	15.38 ± 0.90	15.62 ± 1.23	15.10 ± 0.88
LDH1	6.54 ± 0.86	9.43 ± 0.80	7.30 ± 0.49	8.47 ± 0.48	7.43 ± 0.39	7.92 ± 0.23
LDH2	1.20 ± 0.22	1.11 ± 0.05	1.08 ± 0.15	0.92 ± 0.07	1.17 ± 0.16	1.00 ± 0.08
LDH3	4.72 ± 0.55	4.49 ± 0.54	4.57 ± 0.40	3.82 ± 0.33	4.34 ± 0.34	3.74 ± 0.39
LDH4	0.98 ± 0.27	1.18 ± 0.19	1.38 ± 0.51	1.04 ± 0.05	1.64 ± 0.76	1.11 ± 0.07
LDH5	1.52 ± 0.45	1.41 ± 0.38	0.90 ± 0.17	1.14 ± 0.14	1.04 ± 0.13	1.34 ± 0.21
ALP	1.57 ± 0.18	1.53 ± 0.19	1.56 ± 0.14	1.85 ± 0.32	1.55 ± 0.12	1.98 ± 0.14

Table 5. Mean ± SEM concentrations of beta-hydroxybutyrate (BHB), glucose, and lactate of ewes fed and not fed with lupin groats for 8 days after transport stress

Item [mmol.l^{-1}]	Day 0		Day 6		Day 11	
	Control	Lupin	Control	Lupin	Control	Lupin
BHB	0.24 ± 0.02	0.24 ± 0.04	0.33 ± 0.02	0.36 ± 0.04 [a]	0.31 ± 0.06	0.22 ± 0.03 [a]
Glucose	6.54 ± 0.89[a, b]	7.94 ± 1.66[a]	3.86 ± 0.38[b]	4.13 ± 0.17[a]	3.94 ± 0.30[a]	3.82 ± 0.18[a]
Lactate	5.32 ± 1.12	7.12 ± 0.98[a, b]	4.29 ± 0.69	3.20 ± 0.65[a]	2.55 ± 0.52	2.26 ± 0.31[b]

[a] — $P < 0.05$; [b] — $P < 0.01$; values within rows, Day 6 and/or Day 11 compared to Day 0

in both groups ($P < 0.05$ and $P < 0.01$ in control; $P < 0.05$ in lupin-fed), but there was no significant difference between the groups. Similarly, serum lactate concentration decreased significantly after transport, both during lupin feeding ($P < 0.05$) and 2 days after lupin withdrawal ($P < 0.01$).

DISCUSSION

Short-term lupin feeding showed no significant effect on body weight and body condition score after 1-h transport stress, which is consistent with the observations of Viñoles et al. [44], who observed similar results in ewes fed with lupin for short time, but not after transportation. Kadim et al. [10] reported that 2-h transportation decreased body weight in lambs due to dehydration and food deprivation.

In our study, lupin feeding for 8 days after 1-hour transport did not affect the serum concentration of total protein and/or its fractions, but significantly increased the albumin : globulin ratio. The ewes had free access to water, so the ALB : GLB ratio could not have been increased due to dehydration when albumin was increased [12], but to decreased globulin fraction (alpha and beta globulin). This decrease in alpha and beta globulin fractions could be associated with the reduction of serum cholesterol concentration as observed in birds fed on lupin diets [47]. The finding of no effect of lupin on protein parameters is consistent with the observations in lupin-fed pigs [48].

Total cholesterol concentrations were significantly decreased by lupin supplementation in this study, which corroborates the widely-known cholesterol-lowering effect of lupin [7, 9, 20, 40]. The reduction of serum cholesterol results from the effect of lupin fibre [7, 9, 30]. The cholester-

ol-lowering effect of lupin is explained by several theories, including the effect of amino acid composition of dietary protein [6], lower absorption of cholesterol in the small intestine of piglets [20] and hamsters affected probably by lupin phytosterols, and/or it can also be associated with the stimulation of low-density lipoprotein (LDL) receptors by the protein component of lupin seeds [29, 39]. Some studies have reported reduction of LDL [18, 27] and VLDL cholesterol concentrations and tendency of HDL cholesterol to increase in rats [39], which is consistent with our results, although we found no change in VLDL concentration.

The present study showed no effect of lupin feeding on the activity of lactate dehydrogenase and alkaline phosphatase in the blood serum of ewes after transport. These observations are inconsistent with some studies (ALP – 34, 38; LDH – 34) which showed clear impact of transport on the activity of these enzymes, even though the animals were subjected to transport stress for a longer time, and lupin supplementation slightly decreased the activity of LDH [27] in hypercholesterolemic rats. Isoenzyme LDH-5 is an important diagnostic marker of transport stress [35], because it is used mostly by the cells of the liver and skeletal muscle and is released into the blood stream during stress. We suggest that maybe a higher dose of lupin grain can be used to improve the function of the liver and skeletal muscles impaired by transport stress, but this suggestion needs further confirmation. The metabolism of glycogen plays a key role during the muscle-to-meat transition. Lactate dehydrogenase enzyme participates in the metabolism of glycids by catalysing the reversible conversion of pyruvic acid to lactic acid. Lactic acid level reflects quantitative transformation of glycogen and indicates typical or atypical processes of meat ripening [14]. Perimortal situations increasing energy metabolism have considerable effects on factors that influence the ripening of meat. Meat of transported animals, e.g. wild rabbits, has lower concentrations of lactic acid, higher concentrations of phosphates and higher pH than for example hunted wild rabbits [16].

The mobilization of body fat reserves and energy balance can be efficiently determined by measuring serum concentrations of betahydroxybutyrate (BHB) [33] when nutritional stress is indicated and concentrations are over 0.8 mmol.l^{-1}. The present study showed serum BHB to be in the physiological range (0.34—0.68 mmol.l^{-1}); however the concentration of BHB significantly decreased after lupin withdrawal. This could mean that lupin supports production of ketones, but this theory should be further investigated. These observations are inconsistent with previous studies [46] where these concentrations were increased after transport. Lactate concentrations after transport were in the physiological range in both groups, control and lupin-fed. These observations are inconsistent with Mitchell et al. [23] and Schaefer et al. [36], who reported increases in serum lactate concentrations after transport stress. We suggest that the significant decrease in serum lactate as a result of lupin supplementation after transport may support the utilization of lactate for gluconeogenesis in ruminants after transport stress.

In the present study, plasma glucose concentrations in control and lupin-fed ewes reflected the hyperglycaemic effect [1] of increased activity of stress hormones [11], reaching a maximum after 2 hours from loading onto the transport vehicle [2], primarily due to breakdown of glycogen in the liver [25]. There was no difference between the groups, however, so we suggest that lupin had no effect on glucose concentrations after transport. Our findings are in contrast to previous studies which reported that lupin supplementation decreased blood glucose in rats [27], reflecting the effect of dietary fibre from the legumes [28] and saponins [26] which have hypoglycaemic activity, although it increased plasma glucose in ruminants. In goats, plasma glucose concentrations increased and reached the maximum 2 hours after 2.5-h transport and then decreased to pre-loading values.

We conclude that lupin has a cholesterol-lowering effect also when consumed in the short term. Lupin increased the ALB: GLB ratio and BHB concentration and reduced serum lactate, however it had no effect on body weight, BCS, plasma glucose, serum protein, HDL-C, LDL-C, VLDL-C, or ALP. Lupin may therefore be used as a suitable feed supplement for sheep at times of high nutrient requirement.

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ABDOMINAL ULTRASONOGRAPHY IN CATTLE

Munday, K.[1], Mudron P.[2]

[1]Prings Farmhouse, Prings Lane, Maplehurst, Rh13 6GZ
England
[2]Clinic of Ruminants, University of Veterinary Medicine and Pharmacy
Komenskeho 73, 041 81 Košice
The Slovak Republic

pavol.mudron@uvlf.sk

ABSTRACT

The main goal of this study was to design and propose specific abdominal zones that would contain the gastrointestinal organs in healthy cattle when scanned with trans-abdominal ultrasound. The second goal was to measure the intestinal wall thicknesses of the cranial duodenum, jejunum and colon and to compare healthy cattle intestinal wall thicknesses with pathological cases. All of the six healthy cattle had organs located in the zones proposed. Three of the four pathological cases had organs outside of the zones proposed. The six healthy cattle had an average cranial duodenum wall thickness of 2.45 mm, an average jejunum wall thickness of 1.90 mm and an average colon wall thickness of 3.02 mm. Of the pathological cases, three out of four had intestinal walls that were thicker than that of the average values for the healthy cattle. The thickest intestinal walls were found in the paratuberculosis positive cow. This cow had a cranial duodenum wall thickness of 9.5 mm, a jejunum wall thickness of 4.9 mm and a colon wall thickness of 10.0 mm. In conclusion, trans-abdominal ultrasonography has the potential to be an ideal diagnostic tool for the investigation of the bovine gastrointestinal tract and gastrointestinal disorders such as abscesses, peritonitis and displacement of the abomasum. Trans-abdominal ultrasound also has the potential to be a non-painful, non-invasive tool for the diagnosis of proliferative intestinal inflammations in cattle.

Key words: cattle; intestines; paratuberculosis; ultrasonography

INTRODUCTION

Ultrasonography has mainly been used in bovine veterinary medicine in the field of reproduction and lameness. The use of trans-abdominal ultrasound to examine the gastrointestinal tract has been studied to a lesser extent. Initial studies have used ultrasound to evaluate the normal anatomical position and the size of the abdominal organs in cattle with a focus on reticulum anatomy, physiology, and pathology [4]. Other studies have involved using ultra-

sound as a tool for diagnosing abdominal and gastrointestinal disorders such as peritonitis, left and right displaced abomasum, abscesses and ileus [2]. Currently, no studies have been published using ultrasound as a technique for diagnosing *Mycobacterium avium* subsp. paratuberculosis (Johne's disease) in cattle. Previous studies have used ultrasound to study *Mycobacterium avium* subsp. paratuberculosis in camels and goats [10]. Ultrasound may provide the opportunity to establish a diagnosis and sometimes a prognosis of abdominal disorders and can be used to monitor the progression of therapy of some of the major diseases encountered in a bovine practice. Ultrasound allows a "cow-side" diagnosis and can reduce time and money for the producer and spare pain for the animal [3].

The main goal of this study was to design and propose specific abdominal zones that would contain certain gastrointestinal organs in healthy cattle when scanned with trans-abdominal ultrasound. In addition, the study was designed to measure the thickness of the cranial duodenum, jejunum and colon walls.

METHODS AND MATERIALS

In this study, six healthy 500—600 kg Holstein × Friesian cattle were examined trans-abdominally with ultrasound. Four pathological cases were scanned, which were; left displaced abomasum (LDA), paratuberculosis, a cow post percutaneous abomasopexy and local peritonitis. The ultrasonographic examinations were performed on non-sedated, standing cattle using a 3.5—5.0 MHz convex transducer (SonoScape A5V) and was mainly focused on the examination of peritoneum, abomasum, duodenum, jejunum, and colon. The paratuberculosis in patient 2, was diagnosed according to history, clinical signs and positive results of blood examination by PCR [8].

The abdominal zones were designed to be able to easily identify the abdominal organs and their locations and to help in the diagnosis of pathological cases. The determination of the designed zones were based on bovine morphology in healthy animals [9]. Zone one (Fig. 1) is the left side of the abdomen, from the tuber coxae to the 6—8th intercostal space (ICS). The rumen and reticulum are located in this zone. Zone two is split into zone 2L and zone 2R (left and right side of the sternum). Zone two is located on the left and right side of the sternum in the ventral third of the abdomen from the 6—9th ICS. The dorsal border of zone 2L and 2R is at the level of the olecranon and the ventral border is the base of the abdomen. The reticulum is located within zone 2L and zone 2R. Zone three is located on the right side of the abdomen. Its dorsal border is at the level of ten cm over the olecranon and the ventral border is at the level of the olecranon. Zone three runs from the 7—10th ICS. The omasum is located within zone three. Zone four is the area 10 cm caudal to the xyphoid process of the sternum and to the right paramedian region of the ventral midline to the level of the umbilicus. The dorsal border of this zone is the level of the olecranon and the ventral border is the bottom of the abdomen. Zone four contains the majority of the abomasum, pylorus and part of the cranial duodenum. Zone five is located in the dorsal half of the abdomen. The dorsal border of zone five is processus transversus of the

Table 1. Thickness of intestinal walls (mm) in the control cattle (mean ± SD) and four pathological cases

Animal	Cranial Duodenum	Jejunum	Colon
Control cattle (n = 6)	2.45 ± 1.19	1.9 ± 0.4	3.02 ± 0.58*
Patient 1 (LDA)	6.1	2.8	7
Patient 2 (paratuberculosis)	9.5	4.9	10
Patient 3 (post abomasopexy)	5.5	2.2	7
Patient 4 (peritonitis)	1.8	1.4	2.1

* — Colon differs from jejunum ($P < 0.01$)

Fig. 1. Zone one (left abdomen: reticulum and rumen)

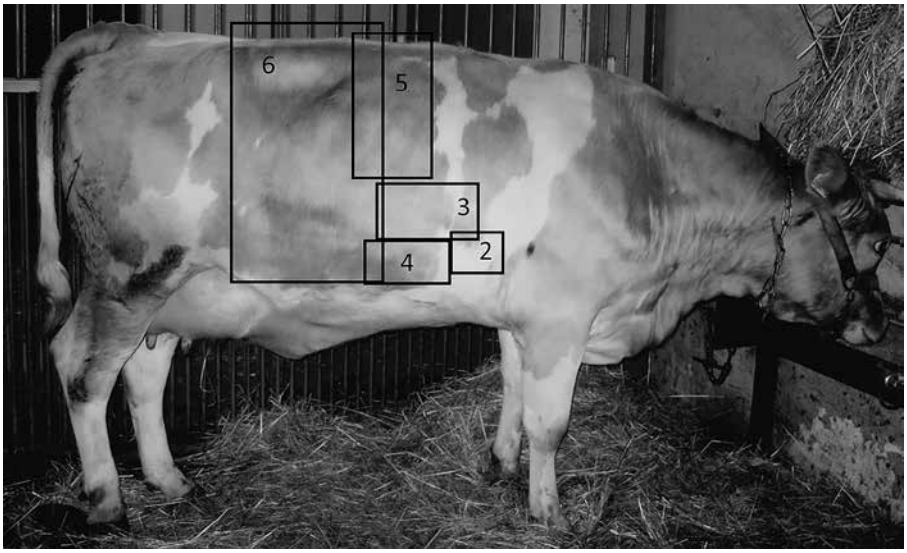

Fig. 2. Zones 2—6 (right abdomen: reticulum, omasum, abomasum, duodenum, jejunum, and colon)

thoracic vertebrae and the ventral border is at the level of the articulatio humeri. Zone five is located from the 10—12th ICS. The liver and cranial portion of the duodenum is located in zone 5. Zone six is divided into 3 subzones; 6A, 6B and 6C. Zone six extends from the 10th ICS to the tuber coxae. The dorsal border of zone 6A is the processus transversus of the lumbar veterbrae, the ventral border is at the level of the articulation humeri. Zone 6A contains the colon. The dorsal border of zone 6B is at the level of the articulation humeri and the ventral border is at the level of the articulation cubiti. Zone 6B contains loops of the jejunum and the parts of the colon may be visualised. The dorsal border of zone 6C is at the level of the articulation cubiti and continues ventrally to the linea alba. Within zone 6C, loops of the jejunum can be imaged (Fig. 2).

The intestinal dimensions and intestinal wall thicknesses were measured within the ultrasound examination after scan freezing.

The differences in the wall thicknesses among the duodenum, jejunum, and colon were statistically analysed by an unpaired *t*-test. The level of significance was set at $P < 0.01$.

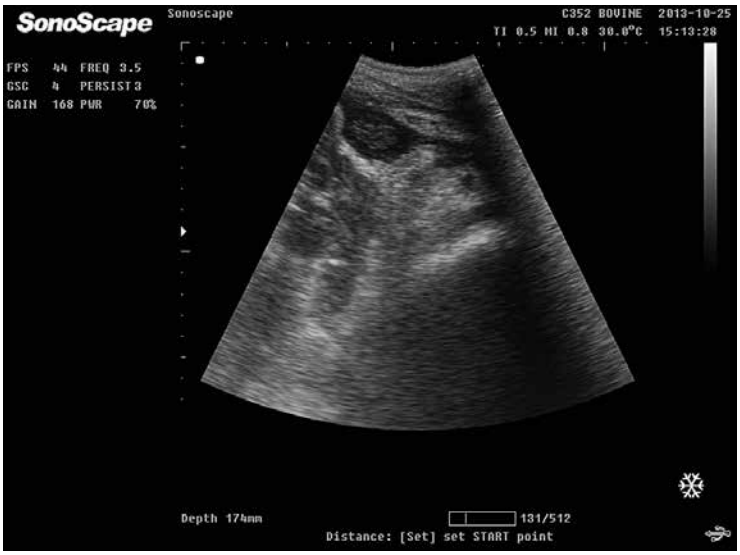

Fig. 3. Ultrasound of zone four in patient 3 with post abomasopexy complications. Abscess cranial to the umbilicus and thickening of the abomasal wall are visible. The abscess measured 19.3 × 37.7 mm

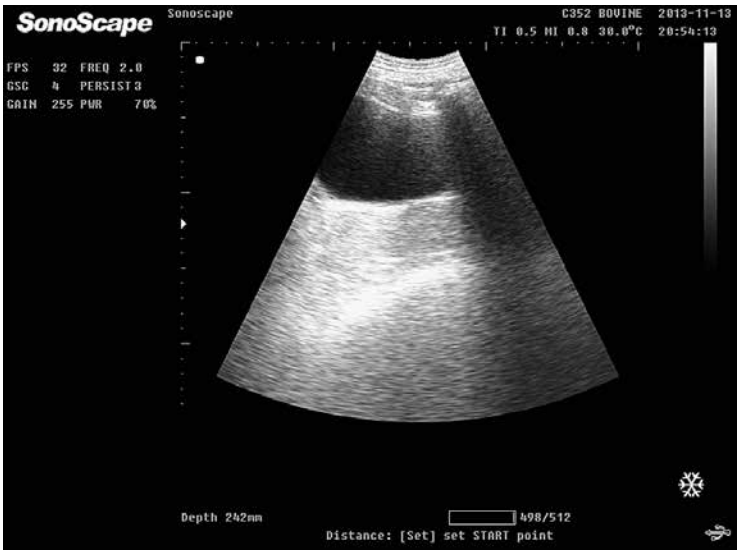

Fig. 4. Ultrasound of the hypoechogenic pocket of fluid within the abdominal cavity at the 9–10th ICS

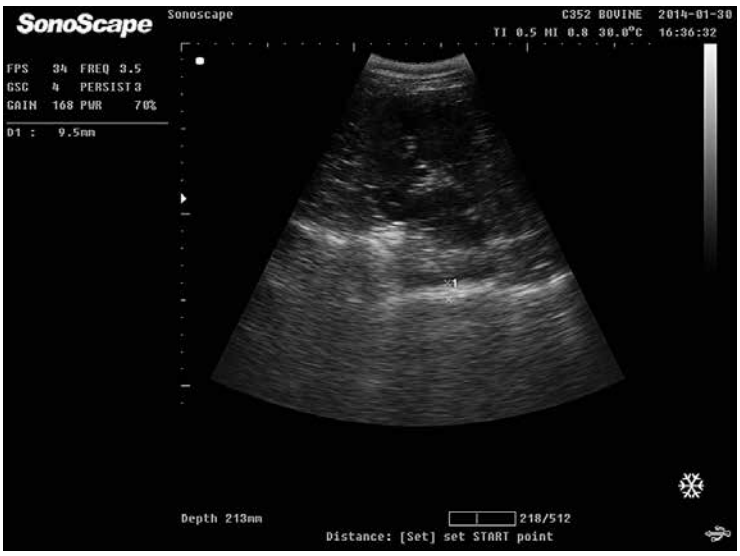

Fig. 5. Ultrasound of the cranial duodenum wall thickness (white line with two x) located in zone five of patient 2

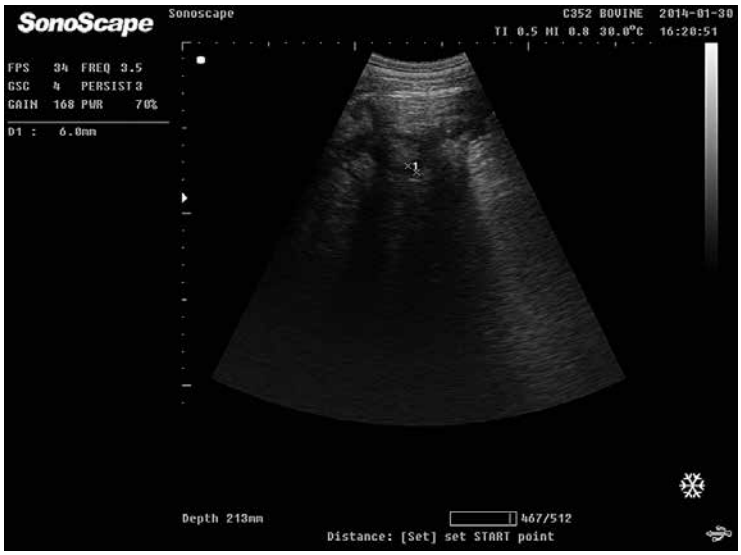

Fig. 6. Ultrasound of the jejunum wall thickness (dark line with 2×) imaged in a more cranial location than normal, at the level of the 8th ICS in zone 4, as well as zone 6B and 6C

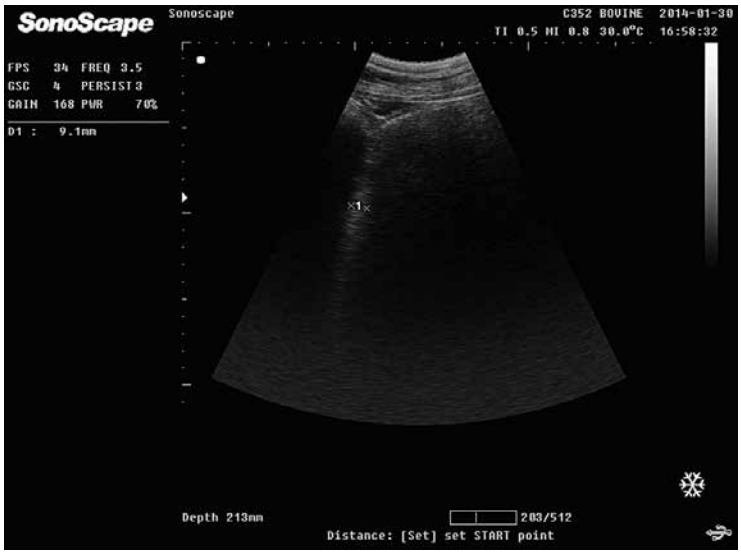

Fig. 7. Ultrasound of the colon wall thickness (light line with two ×) imaged in zone 6C of patient 2

RESULTS

All of the proposed zones were scanned in each animal. All six healthy cattle had their organs located in the specific zones proposed. Only patient 3, post abomasopexy had all the organs within the proposed zones. All the other pathological cases had their organs outside the described abdominal zones.

Patient 1 had LDA and its abomasum was imaged in zone one at the 11—12th ICS. All the other abdominal organs were displaced cranially and ventrally across the right abdomen due to the dilation and displacement of the abomasum to the left. Patient 2 was positive for paratuberculosis. Ultrasound was performed and it was found that the jejunum was in a more cranial position than normal. It was imaged in zone three at the 8—9th ICS. The ultrasound of patient 3, after abomasopexy surgery, revealed all of their organs to be within the zones previously proposed. The ultrasound of this patient also revealed an abscess located caudally to the fixation site, and cranially to the umbilicus (Fig. 3). Patient 4 was admitted to the clinic with a drop in feed consumption, drop in milk production, high tem-

perature and generally ill thriftiness and depression. Ultrasonography was performed and fluid, fibrin and purulent material was imaged at 9—10th ICS. Local peritonitis was diagnosed (Fig. 4).

The six healthy cattle had an average cranial duodenum wall thickness of 2.45 mm, an average jejunum wall thickness of 1.90 mm, and an average colon wall thickness of 3.02 mm (Table 1). The statistical analysis revealed a significant difference between the jejunum and the colon wall thickness ($P < 0.01$). Of the pathological cases, three out of four had intestines that were thicker than the average value from the healthy cattle (Table 1). The thickest intestines were found in patient 2 suffering from paratuberculosis (Fig. 5, 6, 7).

DISCUSSION

The proposed abdominal zones were scanned in all healthy cattle. All of the control cattle had their organs within the zones that were proposed. The only pathological case to have their gastrointestinal organs within the proposed abdominal zones was patient 3 that was post abomasopexy surgery. All of the other pathological cases had abdominal organs outside of the proposed zones. Patients with abomasal displacement are characterised by a changed position of the abdominal organs, due to the enlarged and displaced abomasum. The patient in our study suffered from a left displacement of the abomasum that led to displacement of intestines and liver cranially and ventrally in the right half of the abdomen which became "empty" due to displacement of the abomasums [6]. In the patient with paratuberculosis, the ultrasound scanning revealed that the forestomachs and liver were all within the proposed zones. However, the jejunum was located in zone 3, adjacent to the omasum at the 8—9th ICS, instead of zone 6B and C. These changes are thought to be due to inflammation within the intestines. This inflammatory process could be responsible for making intestines enlarged and heavier and thus they extended more cranially throughout the abdomen. When the size and position changes of the organs are not very strong, no changes in their location and borderlines can be observed like in the patient with post abomasopexy abscess in the abdominal cavity. However, without ultrasonography the abscess would not have been identified and therefore may not have been treated, which may have caused other systemic effects [4]. Moreover, ultrasound imaging of the abomasum in the post abomasopexy patient revealed inflammation, fibrous tissue and part of the "toggle" fixation from the abomasopexy operation within the parenchyma of the abomasum. All these findings can occur when the fixation of the abomasum is not performed correctly and belong to the most frequent complications of the percutaneous abomasopexy [7]. Pathological processes associated with diffuse peritonitis can also lead to changes in the positions of the abdominal organs. This is due to the changed volume of the organs, as both abomasum and intestines are usually suffering from decreased motility, leading to the accumulation of ingesta and gas within the lumen [3]. Such a situation could be seen in the patient with peritonitis in our study. The abomasum was located more caudally in zone 6C instead of zone 4. The abomasum wall had a hyperechogenic appearance and was thicker when compared to the healthy cattle. This was thought to be due to inflammation from the peritonitis. The liver and the cranial duodenum were imaged more cranially at the level of the 9—10th ICS. This was because the left kidney was found to be considerably enlarged, therefore displacing the other organs cranially and ventrally.

The intestinal wall thickness was chosen to measure because it has been proposed to be a potential parameter of intestinal health [3]. The wall of the normal small intestine is 2 to 3 mm thick and its luminal diameter is 2 to 4 cm in adult cattle [5]. The mean thickness of the intestinal walls in the six healthy cattle in our study was nearly within this range measured as: 2.45 mm (cranial duodenum), and 1.90 mm (jejunum). The mean thickness of the colon wall was thicker (3.02 mm) in comparison to the small intestine. Even if all the patients in our study suffered from abdominal diseases, only three of them demonstrated thicker intestinal walls than the six healthy cattle scanned. Surprisingly, the animal with the left displacement of the abomasum had thicker intestinal walls than the animal with peritonitis. Thus, individual reaction of the intestinal structures to pathological processes in the abdominal space can be suggested. The thickest intestinal walls when compared to the other pathological cases and the control cattle, was seen in the patient with paratuberculosis. The duodenum wall of this patient measured 9.5 mm, the jejunum measured 4.9 mm and the colon wall measured as 10.0 mm. This was three to four times thicker than the mean values from the control cattle. This correlates with information stating that

cattle positive for *Mycobacterium avium* subsp. Paratuberculosis, have three to four times thicker intestinal walls, especially the jejunum and ileocaecal valve section [1]. There is currently no data using ultrasonography to measure the thickness of the intestinal walls in cattle with intestinal disorders such as paratuberculosis. The only data available for ultrasound diagnosis of the thickness of the intestinal walls due to paratuberculosis is that from goats and camels [10].

In conclusion, trans-abdominal ultrasonography is an ideal non-invasive and economical diagnostic tool for the investigation of the bovine gastrointestinal tract and gastrointestinal disorders. This study has demonstrated the ability of the use of ultrasound to diagnose diseases such as local peritonitis, the presence of intra-abdominal abscesses, and the confirmation of displaced abomasum. Moreover, this technique may be a promising method in the diagnostics of clinical forms of paratuberculosis in cattle.

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BASIC ROLES OF SEX STEROID HORMONES IN WOUND REPAIR WITH FOCUS ON ESTROGENS (A REVIEW)

Čriepoková, Z.[1], Lenhardt, Ľ.[1], Gál, P.[2, 3, 4, 5]

[1]Department of Pathological Anatomy, University of Veterinary Medicine and Pharmacy, Košice
[2]Department for Biomedical Research, East-Slovak Institute of Cardiovascular Diseases, Inc., Košice
[3]Department of Pharmacology, Faculty of Medicine, P. J. Šafárik University, Košice
[4]Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University, Bratislava
The Slovak Republic
[5]Institute of Anatomy, 1st Faculty of Medicine, Charles University, Prague
The Czech Republic

pgal@vusch.sk

ABSTRACT

Previously, it has been shown that sex hormones, in particular estrogens, play an important role in the regulation of biological processes involved in tissue repair and regeneration. Accordingly, several studies have supported the beneficial properties of hormone replacement therapies (HRT) in postmenopausal models. The present review paper explores the potential for targeted sex steroid HRT as a new therapeutic option for the surgical management of wounds in postmenopausal women and animals.

Key words: androgens; estrogens; wound healing

INTRODUCTION

Wound healing, a biological process in the body, is achieved through four continuously overlapping phases: hemostasis, inflammation, proliferation, and remodeling [14, 15]. For a wound to heal successfully, all four phases should occur in a proper sequence with exact timing, and continue for a specific duration at optimal intensities [14, 15, 25]. Wound healing begins at the moment of injury and involves the interplay of many cell types, such as: neutrophils, macrophages, lymphocytes, keratinocytes, fibroblasts, endothelial cells, and adult stem cells. This process is regulated by several cytokines and chemokines and also includes extracellular matrix (ECM) depositions [15, 23, 27, 34].

Interruptions, aberrancies, or prolongation of selected phases involved in wound repair can lead to healing impairment and/or to development of non-healing chronic wounds [15, 34]. Such wounds generally have failed to progress through the normal stages of healing and frequently enter a state of pathologic inflammation or lodge in the proliferative phase due to a postponed, incomplete, and/or uncoordinated inflammation and granulation tissue formation [11, 15, 33, 34].

Multiple factors can cause impaired wound healing by affecting one or more phases of the process and in general

may be categorized into local and systemic. The influences of these factors are not mutually exclusive [15]. Single or multiple factors can play a role in any of individual phases, contributing to the overall outcome of the healing process [15].

Important local factors that influence the wound healing include: venous insufficiency, oxygenation, presence of infection and foreign body in the wound as well as localization, size, deepness, and the bed of the wound [15, 29]. Systemic factors affecting wound healing are: nutrition, sex steroid hormones, age, gender, stress, central hypoxia, hematologic disorders, obesity, diseases, such as diabetes, hereditary healing disorders, uremia, etc. [15, 29]. In addition to the above mentioned systemic factors; immunocompromised conditions during radiation therapy and/or administration of drugs such as glucocorticoids, NSAID and anti-tumor chemotherapy, also frequently lead to impaired wound repair [15, 29].

At present, it is widely accepted that aging is associated with delayed wound healing and that primary causative factor of this condition is not ageing alone, but a decline of estrogen circulating levels, as well as maintenance of circulating levels of testosterone and dihydrotestosterone. Hardman and Ashcroft [16] studies in the area of genetic human research have suggested that estrogen deprivation is the major factor controlling delayed healing in elderly humans. This study which wasconducted with the help of microarray techniques has shown that 78% of genes that are differentially expressed during wound healing in the young and elderly men are estrogen-regulated, while only 3% are age-associated, strongly implicating reduced estrogen, and not known geronto-genes, as the primary regulator of delayed healing in aged subjects. From this point of view, the sex steroid hormones (Table 1), in particular estrogen, are one of the main systemic factors affecting wound healing. In female animals, the chronic estrogen deprivation has been achieved by castrations and many researchers have corroborated impaired wound healing of the skin in the presence of the hypoestrogenic state.

EFFECT OF ESTROGENS ON WOUND HEALING

Ashcroft et al. [6] demonstrated that estrogen deprivation in ovariectomized rats led to delayed and impaired wound healing as determined by: re-epithelialization time, wound width, and collagen deposition. However, topical estrogen application accelerated the healing rate as measured by: decreased re-epithelialization time, decreased wound width, and increased collagen deposition [6]. Such a situation may be found similar in humans. In this context, postmenopausal women with chronic estrogen deprivation have shown reduced rates of collagen deposition and re-epithelialization of wounds compared with premenopausal women. Nonetheless, postmenopausal women taking HRT showed a similar rate of collagen deposition and re-epithelialization of wounds compared to premenopausal women, indicating that systemic estrogen replacement reversed delays in wound healing [6]. The role of estrogen in the acceleration of cutaneous healing was observed also in others studies conducted on OVX female mice where results were based mainly on the cytokine profile [8, 10, 17].

OBESITY AND ESTROGEN DEFICIENCY IN WOUND HEALING

Obesity is a serious factor that negatively influence skin wound healing due to a relative hypoperfusion and ischemia that occurs in subcutaneous adipose tissue [1]. Estrogen is in some way implicated in the body metabolism because females with an absence of estrogen, lead to increased body weight gain [22]. Furthermore, in rodent studies, the systemic estrogen treatment of OVX mice has decreased the risk of gaining weight and body fat [36]. Holcomb et al. [18] showed that obesity in the absence of estrogen inhibited wound healing; however, obesity in the presence of ovarian hormones did not inhibited wound healing. Based on these data, it may be suggested that estrogen must have a direct regulatory role in wound repair, rather than only a protective action in developing obesity. On the other hand, in the same study, the authors have revealed that the lean phenotype is associated with improved wound healing regardless the estrogen status [18]. Implication of other research methods such as PCR, histopathology, and wound tensile strength measurement would probably better explain these tricky roles of estrogens in wound healing in the case of the lean phenotype.

Table 1. Influence of sex steroid hormones on wound healing

Species	Gender	Age	Hormonal status	Treatment	Wound healing	Wound type	References
rats	female	young	OVX		delayed	acute	6
rats	female	young	OVX	local application of estrogen	accelerated	acute	6
human	women	aged	postmenopausal		impaired	acute	6
human	women	aged	postmenopausal	HRT	accelerated	acute	6
mice	female	young	OVX coexisting obesity		inhibited	acute	18
mice	female	young	OVX lean phenotype		not inhibited	acute	18
mice	female	young	not OVX coexisting obesity		not inhibited	acute	18
mice	female	young	OVX	DHEA	accelerated	acute	26
mice	male	old	intact	DHEA	accelerated	acute	26
mice	male	young	intact	DHEA	without effect	acute	26
mice	?	?	?	DHEA	limited extend of postburn tissue necrosis	thermal injury	4
mice	?	?	?	estrogen	without effect	thermal injury	4
mice	male	young	castrated		markedly accelerated	acute	7
human	men	elderly	elevated serum testosterone level		delayed	acute	7
rat	male	young		local application of estrogen	accelerated	acute	32
mice	male	young	castrated	systemic 17- beta estradiol	delayed	acute	13
mice	male	young	intact	systemic 17- beta estradiol	impeded	acute	13
mice	male	young	intact	local application of estrogen	delayed reepithelialisation of cornea	acute	35

OVX — ovariectomized; HRT — hormonal replacement therapy; DHEA dehydroepiandrosterone

RISKS OF ESTROGEN REPLACEMENT THERAPY

Although estrogen replacement is beneficial for cutaneous wound healing, its long term use is unfortunately associated with serious health risks, such as, increased risks of cerebral vascular accidents, breast cancer, venous thromboembolism, etc. [2, 30]. Therefore, further studies need to be conducted to find a safer way of estrogen replacement therapy with less side effects.

PRECURSOR OF SEX HORMONES REPLACEMENT THERAPY

Dehydroepiandrosterone (DHEA) is a precursor of both androgenic and estrogenic effector molecules and is synthesized in the adrenal cortex [5]. In humans, circulating levels of DHEA and its sulfate ester, DHEA sulfate (DHEAS), decrease progressively with age [20]. This is in contrast to other adrenal steroids such as glucocorticoids, in which serum levels remain relatively well-preserved with age [20]. However, adrenal DHEA production is very modest in rodents [31]. Despite this fact, rodents possess the necessary enzymatic machinery to convert exogenous DHEA to sex steroids [21]. Numerous animal studies have demonstrated several beneficial effects of DHEA administration in preventing obesity, diabetes, and heart disease, in enhancing the immune system, and even in prolonging the life-span [3]. Moreover, DHEA has been shown to inhibit breast cancer and to stimulate positive estrogenic actions, such as increased bone mineral density without predisposing to endometrial cancer [26]. Thus, the advantage of DHEA treatment compared to estrogen and androgens systemic replacement is that DHEA is only converted to its active metabolites in the specific target tissues where the appropriate enzymatic machinery does exist; thus, the adverse effects of systemic hormone treatments are eliminated [26]. In the context of skin wound healing, the systemic treatment with DHEA accelerates wound healing in young OVX female mice and old male mice [26]. Since the blockade of DHEA conversion to estrogen by aromatase inhibitor stopped wound healing improvement and the administration of androgen receptor antagonist did not, Mills et al. [26] have suggested that DHEA acts via its conversion to estrogen. Of note, DHEA has no observable effects in young animals. This may reflect an adequate level of circulating estrogens in young animals as well as that a supra-physiological local estrogen levels via DHEA conversion exert no effects on wound healing [26]. However, in study conducted on mice subjected to thermal injury, the subcutaneous administration of DHEA dramatically limited the extent of tissue necrosis. DHEA, 17-alpha-hydroxypregnenelone, 16-alpha-bromo-DHEA, and androstenediol, demonstrated comparable level of protection. On the other hand, other forms of steroids, including DHEA sulfate, androstenedione, 17-β-estradiol, or dihydrotestosterone, exhibited no protective effects [4]. These results have suggested that DHEA possess regulatory functions of its own in the context of cutaneous wound healing at least in the model of thermal injury [4].

EFFECT OF ANDROGENS ON WOUND HEALING

Whereas estrogen and DHEA positively influence skin repair, androgens rather seems to delay the healing process. The healing of full-thickness incisions is markedly accelerated in young castrated male mice compared with age-matched controls [7]. Gilliver et al. [12] have also revealed that castration or androgen receptor (AR) blockade improves healing in rodents. In the clinical setting in human medicine, elevated serum testosterone levels were correlated with delayed healing of excisional punch wounds in a group of health status-defined elderly men [7]. Because studies using androgen ablation through castration or AR blockade provide no information on the specific androgen species influencing repair, Gilliver et al. [12] subjected rats treated with the 5-a-reductase inhibitor MK-434, which blocks conversion of testosterone to dihydrotestosterone (DHT), to incisional wounding in parallel with castrated animals, in which systemic production of both DHT and testosterone is abolished. Since wound repair has been accelerated to a similar extent in castrated and MK-434-treated animals, it has been suggested that DHT, rather than testosterone, is responsible for the apparent inhibition of healing by androgens [12].

SEX HORMONES AND CHRONIC WOUNDS

At present, the exact roles of sex steroid hormones in the development of chronic wounds remains unclear, but current evidence suggest on the one hand that being male is a risk factor for venous ulceration and on the other hand, that the use of HRT by elderly women reduces the risk of ulceration [24]. Moreover, Gilliver et al. [12] recorded that circulating DHT levels have been shown to be significantly increased in a group of elderly male patients with venous ulcers compared with healthy age-matched control subjects. From this point of view it may be suggested that DHT have important roles in delayed cutaneous wound healing in males.

CONCLUSIONS

Cutaneous wound healing is influenced by many factors and the importance of sex steroid hormones is evident. This fact supports, in addition to clinical observation and animal research, the localization of androgen and estrogen receptors in the skin [28]. In general, the hypoestrogenismus in female is associated with impaired wound healing and in male the maintenance of testosterone and DHT along with a decline of estrogen level is associated with impairment of skin repair [19]. Possibly this issue has led to experimental estrogen therapy also in males alone or with castration. Otherwise, the topical application of estrogen accelerates skin wound healing also in males [32]. On the contrary, Gilliver et al. [13] reported that systemic 17-β estradiol treatment delays both wound re-epithelialization and the progressive reduction of wound area in castrated males. Estrogen similarly impeded healing in intact animals; even though, they declared that sex steroids masked the fundamental underlying differences in the ways that males and females heal acute wounds. These differences include marked dimorphism in the responses to macrophage migration inhibitory factor (which inhibits repair in females only) and testosterone (an inhibitor of repair in males but not females) [13]. The study of the effect of the local estrogen therapy on healing the cornea has revealed similar detrimental results on re-epithelialization [35]. Accordingly, the estrogen therapy of skin repair in male seems to be rather inappropriate. Nevertheless, it must not mean that estrogen is not implicated in the process of wound healing in males. Of note, not all aging individuals with severe venous reflux, which is considered as a causative factor of venous ulcer development, go on to develop a venous ulcers, and recent research has identified a genetic component on ER-β gen associated with this disease [9].

Many studies, on the rodent model, have been done in the wound healing research for human requirements, but current data suggest that further work need to be performed to better understand the underlying gender-specific mechanism of wound healing. In the field of small animal veterinary medicine, the information about the effects of sex steroid hormones on the wound healing is lacking despite the fact that many young companion animals are castrated, even sometimes they are castrated during prepubertal age. Unfortunately, interest of the effects of castration is focused on cancer, obesity and incontinence occurrence, and hair coat quality rather than changes in skin properties, impaired wound healing and chronic wound occurrence.

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ANALGESIC EFFECT OF TRAMADOL AND BUPRENORPHIN IN CONTINUOUS PROPOFOL ANAESTHESIA

Capík I.[1], Nagy O.[2]

[1]Small Animal Clinic
[2]Clinic for Ruminants
University of Veterinary Medicine and Pharmacy
Komenskeho 73, 041 81 Kosice
The Slovak Republic

capik.igor@uvlf.sk

ABSTRACT

The objective of this study was to compare in clinical patients the analgesic effect of the centrally acting analgesics tramadol and buprenorphine in continuous intravenous anaesthesia (TIVA) with propofol. Twenty dogs undergoing prophylactic dental treatment, aged 2—7 years, weighing 6—27 kg, were included in ASA I. and II. groups. Two groups of dogs received intravenous (IV) administration of tramadol hydrochloride (2 mg.kg^{-1}) or buprenorphine hydrochloride (0.2 mg.kg^{-1}) 30 minutes prior to sedation, provided by midazolam hydrochloride (0.3 mg.kg^{-1}) and xylazine hydrochloride (0.5 mg.kg^{-1}) IV. General anaesthesia was induced by propofol (2 mg.kg^{-1}) and maintained by a 120 minutes propofol infusion (0.2 mg.kg^{-1}min^{-1}). Oscilometric arterial blood pressure (ABP) measured in mm Hg, heart rate (HR), respiratory rate (RR), SAT, body temperature (BT) and pain reaction elicited by haemostat forceps pressure at the digit were recorded in ten minute intervals. The tramadol group of dogs showed significantly better parameters of blood pressure ($P < 0.001$), lower tendency to bradycardia ($P < 0.05$), and better respiratory rate ($P < 0.001$) without negative influence to oxygen saturation. Statistically better analgesia was achieved in the tramadol group ($P < 0.001$). Tramadol, in comparison with buprenorphine provided significantly better results with respect to the degree of analgesia, as well as the tendency of complications arising during anaesthesia.

Key words: analgesia; buprenorphine; dog; propofol; TIVA; tramadol

INTRODUCTION

Tramadol is marketed as a racemic mixture of both R and S stereoisomers. This is because the two isomers complement each other's analgesic activity [3]. Tramadol is a reuptake inhibitor of norepinephrine and serotonin and a weak μ-opioid receptor agonist [10]. Tramadol is metabolised to O-desmethyltramadol, a significantly more potent opioid. It is thought that the effects on the central catecholaminergic pathways contribute significantly to the drug's

analgesic effect. Tramadol is recommended for the management of chronic and acute pain of moderate to moderately sever intensity [12]. Administered intravenously (IV), its analgesic potency is the same as meperidine and one-tenth that of morphine [5].

Tramadol and morphine administered preoperatively were compared with the aim to assess early postoperative pain in canine ovariohysterectomy [7]. No differences were found between the two groups with regard to: analgesia, sedation, SpO_2, pH and blood gases, cardiovascular variables, glucose, catecholamine, and cortisol concentrations. Tramadol (1 mg.kg^{-1}), administered after the induction of anaesthesia, offered equivalent postoperative pain relief, and similar recovery times and postoperative patient controlled analgesia (PCA) morphine consumption, compared with giving morphine at 0.1 mg kg^{-1}. These results also suggest that pre-surgical exposure to systemic opioid analgesia may not result in clinically significant benefits [15].

Buprenorphine is a semi-synthetic partial opioid agonist that is used: to treat opioid addiction in higher dosages, to control moderate acute pain in non-opioid-tolerant individuals in lower dosages, and to control moderate chronic pain in even smaller doses [12]. Buprenorphine has produced excellent analgesic results in broad clinical applications for cats, dogs, exotic species and laboratory animals. It provides analgesia for: the management of perioperative/postoperative pain, painful joint injuries, fractures, tissue inflammation due to infections, tissue necrosis and trauma resulting from wounds. The amelioration of postsurgical pain has been substantiated in a variety of species [13]. A study comparing an analgesic effect of butorphanol and buprenorphine in parrots, confirmed a significantly increased threshold to electrical stimuli in the case of butorphanol. Buprenorphine at the dosage used did not change the threshold to electrical stimulus. Butorphanol provided an analgesic response in half of the birds tested. It would be expected to provide analgesia to African grey parrots in a clinical setting [9]. Buprenorphine should be used with caution in animals with: head trauma, compromised cardiovascular function, liver disease and geriatric or severely debilitated animals. A rare, but possible side effect of buprenorphine, is a slowed breathing rate in some dogs, so it should not be used to treat a dog with heart failure, head trauma or respiratory issues.

Common adverse drug reactions associated with the use of buprenorphine are similar to those of other opioids and include: nausea and vomiting, drowsiness, dizziness, headache, memory loss, cognitive and neural inhibition, perspiration, itchiness, dry mouth, miosis, orthostatic hypotension and urinary retention. Constipation and CNS effects are seen less frequently than with morphine [2]. Due to the mainly hepatic elimination, there is no risk of accumulation in patients with renal impairment [8].

The objective of this study was to compare the analgesic effect of buprenorphine and tramadol in dogs premedicated with midazolam, and xylazine IV. Induction into general anaesthesia was achieved by propofol IV and anaesthesia was maintained for 120-minutes by the infusion of propofol.

MATERIALS AND METHODS

Twenty dogs undergoing dental prophylactic treatment were used in the study and the owners were informed and agreed with the anaesthetic protocol.

Intravenous catheters were placed into v. cephalica antebrachii sinistra and dextra, one for a constant rate infusion based total intravenous anaesthesia and the second one for blood collection. In the buprenorphine group (B), analgesia was achieved by 0.02 mg.kg^{-1} of buprenorphine IV, 30 minutes prior to sedation. In the tramadol group (T), tramadol in the dose of 2 mg. kg^{-1} IV was used as an analgesic the same time before sedation. All dogs were sedated using 0.3 mg.kg^{-1} midazolam given intravenously, fractionated into three, two minute intervals followed by 0.5 mg.kg^{-1} xylazine IV. The dogs were intubated when the degree of relaxation and reflex activity allowed this procedure. Aesthetic induction with propofol at 2 mg.kg^{-1} was followed by a 120 min propofol infusion at 0.2 mg.kg^{-1}.min^{-1} by an infusion system IPB 2050 (Polymed). The age of dogs ranged between 2—7 years (4.16 1.6) with weight range of 6—27 kg (12.6 ± 2.9). The dogs under examination were included into ASA I. and II. groups. They were divided into two groups (B and T) ten animals in each. The study was approved by the local Ethics Committee (CZ). During anaesthesia, the dogs were breathing room air.

The dogs were monitored throughout the aesthetic and recovery periods. The ambient temperature was kept at 26 °C. Arterial blood pressure (ABP), heart rate (HR), respiratory rate (RR), saturation of hemoglobin with oxygen (% SAT), body temperature (BT), were recorded by a Patient Monitor-9000Vet (Hamburg, DE) and pain responses were recorded in ten minute intervals. The pain was in-

duced by intermittent haemostat pressure at the digits during one minute. In the case of the appearance of decreased depth of anaesthesia, xylazine in the dose of 0.125 mg.kg^{-1} or propofol 2 mg.kg^{-1} IV was administered. In the case of an increased plane of anaesthesia, propofol infusion was stopped. The pain response was classified in three grades: Grade 1 — no response to the pain; Grade 2 — increased heart and respiratory rate; Grade 3 — movement. During the recovery period, the time of extubation followed the presence of the swallowing reflex, the ability to lift up the head, taking a sternal position and first walking were recorded.

Statistical analysis was based on the mean values (x) and standard deviations (SD). Means obtained for the observed parameters were statistically analysed using one way analysis of variance (ANOVA). Analyses were done using MS Excel software.

RESULTS

Comparing tramadol and buprenorphine following their IV administration, the buprenorphine group (B) produced deeper sedation seen as unstable gait and lying within 5—8 minutes following administration. Fifty percent of dogs in the B group were intubated before induction of the general anaesthesia with no or minimal tracheal irritation during the procedure. This was not seen in the tramadol (T) group of dogs. In this group, following midazolam administration, dogs experienced some degree of incoordination disappearing after xylazine applications. Dogs in the T group were able to intubate following propofol administration. The average values of the mean blood pressure showed significant differences between groups ($P < 0.001$) with higher blood pressure in the T group (Tab. 1). The heart rate did not differ significantly between the groups (Tab. 2).

Table 1. Changes in the mean blood pressure in the groups of dogs (mean ± SD)

Group of dogs		Time [min]												
		0	10	20	30	40	50	60	70	80	90	100	110	120
T	X	92.7	98.0	93.8	89.5	88.3	84.8	83.5	90.0	90.3	90.3	92.3	88.8	90.3
	SD	8.1	13.6	11.2	6.2	6.7	5.6	7.9	15.1	12.3	11.7	9.3	5.3	3.9
B	X	89.0	94.2	87.2	82.0	71.7	78.7	75.8	74.0	86.5	79.2	75.3	86.8	76.5
	SD	27.7	17.6	13.7	11.1	18.2	17.1	20.1	13.8	17.1	14.4	18.4	13.3	15.4

Table 2. Changes in the mean heart rate in the groups of dogs (mean ± SD)

Group of dogs		Time [min]												
		0	0	20	30	40	50	60	70	80	90	100	110	120
T	x	64.5	89	79	75.7	75	74.7	75.3	72.7	75.2	74	70.3	71.3	71.3
	SD	14.6	43.2	25.9	17.7	10.8	7	6.1	12.2	12.1	13.1	7.8	7.4	7.9
B	x	68.5	75	86.8	78.7	71.3	74.7	72.5	71.2	82	74.8	79.3	66.8	64.2
	SD	10.3	19.7	45.9	25	13.5	12.1	8.8	9.6	32.1	22.4	16.6	11.1	12.4

Table 3. Changes in the respiratory rate in the groups of dogs (mean ± SD)

Group of dogs		Time [min]												
		0	10	20	30	40	50	60	70	80	90	100	110	120
*T	x	14.7	11.7	14.2	14.7	14.5	15.8	15.7	16.3	18.8	15.8	16.2	16.2	17.0
	SD	4.8	3.7	5.2	5.9	5.0	3.9	5.4	6.9	6.2	6.9	6.0	6.0	5.8
B	x	15.2	16.0	15.3	15.8	29.3	25.2	28.2	23.8	26.0	21.5	22.3	22.7	26.3
	SD	12.6	12.5	9.5	10.0	33.8	30.3	38.0	24.8	29.4	20.4	21.1	23.6	29.6

* — Statistically significant differences between the groups (P < 0.001)

Table 4. Changes in pain response in the groups of dogs (mean ± SD)

Groups of dogs		Time [min]												
		0	10	20	30	40	50	60	70	80	90	100	110	120
*T	x	1	1	1	1	1.3	1.3	1.5	1.5	1.8	1.7	1.2	1.2	1.8
	SD	0	0	0	0	0.5	0.5	0.5	0.5	0.8	0.8	0.4	0.4	0.4
B	x	1.3	1.8	1.8	1.8	2.2	2.2	2.2	2.2	2.0	2.2	2.2	2.0	2.8
	SD	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0	0.4	0.4	0.0	0.4

* — Statistically significant differences between the groups (P< 0.001)

However, this result was obtained using atropine at a dose of 0.01 mg.kg^{-1} IV in four patients (40 %) of the group B with the aim to correct the developing bradycardia. In two dogs, it was necessary to apply it two times and in another two, three times during anaesthesia. In dogs of the T group, bradycardia occurred in one patient immediately after propofol induction. A single dose of atropine adjusted the heart rate for the rest of the anaesthesia. A significant difference was confirmed between the T and B groups (P < 0.05) in relation to the frequency of the use of atropine. In three dogs in the B group, the tachypnoea ranging from 45—120 breaths per minute were observed between buprenorphine administration and sedation. During TIVA, these dogs showed also tachypnoeic breathing of the costal type in the range of 28—132 breaths per minute. Other dogs in this group had a tendency for bradypnoea with a respiratory rate in the range of 7—9 breaths per minute. The statistical analysis between the average respiratory rate confirmed significant differences between the groups (P < 0.001, Tab. 3). The SAT ranged from 87 to 98 % with insignificantly lower saturation in the B group. The pain response showed better results in the T group, where during the first 30 minutes of anaesthesia, no pain responses were noted (Tab. 4). Statistically better analgesia was achieved in the T group (P < 0.001). In the recovery period, the average time of the extubation was 10 minutes and 50 seconds in the B group and 13 minutes in the T group. Head lifting (17.3 *vs.* 18.5 min.), sternal position (25 *vs.* 30.3 min.) and walking (27.7 *vs.* 32.3 min.) appeared sooner in the T group. The body temperature did not show any significant changes during the general anaesthesia. The recovery times have not been statistically significant between groups.

DISCUSSION

The comparison of the perioperative analgesic effects of the two analgesics was the main reason of this study. Buprenorphine is regulated by the Drug Enforcement Agency, while tramadol is not. This was also one of the reasons to compare these two analgesics, to facilitate the administrative work. Buprenorphine in human anaesthesia plays a role in regional epidural anaesthesia and is not used as perioperative analgesia. Tramadol is a much weaker pain medication when compared to morphine; equally about 10:1. Studies have shown that tramadol does not cause the breathing problems. For pain moderate in severity, its effectiveness is equivalent to that of morphine, for severe pain, it is less effective than morphine [12].

Tramadol (1 $mg.kg^{-1}$), administered after the induction of anaesthesia, offered equivalent postoperative pain relief, and similar recovery times and postoperative patient controlled analgesia (PCA) morphine consumption compared with giving morphine 0.1 $mg.kg^{-1}$. These results also suggest that pre-surgical exposure to systemic opioid analgesia may not result in clinically significant benefits [15].

Tramadol was not found to be a suitable analgesic for use in human balanced anaesthesia, because of problems with increased intraoperative awareness [11].

Our experience with tramadol in balanced anaesthesia does not confirm this argument. The dogs in the T group had significantly better parameters of blood pressure, respiratory rate and analgesia in comparison with the B group. Some disadvantage seems to be, no sedative effect in the dose used. Buprenorphine produced overt sedation shortly after administration in some patient accompanied with tachypnea. Buprenorphine seems to have potentiating effect to xylazine, as 50 % of the dogs were possible to intubate without propofol induction. It was not seen in the group T, where intubation was possible following propofol induction. The dogs in the B group had significantly higher episodes of bradycardia, corrected by an anticholinergic.

A study comparing tramadol and buprenorphine in cancer pain, confirmed the better tolerance of tramadol than buprenorphine, which caused fewer and milder adverse reactions. Tramadol, although theoretically less potent, nevertheless brought about as much pain relief as buprenorphine. In conclusion, for this class of drug, tramadol provided an excellent balance between efficacy and tolerability, confirming preliminary studies [1]. The experimental study by Kögel et al. [6] did not confirm antinociception of tramadol in beagles explaining it as marginal amounts of the M1 metabolite of tramadol.

Our results found in balanced anaesthesia in dogs where tramadol was used, confirmed significantly better monitored parameters in comparison with the buprenorphine group.

Buprenorphine, in our study, has produced sufficient sedation, but the analgesic effect was inferior to tramadol as seen in our results. Taylor, Houlton [14] also found that buprenorphine produced more sedation than morphine in dogs and considered that this affected the analgesia assessment. Whereas, in general anaesthesia, there is a loss of consciousness, the sedative effect of analgesics does not play as important a role in pain assessment like in awake animals in the post-operative period. While in the T group, we did not find signs of pain, within the first 30 minutes in the B group some dogs reacted with increased heart and respiratory rates from the beginning of the anaesthesia. Studies in human medicine also state the significantly better analgesic effect of oral tramadol in human oncologic patients suffering from strong/unbearable pain [1, 4]. The time of recovery did not differ significantly between the groups, taking approximately 30 minutes to stand up and walk. It can be concluded that balanced anaesthesia with tramadol achieved significantly better endpoints at a lower frequency of corrective interventions to maintain the observed parameters in physiological range.

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ELECTRORETINOGRAPHY (A REVIEW)

Balicka, A., Trbolová, A., Vrbovská, T.

Small Animal Clinic
University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81 Košice
The Slovak Republic

agnieszka.a.balicka@gmail.com

ABSTRACT

Electroretinography (ERG) is a functional test of the outer retina. During an examination, the retina is selectively stimulated. The stimulation of the retina produces a response of the individual retinal cells and reveals information about its function. The ERG examination requires very specific conditions in order to avoid undesirable factors which may adversely affect the recordings. The electroretinography examination may be performed for a short period ("rapid protocol"), commonly used to access retinal activity. The "long protocol" is used for the differential diagnosis of retinal disorders. It is mainly used in diagnosing and evaluating retinal dysfunction when there are no ophthalmic lesions present. The main indications for electroretinography are the pre-operative examination of cataract patient and the early diagnosis of inherited retinal diseases. In veterinary ophthalmology, ERG is performed under general anesthesia. The ERG results have wave forms with characteristic components depending upon several factors. Its interpretation requires knowledge of retinal pathology and electrophysiology.

Key words: electroretinography (ERG); ophthalmology; retina; retinopathy

INTRODUCTION

Electroretinography (ERG) is an electrodiagnostic technique assessing retinal function in dogs and many other animals. The first ERG examination in veterinary medicine was performed by Perry, Thomson and Tansley in 1953 [25], although the history of ERG begins 100 years earlier when DuBois-Reymound discovered the resting potential between the anterior and posterior pole of the non-stimulated eye [4]. In 1865, Holmgren measured the electrical potential over the eye as a response to the illumination of the retina which was considered as the beginning of electroretinography [9]. The first ERG examination on an animal took place in 1873 [3]. In 1953 a dog was examined for the first time [25]; feline ERG was 10 years later [29].

ERG is a non-invasive examination that provides objective information about the function of individual retinal cells [16]. There are dual protocols used in veterinary

ophthalmology. The rapid protocol (Yes/No protocol) gives information about the function of the retina. The rapid protocol is commonly used as a pre-surgical evaluation of the retina in patients being prepared for cataract surgery (before the phacoemulsification full protocol of the ophthalmic examination is required). The long protocol is used in the differential diagnosis of various retinal problems, and also when ophthalmic lesions are absent. It is the method used for the early diagnosis of hereditary retinal disorders as well. The long protocol produces information about the different retinal cell types, while the brief protocol (rapid protocol) reveals the mixed rod-cone responses [20]. The examination is based on the active responses of the photoreceptors stimulated by a light of various intensities [5]. Electroretinography requires very specific conditions during examination in order to avoid adverse factors affecting the results of the examination. All of the factors affecting the wave form recordings of the ERG can be divided into physiological or instrumented-related [17]. The proper interpretation of the results requires collecting a data base in which all of the physiological and instrumental aspects have been taken into consideration [20].

RETINA

The retina is the organ transducing light into neuronal signals that are perceived as a visual image. The retina is divided into ten layers or can be subdivided into the neuroretina and the retinal pigment epithelium which is responsible for the nutrition of the outer layers. The neuroretina, with several cell layers, consists of the photoreceptors in one of the outer layers and is responsible for vision [15, 22].

At the moment of birth, until 6-9 week of age, the retina consists of an immature- neurosensory layer which is separated from a pigmented epithelium. The immaturity of the retina is specific for different species and can be noticed in the histopathological examinations or by electroretinography [15].

Rods and cones are the photoreceptors responsible for vision. The number of photoreceptors and their type varies depending upon the different species and the specifics concerning the nature of their external environment and ecology. Humans, as with dogs and cats, have a rod-cone type of retina. However, cats have a much higher concentration of rods than humans. This difference means that cats have superior night vision, but their visual acuity is relatively limited [24]. Rods are sensitive to low levels of light and to small changes of illumination. Rods are responsible for scotopic vision – dim environments. Rods have low visual resolution in various grey tones. They are useful in the detection of movement. Cones are responsible for photopic vision, in high level of illumination — daylight. They determine high-resolution vision that is important while observing the details. Cones contain pigments for color vision which makes them responsible for general color vision and they are different in different animals according to their cone types [15, 22].

In photoreceptors containing photopigments, after exposure to light, chemical energy is produced which is converted into electrical energy, which is transmitted by the optic nerve, via the optic chiasm, optic tracts, lateral geniculate body, and optic radiations, to the visual cortex [22].

During an ERG examination, the retina is illuminated with various intensities of light. Light induces electrical charges in the cells of the retina which cause an electrical response. The response is the summation of the electrical potentials that result from light induced changes in the movement of sodium and potassium ions within the extracellular space. This response, as wave forms in the electroretinograms, are measured and analyze during the ERG examination [20].

ELECTRORETINOGRAPHY

Electroretinography is a non-invasive diagnostic method of measuring the electrical response of retinal photoreceptor cells [16]. This specific response is caused by the standardized selective stimulation of individual retinal cells types. Every few years, updated guidelines for ERG examination are published which define the conditions for the reproducible recordings based on the anatomy and physiology of a patient and allow for the possibility of comparing results worldwide [23]. According to the guidelines for the clinical electroretinography in the dog updated in 2012, the full examination should consist of four stages.

The recommended protocol:

1. Dark adapt for 20 min while evaluating the rod function and the dynamic process of dark adaptation every 4 min (for practical reasons, the first flash (at 0 min) may be

delivered after 10 s of dark adaptation and the subsequent flashes after 4, 8, 12, 16 and 20 min); with flash intensity preferably 0.01 cd s.m^{-2} or 0.02 cd s.m^{-2}. Optional: perform the dark-adapted intensity/ response series; with flash intensity increasing in at least 7 steps from below b-wave threshold (B0.001 or B0.002 cd s.m^{-2}) to C3 cd s.m^{-2}.

2. Test the mixed rod–cone response to a 3.0 cd s.m^{-2} flash in the dark. Optional: perform the dark-adapted, high-intensity flash test; with flash intensity 10 cd s.m^{-2}.

3. Test the cone function after 10 min of light adaptation (background light at 30 cd s.m^{-2}); with flash intensity of 3 cd s.m^{-2}.

4. Perform the 30 Hz flicker test at 3 cd s.m^{-2} with a rod suppressing background light of 30 cd s.m^{-2} [6].

The following protocol guarantees the separate examination of rods and cones. According to the knowledge of the photoreceptor's physiology and biochemistry, in order to examine the cones in light adaptation, a high flicker rate and high intensity of stimulation is required. In order to examine the rods in dim stimulation, a low flicker rate and dark adaptation at the beginning are needed. To illustrate the rod's sensitivity for dim light in comparison to the required energy of cones, rods are activated by 1—5 photons, while cones need 1,000 photons [23]. Various intensities of light cause various nerve cell reactions in the retina. Light intensity should be determined according to the transparency of the cornea and the lens.

According to the recording electrode type and the distance of the reference electrode from the eye, differences have been noticed in the ERG of the dog that may have influence on the interpretation of the ERG results and assessment of the retinal function evaluation. The results obtained using these different types of electrodes cannot be directly compared [17]. Four electrodes are used to perform the ERG examination. One corneal lens electrode is placed on the cornea; two reference electrodes are placed in the temporal cantus of the eye; and a grounded electrode is positioned on the top of the scull [6, 17]. The space between the corneal electrode and the cornea should be filled with a solution. The ionic solution used in the ERG has to be nonirritating and with a viscosity of less than 0.5% of a methylcellulose solution [6]. The retina has to be illuminated evenly; the position of the pupils and its dilatation has to be equal in both eyes [6, 20]. After retinal stimulation, the electroretinogram should be received as curves with all its component: a, b, c, and d waves.

The a-wave consists of the first negative deflection from the photoreceptors. It corresponds to the hyperpolarization of the photoreceptors after the light stimuli [7].

The b wave is the first positive deflection of the depolarization from non-neuronal glia cells in the inner nuclear layer [7]. It is known that the b-wave is associated with Muller cells and bipolar ON cells. According to Kofuij et al., the connection between the b wave and the Muller cells has been excluded [12]. On the ascending limb of the b-wave there are small wavelets, i.e. rhythmic waves known as oscillatory potentials (OPs) [32].

The c-wave is a slow positive, deflection from the pigmented epithelium and the Muller cells hyperpolarization [6, 7].

The d-wave is a positive peak and a late off type response [33].

The i-wave is the following positive peak [14].

Every retinal disorder determined by the results of the examination can be interpreted from the electroretinogram by the analysis of the implicit time and amplitude of the waves. The amplitude of the a-wave is measured from the pre-stimulus baseline to the trough of the a-wave, and the amplitude of the b-wave is measured from the trough of the a-wave to the peak of the b-wave. The implicit time of the a-wave is measured from the onset of the stimulus to the trough of the a-wave and the implicit time of the b-wave is measured from the onset of the stimulus to the peak of the b-wave [17].

FACTORS AFFECTING THE ELECTRORETINOGRAM

The ERG examination requires specific conditions due to various factors having an impact on the recording. Not only anatomical and physiological differences between species have influence on the ERG, but also noise and surrounding light are factors [21]. Differences between breeds [6, 1] and age groups [26, 30] cause differences in recordings like a decrease of the amplitude in older patients [27]. All parameters like the patient's body temperature 38—39 °C and oxygenation should be kept stable to avoid affecting factors [6, 27]. The preparation of the patient and technical aspects of examination, like the placement or type of electrode used are very important. To prevent pre-exposure of light from instruments during pre-surgical ophthalmic

examinations, the ERG patient should spend minimally 60 minutes in a normally illuminated room [6].

The ERG examination needs to be performed in a dark dimly lit room with red light that allows for the simplified monitoring of the patient during the examination. In veterinary ophthalmic electroretinography examinations, the patient needs to be placed under general anesthesia to exclude blinking, movement of globe and other disturbances that may have an adverse influence on the recordings. The position and size of the pupils must be proper to ensure equal exposure to the light on the retina. It is necessary to use a lid speculum to retract the eyelids and stay sutures to position eye globe [6, 17, 28]. A stable depth of anesthesia is necessary during the examination [6]. To avoid all affecting factors, the anesthetic protocol has to be taken into consideration. According to the collected database for anesthetics, diazepam reduces the a-wave amplitude, while barbiturates decrease it in high doses but also increase it in low doses [23]. It has been demonstrated that a xylazine and ketamine combination has not only low impact on implicit time and amplitudes but also do not cause eye globe rotation and miosis [11, 13].

CLINICAL ASPECT

The electroretinography examination is useful even during an ophthalmic examination of the fundus when the optic system is not transparent — in cases of corneal edema, hyphaema, hypopion, or vitreous hemorrhage [20].

The ERG is a basic examination before cataract surgery, as it determines the eventual therapeutic strategy. Cataracts often occurs in geriatric patients along with other ocular abnormalities. The revealed abnormalities in pre-surgical evaluation of the retina excludes patient from phacoemulsification [8, 16, 20, 22].

In dogs, some of the retinal disorders are congenital and often not diagnosed until the onset of sever visual problem in older patients. It is recommended to perform an ERG examination prior to breeding in order to prevent genetic defects. The ERG gives the possibility of an early diagnosis of hereditary eye diseases that is significant in selective breeding [16, 20, 22, 27]. The analysis of the electroretinogram of patients with progressive developing diseases like PRA gives knowledge about the pathogenesis of retinal disorders and its stages which is important in veterinary ophthalmology [20]. It has been found in Abyssinian crossbred cats that progressive retinal degeneration in heterozygous carrier cats could be differentiated from homozygous affected cats prior to clinically evident retinal degeneration by comparing the ERG b-wave to a-wave ratio [10]. Using ERG examination we can differentiate vision defects caused by retinal damage from optic nerve disorder like optic neuritis. Blindness in an early stage of SARD typically is not accompanied by any ocular findings, especially in the retinal layers [18]. In the absence of funduscopic abnormalities, ERG is required to detect the disease [16, 20]. Glaucomatous optic neuropathy causes potentially detectable progressive loss of retinal ganglion cells; electroretinography may be useful in the diagnosis and evaluation of glaucoma [2].

In some cases, like PRCD in English Cocker Spaniels, and Labradors, or CRD in short haired Dachshund, changes in the ERG can be found even 3 years before clinically observed fundic changes appear. This examination over reaches real symptoms of retinal diseases and gives the possibility of treatment. In human medicine, but also veterinary medicine, thanks to ERG, progress in gene therapy helps to prevents development of diseases, for example in the case of successful gene therapy in older Rpe65-deficient dog [19].

ERG/MRI/ COLORIMETRIC PRL DEVICE

In veterinary ophthalmology there are many possibilities (colorimetric PRL Device, ERG, basic ophthalmoscope examination or even MRI) for detecting the function of the retina or loss of vision. Some of these methods require sedation of the patients or general anesthesia. All of these methods requires specialist equipment, specialist conditions and knowledge for the interpretation of the results. According to research published in 2013, colorimetric PLR may be a useful method for determining whether electroretinography (ERG) or magnetic resonance imaging (MRI) should be performed on dogs with acute blindness. Electroretinography is the final examination that needs sedation and special conditions, but in objective ways, provides detailed information about photoreceptor function [31].

CONCLUSIONS

In order to perform ERG examination properly, it is necessary to consider all physiological and instrumental

affecting factors. It is a basic pre-surgical examination before cataract surgery. It is used worldwide in the differential diagnosis of retinal disorders, also for the early hereditary retinal diseases with absences of ophthalmic lesions. The standardized selective stimulation of the ERG examination provides information about the function of the retina, its cells, and eventual problems. In addition ERG can determine the separate evaluation of rods and cones function. The ERG examination is based on the electrical stimulation of the retina that is recorded as curves with specific components. The ERG curves are filtered, averaged and graphically represents the response of the retina. The retinal disorders determined are the results of the examination of the data interpreted from the electroretinogram by the analysis of the implicit time and amplitude of the waves. Every few years, updated guidelines for ERG examinations are published to standardize the examination all over the world. The last guidelines for dog's electroretinography with full standardized protocol were published in 2012.

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ARTERIES OF THE CERVICAL SPINAL CORD IN THE EUROPEAN HARE

Maženský, D., Flešárová S.

Department of Anatomy, Histology and Physiology
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
The Slovak Republic

david.mazensky@uvlf.sk

ABSTRACT

The aim of this study was to describe the blood supply to the cervical spinal cord in the European hare using the dissection technique. This study was carried out on 10 adult European hares. The arterial system of the cervical spinal cord was injected using Batson's corrosion casting kit No. 17. The presence of the ventral radicular branches of the *rami spinales* entering the ventral spinal artery in the cervical region was observed in 62.1 % as left-sided and in 37.9 % of the cases as right-sided. There were two dorsal spinal arteries located on the dorsal surface of the cervical spinal cord in 70 % of the cases, and no dorsal spinal artery in 30 % of the cases. The presence of the ventral radicular branches of the *rami spinales* that reached the spinal cord, was observed in 66.2 % of the cases on the left side and in 33.8 % of the cases on the right side. Based on our results, we can conclude that there is high variability of the blood supply to the cervical spinal cord in the European hare.

Key words: European hare; cervical spinal cord; dorsal spinal artery; ventral spinal artery

INTRODUCTION

The European hare is one of the most frequently occurring wild animals in Slovakia. Despite its relatively abundant occurrence, there is a significantly gap of information about its anatomy in the literature.

The unique anatomy of each region of the cervical spinal cord demands a study of this nature. The domesticated rabbit has been widely used as a laboratory animal to test the effects of neuroprotective drugs and to examine the pathophysiology of spinal cord injury and, for this reason, we studied the arterial arrangements of the cervical spinal cord in the rabbit [6]. The arterial supply to the cervical spinal cord has been described in several investigations [1, 3, 10]. Research on the arrangements and variability of the feeding arteries of the spinal cord in several species of laboratory animals [4, 9, 11, 12] and in man [7] are more common. Until now, the arterial arrangements of the cervical spinal cord in the European hare has not yet been described.

The aim of this study was to describe the blood supply of the European hare spinal cord with a focus on the cervi-

cal section. We describe some variations in the arterial pattern of the segmental branches of the cervical spinal cord in the European hare.

MATERIALS AND METHODS

This study was carried out on 10 adult European hares (*Lepus europaeus*, L. 1758), aged 140 days. We used hares (obtained from ISFA APRC, Nitra, Slovak Republic) of both sexes (female n = 5; male n = 5) with a weight range between 1.5—1.8 kg in an accredited experimental laboratory of the University of Veterinary Medicine and Pharmacy in Kosice. The animals were kept in cages under standard conditions (temperature 15—20 °C, relative humidity 45 %, 12-hour light period), and fed with a granular feed mixture (O-10 NORM TYP, Spišské kŕmne zmesi, Spišské Vlachy, Slovak Republic). The drinking water was available to all animals *ad libitum*. The animals were injected intravenously with heparin (50 000 IU.kg^{-1}) 30 min before they were sacrificed by intravenous injection of embutramide (T-61, 0.3 ml.kg^{-1}). Immediately after euthanasia, the vascular network was perfused with a physiological solution. During manual injection through the ascending aorta, the right atrium of the heart was opened in order to lower the pressure in the vessels to ensure an optimal injection distribution. Batson's corrosion casting kit No. 17, using a volume of 50 ml (Dione, České Budějovice, Czech Republic) was used as the casting medium. After polymerization of the medium, 4 % formaldehyde was injected into the vertebral canal between the occipital bone and the first cervical vertebra, and between the sixth and seventh cervical vertebra to fix the spinal cord. After 1-week of fixation, the vertebral canal was opened by removing the vertebral arches in the cervical spinal region. Also, the occipital bone was partly removed. The prepared spinal cords were fixed in 10 % formaldehyde. This study was carried under authority decision No. 2647/07-221/5.

RESULTS

The ventral spinal artery was present as a single trunk located on the ventral surface of the spinal cord. On the dorsal surface, there were either two dorsal spinal arteries or no dorsal spinal artery at all. The ventral spinal artery had a rostral connection with the vertebral artery. The dorsal and ventral arteries received, along their course, several dorsal and ventral radicular branches entering the intervertebral foramina. Some radicular branches significantly contributed to the spinal cord blood supply.

The ventral spinal artery was located along the ventral median fissure of the spinal cord. *Rami spinales* arising from the bilateral vertebral arteries entered the vertebral canal through the intervertebral foramen. After entering the vertebral canal, they sent to the spinal cord, ventral and dorsal radicular branches. Some ventral radicular branches entered the ventral spinal artery. The frequency of occurrence of individual ventral radicular branches reaching the spinal cord is shown in Table 1. The left-sided ventral radicular branches entering the ventral spinal artery were present in 62.1 % of the cases, the right sided ventral radicular branches were present in 37.9 % of the cases.

Two dorsal spinal arteries located on the dorsal surface of the cervical spinal cord were present in 70 % of the cases (Fig. 1), and not any dorsal spinal artery in 30 % of the cases (Fig. 2). Two longitudinal dorsal spinal arteries were formed by the fusion of the small cranial and caudal branches arising from the dorsal radicular branches of spinal arteries. We found no rostral origin of dorsal spinal arteries in the area of formation of the basilar artery. In the case of the absence of the dorsal spinal arteries, the cervical part of spinal cord received the blood by means of dorsal radicular branches of spinal arteries with very irregular arrangements (Fig. 2). The frequency of occurrence of individual dorsal radicular branches reaching the spinal cord is shown in Table 2. The left-sided dorsal radicular branches were present in 66.2 % of the cases, the right-sided ventral radicular branches were present in 33.8 % of the cases.

DISCUSSION

Based on our results, we can conclude that there is a high variability of the blood supply to the cervical part of the spinal cord in the European hare. Until now, the arterial blood supply of the cervical spinal cord in the European hare has not been described. In the study of cervical spinal cord arteries, dogs, rats, pigs and rabbits have often been used. In the dog, the blood supply of the cervical spinal cord have been studied in detail, with variations in the origin of the ventral spinal artery and the frequency of the occur-

Table 1. Frequency of occurrence of ventral radicular arteries of the cervical spinal cord that contributed significantly to the spinal cord blood supply

	Occurrence of arterial spinal branches [%]	
Level	**Right**	**Left**
C1	0	0
C2	0	40
C3	20	40
C4	30	60
C5	60	70
C6	60	30
C7	30	80
C8	20	40
	62.1%	**37.9%**

C — Cervical segment of the spinal cord

Table 2. Frequency of occurrence of dorsal radicular arteries of the cervical spinal cord that contributed significantly to the spinal cord blood supply

	Occurrence of arterial spinal branches [%]	
Level	**Right**	**Left**
C1	0	50
C2	40	50
C3	30	60
C4	50	50
C5	30	70
C6	30	80
C7	0	30
C8	50	60
	66.2%	33.8%

C — Cervical segment of the spinal cord

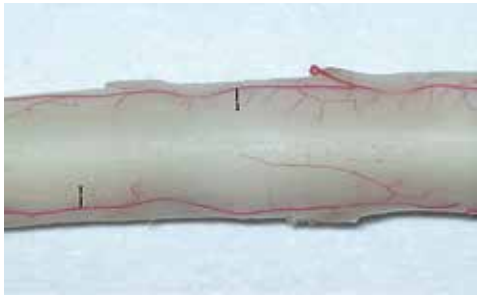

Fig. 1. Presence of two longitudinal dorsal spinal arteries
(1) dorsal spinal artery. Dorsal view. Magn. ×12.5

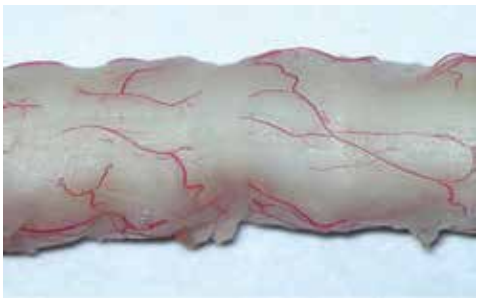

Fig. 2. Absence of dorsal spinal arteries
Dorsal view. Magn. ×20

rence of the spinal arteries described[8]. The blood supply of the rat spinal cord was probably the most profusely documented, but the results were often very different [2, 3, 9, 10, 13]. In the pig, only the variations and the presence of extrasegmental arteries in the spinal cord blood supply have been described [11]. The frequency of the occurrence of the segmental spinal arteries was higher on the left than on the right side; the opposite for the case in dogs [8]. The presence of ventral branches of spinal branches entering the ventral spinal artery in the cervical region was more frequently on the left than on the right side. A similar arrangement was found in the rabbit [6]. We did not find any connections of the dorsal spinal arteries in the area of formation of the basilar artery in our study. On the dorsal surface we found two longitudinal dorsal spinal arteries receiving dorsal branches of spinal arteries or they were absent. A similar situation was described in the rabbit [6]. The presence of dorsal branches of the spinal branches was more frequently on the left than on the right side, like in the rabbit [6].

CONCLUSIONS

Study of the arterial patterns of the spinal cord provides additional information concerning the manner of vascularization of the central nervous system in general [5, 13].

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PERIPARTURIENT PERIOD IN TERMS OF BODY CONDITION SCORE AND SELECTED PARAMETERS OF HORMONAL PROFILES

Vargová, M., Kováč, G.[1]

Department of the Environment, Veterinary Legislation and Economy
[1]Clinic of Ruminants University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81 Košice
The Slovak Republic

mariavargova24@gmail.com

ABSTRACT

The majority of all diseases in dairy cows occur during the period from three weeks before parturition to three weeks after parturition, in the periparturient or transitional period. The objective of this study was to evaluate the dynamics of selected parameters of: the hormonal profiles, the body condition score (BCS) and their interrelationships. The study was carried out on 15 dairy cows of the Slovak Pied Cattle, from three weeks before to nine weeks after parturition, which were divided into six groups. The concentrations of leptin during *ante partum* increased from 23.08 ± 10.58 ng.ml^{-1} to 26.80 ± 11.47 ng.ml^{-1}, then gradually decreased ($P > 0.05$), and conversely, the concentrations of ghrelin before parturition were found to be decreasing and during the postpartal period, the concentrations increased, with the highest value of 35.94 ± 16.85 pg.ml^{-1}. In the case of insulin, we found the opposite tendency of ghrelin. We observed significantly higher values of BCS in dry cows than in cows after parturition ($P < 0.001$). Comparing the BCS and the parameter of the hormonal profiles, we found both positive and negative correlations: leptin and ghrelin ($r = -0.235$, $P < 0.05$), and BCS and insulin ($r = 0.232$, $P < 0.05$), and BCS and leptin ($r = 0.360$, $P < 0.001$). The interrelationships between the hormones and the body condition score, provided evidence that the variations in concentrations of leptin, ghrelin and insulin were related to variations in the BCS.

Key words: body condition score; dairy cow; ghrelin; insulin; leptin; periparturient period

INTRODUCTION

The periparturient period is characterized by a sudden increase in energy requirements imposed by the onset of lactation and by a decrease in voluntary dry mater intake (DMI) which results in a negative energy balance (NEB) [18]. Dairy cows undergo tremendous metabolic and physiological adaptations around the time of parturition in order to support lactation. The dry period, in particular, the transitional period, is characterized by dramatic

changes in the endocrine status [11]. These changes prepare the cow for lactogenesis and parturition. Essentially all dairy cows experience a period of: insulin resistance, reduced feed intake, negative energy balance, hypocalcemia, reduced immune function, and bacterial contamination of the uterus soon before, or within the weeks after calving. Physiological and pathological changes associated with NEB are important factors related to the development of: ketosis, displaced abomasum, and retained placenta [7]. Theses changes may also impact the immune system which can lead to the occurrence of infectious diseases such as mastitis and metritis.

During the last three weeks of pregnancy, nutrient demands by the fetal calf and placenta reach their maximum, yet DMI may decrease by 10 to 30 % compared with the intake during the early dry period. This, in itself, may not be a cause for alarm, as decreased food or feed intake around parturition is a common finding in many mammalian species [9]. Cows lose body condition during early lactation due to NEB. As a result of NEB, cows have their lowest BCS at approximately one to two months *post partum* [28]. Dairy cows, like other mammals, undergo a normal cycle of body energy storage and mobilization, with an increased body fat storage during mid-gestation and increased body fat mobilization during early lactation [9]. Several aspects of dietary management and body condition may affect DMI and increase or decrease the susceptibility to periparturient health problems. Overfeeding energy during the dry period is a prominent risk factor. Management or environmental circumstances that force cows away from their optimal body condition may result in increased risk for health problems [15]. It is well known that overconditioned cows are at a greater risk for the development of metabolic problems [26], and have poorer DMI after calving and readily break down their excessive stores of body fat [24], and they lose more muscle fiber area after parturition than thin cows, suggesting a greater mobilization of body protein as well as fat. Obesity leads to increased susceptibility to the complex of metabolic disorders and infectious diseases known as the "fat cow syndrome". Overconditioning results in the impairment of the immune system [16] and also results in greater indices of oxidative stress [3].

A number of metabolic hormone concentrations also change during this critical period. Leptin may have a role in the activation of lipid oxidation and may protect non-adipose tissue from excessive fat accumulation. Leptin is involved in the central and/or peripheral regulation of: body homeostasis, energy (intake, storage and expenditure), fertility, and immune functions [6]. The reduction of plasma leptin to the NEB is caused by the initiation of copious milk secretion. Temporal changes of plasma leptin and energy balance near calving are parallel, and plasma leptin and NEB nadirs seem to coincide. Undernutrition, or even short-term restriction of access to food, results in a significant reduction in leptin concentrations in ruminants [1]. Leptin acts in opposing fashion to ghrelin by signaling satiation.

Ghrelin has a role in signaling the deposition of fat tissue by increasing food intake and reducing fat utilization [5]. This causes ghrelin to be expressed at lower concentrations during states of positive energy balance (EB) and increased during NEB [21]. It has been shown that ghrelin may play a role in regulating the EB.

Changes in insulin play an important role in the metabolic adaptation of cattle to changes in weight and body condition [17]. Insulin is also a putative mediator of the nutritional status. However, the process of adaptation to the NEB in dairy cows usually is accompanied with a decrease of blood insulin [31]. The genetic selection for milk production has been associated with a decline in circulating insulin levels in dairy cows in early lactation. In addition, adipose and muscle become insulin resistant in late gestation and develop an increased sensitivity to lipolytic agents. Holtenius [13] reported that cows fed a higher energy allowance during the dry period had a greater degree of insulin resistance before and after calving, which allowed for greater non-esterified fatty acids (NEFA) concentrations.

The aim of this investigation was to determine the plasma concentrations of selected parameters of hormonal profiles: leptin, ghrelin and insulin; their changes in relation to the period of *ante partum* and *post partum*; evaluation of BCS and assessment of their interrelationships.

MATERIALS AND METHODS

The monitored parameters of the hormonal profiles: leptin, ghrelin, insulin and body condition score, were evaluated in dairy cows (n = 15) of the Slovak Pied Cattle breed (aged three—five years). Blood samples were taken from three weeks before parturition until nine weeks after parturition by direct puncture of *v. jugularis*. The dairy

cows were classified into six different groups based on the calving date — according to certain phases of *ante partum* (*a. p.*) and *post partum* (*p. p.*):

Group 1 – dairy cows 3 weeks before parturition (3 wk *a. p.*) (n = 15)

Group 2 – dairy cows 1 week before parturition (1 wk *a. p.*) (n = 15)

Group 3 – dairy cows 1 week after parturition (1 wk *p. p.*) (n = 15)

Group 4 – dairy cows 3 weeks after parturition (3 wk *p. p.*) (n = 15)

Group 5 – dairy cows 6 weeks after parturition (6 wk *p. p.*) (n = 15)

Group 6 – dairy cows 9 weeks after parturition (9 wk *p. p.*) (n = 15)

All of the variables were analyzed in blood serum. The blood samples were collected three hours after feeding. The mean production age was 2.5 lactations. The milk yield during the previous lactation was 6668.5 kg of milk. The animals were fed a total mixed ration (TMR) twice daily and had free access to drinking water. The nutrient composition of the TMR varied with the stage of pregnancy and lactation. The concentrations of leptin (ng/ml) and ghrelin (pg.ml^{-1}) were determined by RIA kits from Millipore (St. Charles, Missouri, USA). The insulin (IU.ml^{-1}) was determined by ELISA using commercial assays (Cusabio, China). The body condition score was determined using a 5-point scale and backfat thickness (BFT) measurements were obtained using a 3.5 MHz linear transducer. The body condition score and the BFT were assessed according to Staufenbiel [27]. The evaluation of the results were performed by the assessment of the mean values (x) and the standard deviations (S. D.) in each monitored period. The significance of differences in the average values in relation to several monitored periods were evaluated by the one way analysis of variance (ANOVA). The significance of differences in the average values between the different time of blood taking were evaluated by the Tukey´s Multiple Comparisons Test. The Pearson´s correlation coefficients were calculated to describe the relationships between the variables; the relationships were evaluated by a linear regression analysis, including significance of the correlation at the same time. The statistical analyses were done with the GraphPad Prism 3.0 software.

RESULTS

The components of *pre partum* and *post partum* diets (kg.head^{-1}day^{-1}) are shown in Table 1. The concentrations of the metabolic hormones leptin, ghrelin, and insulin in the blood serum during *ante partum* and *post partum* are presented in Table 2. The values of the BCS are shown in Table 3. Correlation between the concentration of leptin and ghrelin is shown in Figure 1; correlation between the concentration of leptin and BCS is shown in Figure 2 and correlation between the concentration of insulin and BCS is shown in Figure 3.

The concentrations of leptin during a.p. increased, then gradually decreased without any statistical significance. In the case of ghrelin, we found an opposite tendency; the concentrations before parturition decreased and in the early postpartal period the concentrations increased; the highest value was recorded six weeks after parturition. The concentrations of insulin in the time before calving were significantly higher than the values recorded after calving

Table 1. Components of prepartum and postpartum diets
[kg.head^{-1}day^{-1}]

	3 and 1 wk *a.p.*	**1wk *p.p.***	**3 wk *p.p.***	**6 wk *p.p.***	**9 wk *p.p.***
Meadow hay	5.5	1.5	1.5	1.5	1.5
R24	0.3	0.25	0.3	0.25	0.25
Haylage	4	4	6	6	6
Lucerne silage	13	24	22	22	22
Green fodder		25	25	25	25
Soybean meal		0.8	0.8		
Rape meal		2.5	2.5	2.5	2.5
Wheat meal		3	4	2.5	
Limestone		0.2	0.2	0.2	0.2
Flaxseed meal			0.5	1	1
Maize meal				1	
Triticale					3.5

R-24 – mineral supplement
(10.4 % Ca, 9 % P, 11 % Na, 4 % Mg, 7 000 mg Cu, 3 000 mg inorganic Mn, 6 000 mg inorganic Zn, 40 mg Se, 100 mg I, 20 mg Co, 1 000 000 IU vitamin A, 100 000 IU vitamin D3, 2 000 IU vitamin E); wk – weeks before and after parturition; *a.p.* — ante partum; *p.p.* — post partum

Table 2. Concentration of parameters of hormonal profile

Parameter		3 wk a.p.	1 wk a.p.	1 wk p.p.	3 wk p.p.	6 wk p.p.	9 wk p.p.	P
Leptin [ng.ml^{-1}]	x	23.08	26.80	24.98	19.59	19.12	18.91	ns
	SD	10.58	11.47	8.92	6.51	5.03	7.90	
Ghrelin [pg.ml^{-1}]	x	29.25	26.57	30.54	32.73	35.94	35.63	ns
	SD	4.82	5.35	5.40	15.70	16.85	18.02	
Insulin [IU.ml^{-1}]	x	580.8	625.5$^{a\alpha}$	483.3	437.1	375.3^{a}	388.7$^{\alpha}$	< 0.01
	SD	66.30	174.9	289.0	222.7	169.9	172.5	

Results are presented as mean x ± SD. The same indices in lines represent significance of differences in the mean values between the groups: $^{\alpha,\beta}$ — P < 0.05; ^{a} — P < 0.01; P — significance of the differences of the results during monitored time; ns — not significant

Table 3. Mean values of the body condition score

BCS		3 wk *a.p.*	1 wk *a.p.*	1 wk *p.p.*	3 wk *p.p.*	6 wk *p.p.*	9 wk *p.p.*	P
	x	4.42^{ABC}	4.25^{aDE}	3.90$^{\alpha\beta}$	3.48^{Aa}	3.35$^{BD\alpha}$	3.25$^{CE\beta}$	< 0.001
	SD	0.75	0.58	0.45	0.51	0.40	0.30	

Results are presented as mean x ± SD. The same indices in lines represent significance of differences in the mean values between the weeks: $^{\alpha,\beta}$ — P < 0.05; ^{a} — P < 0.01; ^{A,B,C,D,E} — P < 0.001

(P < 0.01). The mean values of BCS during three weeks and one week before parturition were significantly higher than at three weeks after parturition (P < 0.001). By assessment of the correlation between the leptin and ghrelin, we recorded a significant negative correlation (r = –0.235, P < 0.05). The leptin and BCS exhibited a statistical significant positive correlation (r = 0.360, P < 0.001) and the insulin and BCS displayed a positive correlation which was statistically significant (r = 0.232, P < 0.05).

DISCUSSION

The period of transition between late pregnancy and early lactation presents a huge metabolic challenge to the high-yielding dairy cow and the biochemical profiles are important in evaluating the health status of animals during this transition [2]. The health status of dairy cows, especially during the transition period is a limiting factor of production and reproduction. The production diseases of dairy cows are caused by: a level of production inconsistent with nutrient intake, provision of an inadequate diet, an unsuitable environment, an inappropriate breeding policy, or various combinations of these factors. They have a higher incidence during the transitional period [20].

The onset of lactation is generally characterised by a NEB, due to a drastic increase in energy requirements for milk yield and a simultaneous depression in DMI. The energy status of dairy cows is evaluated by energy intake and output [25] and expressed by the BCS [22]. Several studies have shown that the over-conditioned of dry cows have a greater depression of feed intake during the peripartal period and deeper NEB than cows with a lower BCS.

The transition from pregnancy to lactation in dairy cows is associated with a reduction

in the plasma concentration of leptin. We found non-significantly lower concentrations of leptin during the postpartal period, compared with concentrations during the prepartal period. The changes in the plasma concentration of leptin, could also be an important adaptation,

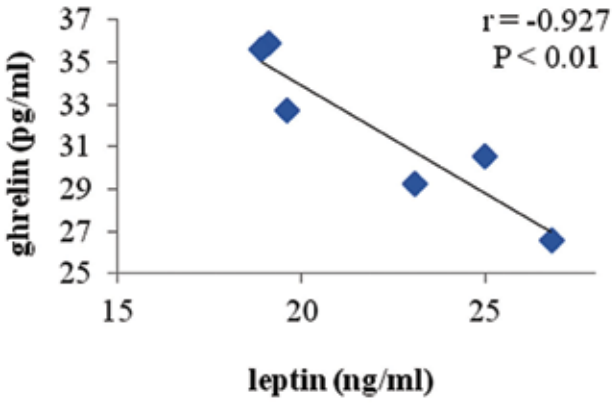

Fig. 1. Correlation between the concentrations of ghrelin and leptin *ante partum* and *post partum*
r — Pearson's correlation coefficient

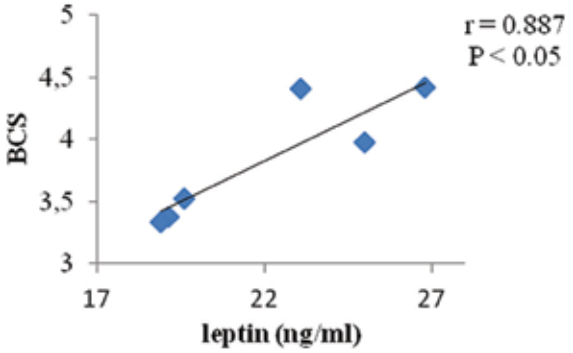

Fig. 2. Correlation between the concentrations of plasma leptin and body condition score *ante partum* and *post partum*

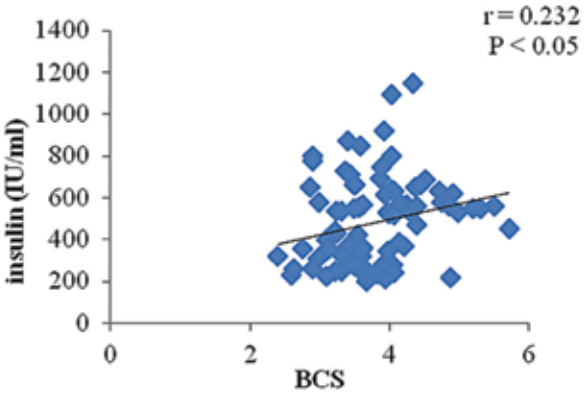

Fig. 3. Correlation between the concentrations of insulin and body condition score a*nte partum* and *post partum*

particularly given the role of white adipose tissue (WAT) in support of early lactation in dairy cattle. It is likely to promote centrally mediated adaptations required during periods of energy deficits. The reduced synthesis of leptin in WAT is largely responsible for the lower concentration of plasma leptin in early lactating dairy cows. This reduction could benefit early lactating dairy cows by promoting a faster increase in feed intake and by diverting energy from non-vital functions such as reproduction [4].

Circulating ghrelin concentrations increase during fasting or NEB in dairy cows, and exogenous administration of ghrelin stimulates feed intake in rats and cattle [5, 32, 33]. We did not detect a significant difference in plasma ghrelin concentrations during the monitored period. The highest concentrations of ghrelin were found six weeks p.p. The highest value of ghrelin immediately after calving has been associated with changes in the feed intake and the initiation of lactogenesis.

Our data indicated that insulin blood concentration decreased from the dry period towards early lactation. The insulin concentration was significantly different in dried cows compared to dairy cows after parturition. In contrast, Illek et al. [14] observed a gradual increase of insulin concentrations during the progressing lactation. A decrease in insulin blood concentration at calving is a metabolic adaptation to cope with the energy demands of lactation [28, 30], as low insulin concentrations favour gluconeogenesis and lipolysis [12].

If valid, the interpretation that leptin negatively regulates ghrelin could imply that the weight reducing effects of leptin are mediated, not only via direct central actions, but also via the peripheral inhibition of ghrelin. In this study, leptin concentrations decreased during the postpartal period, while the concentrations of ghrelin increased.

The data observed in our study confirmed the relationship between leptin and BCS. Our results are in agreement with previous studies showing that plasma leptin was positively correlated with BCS in cows during lactation [8]. Reist et al. [23] also observed a positive relationship between BCS and plasma leptin, whereas Holtenius et al. [13] did not. These results confirm that plasma leptin in ruminants is related to body fat, as previously observed in humans and rodents [19]. We detected a positive relationship between plasma leptin and BCS. This is in agreement with a previous study [29]. However, in the study of León et al. [17], as heifers achieved a higher BCS, the rate of increase in insulin differed.

CONCLUSIONS

In conclusion, our results suggest that selected parameters of hormonal profiles changed throughout the time of *ante partum* and *post partum*, which suggests that they have a physiological role in the dairy cow's energy metabolism. These data provide evidence that the variations in the concentrations of leptin, ghrelin and insulin are related to variations in the BCS. The negative correlation between leptin and ghrelin contributes to the argument that leptin negatively regulates ghrelin. The high rates of the BCS loss in the early postpartum period are associated with a severe NEB alteration in the hormonal profiles. In the period we recorded significant correlations between leptin and ghrelin, leptin and body condition score, as well as between insulin and BCS.

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