Acta Veterinaria (Beograd), Vol. 59, No. 2-3, 147-156, 2009.

DOI: 10.2298/AVB0903147C UDK 619:612.018.2

EFFECT OF SINGLE DOSE DEXAMETHASONE ADMINISTRATION ON METABOLISM RELATED PARAMETERS IN LACTATING FAT TAILED SHEEP

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(Received 15. September 2008)

This study was designed to investigate the effect of a single dose glucocorticoid administration on the parameters related to energy metabolism in sheep. Forty healthy lactating fat tailed ewes, 2-3 years old, were obtained from the Farm of the University of Kafkas. The animals were divided into control (n=20) and treated group (n=20). Ewes in the treatment group (n=20) was parenterally given a single dose of 0.025 mg/kg dexamethasone (Deksavet %0.4 enj.®, Interhas, Istanbul-Turkey) at the beginning of the study. Ewes in the control group (n=20) were parenterally given the same dose of placebo at the beginning of the study. All animals were blood sampled before the drug administration and on the 1st, 2nd, 3rd, 4th, 5th and 7th day of injection. Sera samples were analysed for the determination of concentrations of insulin, β-hydroxybutyric acid (BHB), non esterified fatty acid (NEFA), glucose, triglycerides, cholesterol, aspartate aminotransferase (AST), total protein, albumin, globulin and phosphorus. Cholesterol, glucose (P<0.001) and insulin (P<0.05) concentration obtained on day 1, 2, 3, 4, 5 and 7 were significantly higher than the baseline values on day 0. Concentrations of cholesterol and glucose peaked on day 7 and 3, respectively. NEFA concentration was significantly lower during the experiment except for day 7 when it peaked (P<0.05). Other examined parameters did not significantly change when compared to the baseline values. Comparison of the control and the treated group revealed a statistically significant increase in the concentrations of glucose on day 2, 3, and 4, cholesterol on day 3, 4, 5, and 7, insulin on day 1, 2, 3, 4, 5, and 7 while concentrations of NEFA decreased on day 1, 2, 3, 4, and 7 and phosphorus concentrations decreased on day 4.

The results obtained suggest that a single dose of glucocorticoids may help improving energy metabolism through enhancement of gluconeogenesis during lactation.

Key words: biochemistry, energy metabolism, fat tailed ewes, glucocorticoids

INTRODUCTION

Glucocorticoids, widely used therapeutic agents in the veterinary field (Fürll and Fürll, 1997), take part in the transport of enzymes involved in carbohydrate, lipid and protein metabolism, in the maintenance of fluid and electrolyte balances, and in the regulation of inflammatory and immune reactions. The most important role in carbohydrate metabolism is an increase in blood glucose concentration through induction of gluconeogenesis from aminoacids, inhibition of glycogen destruction, and reduction of glucose usage by transferring glucose into cells. Glucocorticoids block peripheral protein synthesis and stimulate protein destruction in the liver and muscle. Glucocorticoids have also significant effects on lipid hormones (catecholamine, glucagon and STH) through stimulation of lipolysis, inhibition of lipogenesis and decrease of cell glucose intake. However these effects depend on the type of glucocorticoid, animal species (Maddux *et al*., 1998) and physiological status of the animal (Baird and Heitzman, 1970; Maddux *et al.,* 1998; Sandner *et al.,* 1990; Schilinger and Bucher, 1980). Fürll and Knobloch (1994) stated a considerable increase in glucose and insulin concentrations after dexamethasone injection in sheep fasted for five days.

Thanasak *et al*. (2004) also reported an increase in glucose and insulin levels after a single dose in cattle. Sander *et al.* (1990) reported a 200 fold increase in insulin level in fasted goats after dexamethasone administration. Similarly, dexamethasone resulted in glucose rise in cattle (Fürll *et al.,* 1993; Fürll and Knobloch, 1993; Jäckel and Fürll, 1998). Fürll and Leidel (2002) reported that glucocorticoid application had antilipolytic effect and resulted in calcium absorption in the gut and calcium reabsorption in renal tubules in sheep and cattle. Jäckel and Fürrl (1998) reported a marked rise in cholesterol after dexamethasone use. A study involving humans revealed an increase in HDL but not LDL, VLDL and triglycerides after dexamethasone administration (Brotman *et al.,* 2005). Use of Dexamethasone in carbohydrate metabolism disorders such as ketosis or pregnancy toxaemia was proven to improve health status. Studies in cattle have shown that a 5-day application of glucocorticoids did not increase the risk of fatty liver. However it caused antiketogenic and indirect antilipolytic effects (Fürll *et al.,* 1993; Fürll and Knobloch, 1993; Rehage *et al.,* 2002; Sandner *et al.,* 1990; Wittek *et al.,* 2000; Wittek, 2002).

Although the effects of glucocorticoids in cattle have been extensively studied information on their effects in sheep is limited. This study was designed to investigate the effect of a single dose glucocorticoids on energy metabolism related parameters in fat tailed sheep.

MATERIALS AND METHODS

Experimental animals and drug administration

All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Animals in Research" prepared by the National Academy of Sciences and published by the National Institutes of Health.

Forty healthy lactating fat tailed ewes, 2-3 years of age, were obtained from Research Farm of the University of Kafkas. Animals were randomly allotted into the control (n=20) and treatment groups (n=20). The animals were grazed on the farm pasture and water was provided *ad libitum*. The studied animals were clinically examined and treated for endo and ectoparasites a month before the experiment. Healthy animals were included in the study. Ewes in the treatment group (n=20) were parenterally given a single dose of 0.025 mg/kg dexamethasone (Deksavet %0.4 enj.®, Interhas, Istanbul-Turkey) at the beginning of the study. Ewes in the control group (n=20) were parenterally given the same dose of placebo only once at the beginning of the study.

Blood sampling

All animals were blood sampled before the drug administration and at the 1st, 2nd, 3rd, 4th, 5th and 7th day of drug administration. For the analysis of biochemical parameters, 10 mL of blood from each ewe was drawn via jugular vein puncture before feeding. Blood samples were kept at room temperature for one hour and then centrifuged at 3000g for 10 minutes to separate the serum. The sera were stored at –20°C, until analyses.

Biochemical analysis

Spectrophotometric method was utilised to determine concentrations of β-hydroxybutyric acid (BHB), non esterified fatty acid (NEFA) (Randox, United Kingdom), glucose, triglyceride, cholesterol, total protein, albumin and phosphorus (DDS®, Germany) using commercial kits. Insulin concentration was determined by RIA using commercial kits (Roche Diagnostic, Mannheim, Germany). Globulin values were obtained by deducting albumin values from total protein values (Turgut, 2000).

Statistical analysis

Analysis of variance (ANOVA) procedure was used to compare the results between and within groups using SPSS for Windows 6.0. Data were presented as mean±SE. Statistical significance was set at $P<0.05$.

RESULTS

Results are given in the Table 1 and Table 2. Cholesterol and glucose concentrations obtained on day 1, 2, 3, 4, 5 and 7 were significantly higher than the baseline values on day 0 ($P<0.001$). Concentrations of cholesterol and glucose peaked on day 7 and 3, respectively and remained high throughout the study. NEFA concentration was significantly lower during the experiment except from day 7 when it peaked ($P<0.05$). Insulin concentration also began to significantly rise on the first day of dexamethasone application and peaked on day 4 ($P<0.05$) when compared to baseline values. Other examined parameters did not significantly change when compared to the baseline values.

Comparison of the control and treated groups revealed a statistically significant increase in the concentrations of glucose on day 2, 3, and 4, cholesterol on day 3, 4, 5, and 7, insulin on day 1, 2, 3, 4, 5, and 7 while concentrations of NEFA decreased on day 1, 2, 3, 4, and 7 and phosphorus concentrations decreased on day 4 (Table 1 and 2).

Table 1. The concentration of biochemical parameters in the treated (n=20) and control group (n=20) (mean±SE)

	Day	0	1	2	3	4	5	7	P
Cholesterol (mmol/L)	Dexa-methasone	2.45±0.11 d	2.62±0.18 cd	2.74±0.13 bcd	3.07±0.15 A,bc	3.14±0.14 A,b	3.12±0.14 A,b	3.71±0.14 A,a	0.001
	Control	2.39±0.12	2.31±0.16	2.37±0.18	2.45±0.09 B	2.47±0.05 B	2.42±0.15 B	2.38±0.09 B	NS
	P	NS	NS	NS	0.01	0.001	0.01	0.001	
Triglyceride (mmol/L)	Dexa-methasone	0.131±0.01	0.132±0.05	NB	NB	0.143±0.06	0.135±0.06	0.132±0.03	NS
	Control	0.136±0.04	0.133±0.06	NB	NB	0.136±0.07	0.137±0.04	0.135±0.04	NS
	P	NS	NS			NS	NS	NS	
β-hydroxy-butyric acid (mmol/L)	Dexa-methasone	0.63±0.04	0.61±0.02	0.61±0.09	0.58±0.01	0.59±0.02	0.63±0.03	0.64±0.04	NS
	Control	0.58±0.02	0.60±0.04	0.69±0.06	0.65±0.03	0.74±0.09	0.67±0.07	0.63±0.07	NS
	P	NS	NS	NS	NS	NS	NS	NS	
Non esterified fatty acid (µmol/L)	Dexa-methasone	862.1±43.4 a	733.6±61.5 B,b	705.5±44.4 B,b	674.5±49.3 B,b	682.8±41.4 B,b	777.89± 18.90 ab	873.17± 24.98 B,a	0.01
	Control	883.0±47.9	893.0±41.5 A	901.0±60.2 A	964.5±58.1 A	945.9±57.2 A	890.8±69.6 9	1023±61.33 A	NS
	P	NS	0.05	0.01	0.01	0.01	NS	0.05	
Glucose (mmol/L)	Dexa-methasone	3.03±0.12 d	3.27±0.07 cd	3.93±0.17 A,ab	4.09±0.16 A,a	3.81±0.13 A,ab	3.62±0.16 bc	3.73±0.16 ab	0.001
	Control	2.93±0.07	3.16±0.1	2.95±0.16 B	3.27±0.15 B	3.27±0.16 B	3.32±0.17	3.31±0.14	
	P	NS	NS	0.001	0.01	0.05	NS	NS	

Cont. Table 1.

	Day	0	1	2	3	4	5	7	P
Insulin (μIU/L)	Dexa-methasone	0.16±0.01 c	0.27±0.026 A,ab	0.27±0.037 A,ab	0.30±0.055 A,ab	0.35±0.045 A,a	0.27±0.031 A,ab	0.23±0.021 A,bc	0.05
	Control	0.15±0.01	0.14±0.015 B	0.16±0.014 B	0.17±0.09 B	0.18±0.023 B	0.19±0.017 B	0.15±0.083 B	NS
	P	NS	0.001	0.05	0.05	0.01	0.05	0.01	
Aspartate amino-transferase (IU/L)	Dexa-methasone	114.5±2.4	105.8±1.1	93.6±8.2	90.6±3.8	91.7±8.7	82.1±3.6	94.0±9.8	NS
	Control	97.8±2.3	120.3±2.6	112.8±12.4	105.3±15.6	99.6±12.2	84.2±3.4	83.0±3.0	NS
	P	NS	NS	NS	NS	NS	NS	NS	
Bilirubin (mmol/l)	Dexa-methasone	4.60±0.92	4.76±0.34	4.33±0.45	4.11±0.75	3.48±0.50	4.34±0.70	3.90±0.48	NS
	Control	4.96±0.46	4.70±0.55	4.87±0.48	4.52±0.37	4.76±0.42	4.93±0.46	4.53±0.50	NS
	P	NS	NS	NS	NS	NS	NS	NS	

A, B: reflects statistical difference in columns; a, b, c: reflects statistical difference in rows; NS: Not significant

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Table 2. The concentration of biochemical parameters in the treated (n=20) and control group (n=20) (mean±SE)

	Day	0	1	2	3	4	5	7	P
Total Protein (g/L)	Dexamethasone	85.7±1.19	82.3±1.51	NM	NM	83.6±0.86	NM	86.4±1.55	NS
	Control	84.1±0.69	82±1.68	NM	NM	83.9±0.77	NM	84.5±0.93	NS
	P	NS	NS			NS		NS	
Albumin (g/L)	Dexamethasone	36.7±1.28	35.4±1.35	NM	NM	40.1±2.02	NM	39.9±1.21	NS
	Control	35.8±0.99	35.8±1.46	NM	NM	38.6±1.93	NM	37.1±0.99	NS
	P	NS	NS			NS		NS	
Globulin (g/L)	Dexamethasone	49.0±1.83	46.9±1.85	NM	NM	43.5±1.47	NM	46.5±1.85	NS
	Control	48.3±0.95	46.2±1.94	NM	NM	45.3±1.23	NM	47.4±1.69	NS
	P	NS	NS			NS		NS	
Albumin/ Globulin	Dexamethasone	0.76±0.05	0.77±0.05	NM	NM	0.93±0.08	NM	0.86±0.04	NS
	Control	0.75±0.03	0.79±0.05	NM	NM	0.86±0.07	NM	0.79±0.04	NS
	P	NS	NS			NS		NS	
Phosphor (mmol/L)	Dexamethasone	2.08±0.13	1.99±0.12	NM	NM	1.59±0.15 B	NM	2.11±0.13	NS
	Control	2.12±0.09	2.13±0.09	NM	NM	2.05±0.07 A	NM	2.15±0.06	NS
	P	NS	NS			0.05		NS	

A, B: reflects statistical difference in columns; a, b, c: reflects statistical difference in rows; NS: Not significant; NM: Not measurement

DISCUSSION

Glucocorticoids are the most commonly used therapeutic agents in the veterinary field with well known effects on carbohydrate, lipid and protein metabolism. The stabilize of biological membranes and suppress inflammatory and immune reactions. They are widely used in organ and system disorders (allergy; skin, metabolism, liver and bile duct disorders; shock and locomotory system disorders etc.) both topically or systemically (Booth and McDonald, 1988; Lutz, 1998; Melby, 1977; Neugebauer *et al.,* 1995).

Changes in parameters (glucose, insulin, NEFA, bilirubin, BHB, cholesterol and phosphorus) related to energy metabolism after injection of a single dose of glucocorticoid were in agreement with the results previously reported for ruminants (Baird and Heitzman, 1970; Fürll *et al.,* 1993; Fürll and Knobloch, 1993; Fürll and Fürll, 1998b; Jäckel and Fürll, 1998; Rehage *et al.,* 2002; Wittek *et al.,* 2000).

Glucose concentrations in the experimental group was within the reference range reported for sheep (Kraft and Dürr, 1998; Turgut, 2000), but comparison with the control and baseline values disclosed an increase. This finding is in agreement with the report of Fürll and Fürll (1998b) where administration of glucocorticoids resulted in increased glucose values at 24 hours, and peaked on 3rd day of injection. This was attributed to the stimulation of gluconeogenesis and correction of energy balance after administration (Fürll *et al.,* 1993; Fürll and Knobloch, 1993; Wittek, 2002). Increased blood glucose values after dexamethasone administration is related to induced gluconeogenesis through activation of stimulating key enzymes and inhibition of the hexokinase reaction use of aminoacids for gluconeogenesis, and inhibition of glucose consumption by blocking its transfer to the cells (Fürll and Fürll, 1998a).

Insulin and glucose concentrations increased after dexamethasone administration. This was in accordance with earlier studies where glucocorticoid injection resulted in a maked increase in fasted sheep (Fürll and Knobloch, 1994), goats (Sandner *et al.,* 1990) and cattle (Thanasak *et al.,* 2004). Glucocorticoids proven to result in hyperglycaemia through stimulation of gluconeogenesis and reduction of glucose consumption by blocking glucose uptake. Hyperglycaemia results in the stimulation of insulin release (Fürll *et al.,* 1993; Fürll and Knobloch, 1993; Jäckel and Fürll, 1998).

Changes in NEFA, BHB and bilirubin values after a single dose of glucocorticoids are associated with the indirect antilipolytic effects of the drug (Fürll *et al.,* 1993; Fürll and Knobloch, 1993; Rehage *et al.,* 2002; Sandner *et al.,* 1990; Wittek et al., 2000; Wittek, 2002) as was the case in our study where NEFA levels were significantly lower. Glucocorticoids exert effects on lipid metabolism through activation of lipolysis by inhibition of lipolytic hormones (catecholamines, glucagon, somatotropic hormones), blockage of cellular glucose intake and inhibition of lipogenesis. As previously reported hypoglycaemia induces lipolysis in adipose tissues resulting in increased levels of NEFA in ruminants (Rukkwamsuk *et al.,* 1999; Wentink *et al.,* 1997). Obtained BHB values were also within the reference range reported for sheep (Kraft and Dürr 1998; Turgut, 2000).

However, increased BHB is an indication of energy imbalance and poor ratio. Results on BHB are contradictory as dexamethasone use increased BHB (Baird and Heitzman, 1970; Fürll and Fürll, 1998a) or did not alter it (Fürll *et al.,* 1993; Jäckel and Fürll, 1998). In our study the level was low in dexamethasone injected sheep, as reported previously for ruminants (Baird and Heitzman, 1970; Fürll and Fürll, 1998b; Rehage *et al.,* 2002).

Cholesterol was higher in the treated group as reported previously (Jäckel and Fürll, 1998) though the value was also within the reference range (Kraft and Dürr 1998; Turgut, 2000). This increase may be attributed to the enhancing effect of glucocorticoids on liver lipoprotein (VLDL, LDL, HDL) synthesis (Fürll and Fürll, 1998b). Jäckel and Fürll (1998) also reported similar results for ruminants.

A decrease determined in blood phosphorus concentration might have been due to the inhibiting effect of glucocorticoids on intestinal calcium and phosphorus absorption which resulted in lower blood concentrations (Fürll and Knobloch, 1994; Fürll and Fürll, 1998b; Wittek, 2002) though the values obtained were within the physiological range (Kraft and Dürr 1998; Turgut, 2000). AST activity did not significantly change after glucocorticoid injection, as reported earlier (Baird and Heitzman, 1970; Fürll and Knobloch, 1993). Protein parameters (total protein, albumin, globulin, albumin/globulin ratio) were within the normal range (Kraft and Dürr 1998; Turgut, 2000) and did not change during the study. This finding contradicts to that of Fürll *et al*. (1993) and Wittek (2002) where total protein concentration increased, but albumin and globulin concentrations did not change. These differences may be related to the methodology as glucocorticoids were repeatedly used for several days in these studies.

Obtained results may suggest that a single dose of glucocorticoids may help avoiding energy metabolism imbalance in predisposed sheep.

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UTICAJ JEDNOKRATNE APLIKACIJE DEKSAMETAZONA NA NEKE METABOLIČKE PARAMETRE DEBELOREPIH OVACA U LAKTACIJI

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SADRŽAJ

Ova istraživanja su izvedena sa ciljem da se ispita uticaj jednokratne aplikacije glukokortikosteroida na neke parametre energetskog metabolizma ovaca u laktaciji. U ogled je bilo uključeno 40 debelorepih ovaca u laktaciji, starosti dve do tri godine. Ovcama ogledne grupe (n=20) je na početku ogleda parenteralno jednokratno aplikovano 0,025 mg/kg deksametazona (Deksavet, Interhas, Istanbul-Turska) dok su ovcama kontrolne grupe (n=20) aplikovane placebo injekcije. Uzorci krvi su prikupljani pre aplikacije hormona, a zatim 1, 2, 3, 4, 5. i 7. dana ogleda. Nakon izdvajanja seruma, u uzorcima su određivane koncentracije insulina β hidroksibuterne kiseline (BHB), ne-esterifikovanih masnih kiselina (NEFA), glukoze, triglicerida, holesterola, aspartat aminotransferaze (AST), ukupnih proteina, albumina, globulina i fosfora.

Koncentracije holesterola, glukoze ($p<0,001$) i insulina ($p<0,05$) su bile značajno veće u svim posmatranim intervalima u odnosu na vrednosti registrovane pre aplikacije deksametazona. Najveća koncentracija insulina je registrovana sedmog dana a glukoze trećeg dana ispitivanja. Koncentracija NEFA je bila značajno manja tokom ogleda sa izuzetkom vrednosti dobijene sedmog dana kada je bila značajno veća ($p<0,05$) u odnosu na nulti dan. Vrednosti ostalih parametara nisu bile značajno promenjene u odnosu na nulti dan.

Poređenjem vrednosti za oglednu i kontrolnu grupu zapaženo je značajno povećanje koncentracije glukoze (2, 3. i 4. dan), holesterola (3, 4, 5. i 7. dan) i insulina u svim posmatranim intervalima u oglednoj grupi jedinki. Koncentracija NEFA je bila smanjena u svim intervalima, izuzev petog dana a koncentracija fosfora je bila manja u oglednoj grupi samo četvrtog dana.

Ovi rezultati ukazuju da jednokratna aplikacija glukokortikosteroida može da poboljša energetski metabolizam ovaca intenziviranjem glukoneogeneze u toku laktacije.