Oxidative stress and protein catabolism following dexamethasone and isoflupredone administration in Holstein calves

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Abstract:

BACKGROUND: Glucocorticoids have several benefits in large animal medicine but apart from their benefits, there are several disadvantages attributed to the use of these drugs. Among the disadvantages, disturbance in protein metabolism is one of the side effects of glucocorticoids which has been investigated in human and laboratory animals. **OBJECTIVES:** There are no information regarding the effects of glucocorticoids on protein metabolism in large animals. Hence, the present experimental study was performed to evaluate the protein metabolism following glucocorticoids administration in Holstein calves. **METHODS:** Ten clinically healthy Holstein calves (6 to 8 months old) were assigned into 2 equal groups (n=5), containing Dexa and Iso. Dexamethasone (1 mg/kg, intramuscularly) and isoflupredone(1 mg/kg, intramuscularly) were administered in Dexa and Iso groups, respectively, on two consecutive days. Blood samples were taken at days 0 (1st drug administration), 1 (2nd drug dministration), 2, 3, 5 and 7, from all studied animals. Sera were assayed for total protein, albumin, globulin, serum amyloid A and haptoglobin. **RESULTS:** Serum amyloid A and haptoglobinexperience significant increase after administration of both drugs. Isoflupredone induced the synthesis of serum amyloid A and haptoglobin more than dexamethasone ($p<0.05$). Serum concentrations of total protein, albumin and globulin experienced significant decrease after infusion of dexamethasone and isoflupredone ($p<0.05$). Circulating levels of these proteins in Iso group were lower than Dexa one, significantly. **CONCLUSIONS:** Isoflupredone and dexamethasone can induce the protein catabolism. Furthermore, the concentrations of serum amyloid A and haptoglobin, as oxidative stress biomarkers, increased following both drugs administrations due to their oxidation effects on proteins. Finally, the effects of isoflupredone on the metabolism of proteins are significantly higher than dexamethasone in Holstein calves.

Introduction

Glucocorticoids are used for different purposes in large animal medicine. There are several advantages of these drugs, such as organelle and cell-membrane stabilization, improving cellular metabolism and gluconeogenesis, improving microcirculation, decreas-

ing production of endogenous toxins such as myocardial depressant factor, decreasing leukocyte activation and degranulation and minimizing reticuloendothelial depression and histologic organ damage. However, these also have disadvantages apart from their benefits,of which a common one is immunosuppression (Radostits et al., 2007). Among the disadvantages, disturbance in protein metabolism is one of the side effects of glucocorticoids which have been investigated in human and laboratory animals (Tomas et al., 1972; Beaufrere et al., 1989; Horber and Haymond, 1990; Garrel et al., 1995).

Maintenance of optimal body protein status is an essential regulatory process for health (Wajchenberg et al., 1995). Protein mass is lost when there is an increased rate of protein breakdown relative to synthesis or conversely a decrease in synthesis relative to breakdown. Both scenarios result in an increase in irreversible loss of amino acids by oxidation (Burt et al., 2007). The effects of glucocorticoids from endogenous or exogenous sources on protein metabolism are studied in laboratory animals. An increase in the rate of breakdown of muscle protein after treatment of rats with high doses of glucocorticoids has been reported (Tomas et al., 1972).

Several synthetic glucocorticoid hormones such as dexamethasone and isoflupredone are more powerful inducers of protein metabolism, turnover and muscle wasting (McGrath and Goldspink, 1982). Several researchers indicated that glucocorticoids increase protein breakdown relative to synthesis, thereby increasing protein oxidation (Beaufrere et al., 1989; Horber and Haymond, 1990; Garrel et al., 1995).

Glucocorticoids are widely used for large animals, and information regarding their side effects is important for practitioners. According to the knowledge of the authors, all studies on the effects of glucocorticoids have been performed in human and laboratory animals, and information regarding protein metabolism following glucocorticoid administrations in large animals are lacking. Hence, the present experimental study was designed to evaluate the effects of dexamethasone and isoflupredone on protein metabolism in clinically healthy Holstein calves.

Materials and Methods

In October, 2013, 10 clinically healthy Holstein calves (6-8 months old) were selected from two different dairy farms around Shiraz, Iran. The animals were examined prior to study and were proved to be clinically healthy. Calves were assigned into 2 equal groups (n=5), containing Dexa and Iso. Dexamethasone (Vetacoid® 0.2%, Aburaihan Pharmaceutical Co, Tehran, Iran, 1 mg/kg, intramuscularly) and isoflupredone (Vetapredone® 0.2%, Aburaihan Pharmaceutical Co, Tehran, Iran, 1 mg/kg, intramuscularly) were administered in Dexa and Iso groups, respectively, on two consecutive days. Blood samples were taken at days 0 (1st drug administration), 1 (2nd drug dministration), 2, 3, 5 and 7, from all studied animals. Blood samples were collected from all calves through the jugular vein in plain tubes. Immediately after blood collections, sera were separated by centrifugation (10 minutes at 3,000×g) and stored at −22°C until assayed.

Sera were assayed for total protein, albumin, globulin, serum amyloid A (SAA) and haptoglobin (Hp). Total protein was detected by biuret Endpoint method (ZistChem® Diagnostics, Tehran, Iran). Albumin was measured by colorimetric End point method (ZistChem® Diagnostics, Tehran, Iran). Globulin was equal to difference between total protein and albumin. Hp was measured according to the prevention-of peroxidase activity of hemoglobin, which is directlyproportional to the amount of Hp (Tridelta Development Plc, Wicklow, Ireland). SAA wasmeasured by a solid-phase sandwich

enzyme linkedimmunosorbent assay (ELISA) (TrideltaDevelopment Plc).

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using two independent samples t-test to compare mean concentrations of different factors within similar hours between experimental groups. Repeated measures analysis of variance (ANOVA) was also used in order to study the changes in pattern of serum protein profile in each group, statistically. Paired samples t-test was used to determine differences between two different times in each experimental group using statistical package for social sciences (SPSS) software (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois). The level of significance was set at $p<0.05$.

Results

The effects of dexamethasone and isoflupredone on protein metabolism in clinically healthy Holstein calves are presented in Figures 1 and 2. Serum concentrations of Hp and SAA experienced significant increase after the 1st drugs administrations. The high levels of these acute phase proteins remained until the final sampling day. After these corticosteroids administrations, the isoflupredone induced the synthesis of Hp and SAA more than dexamethasone, in all sampling days ($p<0.05$; Figure 1). Serum concentrations of total protein, albumin and globulin experienced significant decrease after the first day of dexamethasone and isoflupredone infusion ($p<0.05$; Figure 2). The decreasing patterns of the protein profile were detected from day 1 up to the final day of blood samplings. Circulating levels of these proteins in Isogroup were significantly lower than Dexaone.

Discussion

Based on the findings, dexamethasone and

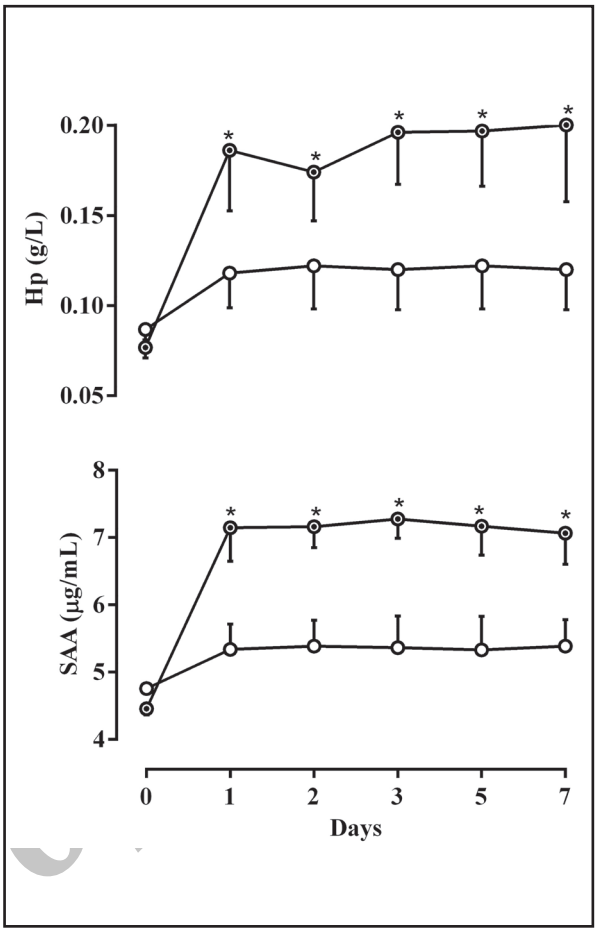

Figure 1. Effects of dexamethasone (1 mg/kg, intramuscularly, on two consecutive days) and isoflupredone (1 mg/kg, intramuscularly, on two consecutive days) on circulating haptoglubin (Hp) and serum amyloid A (SAA) concentrations in clinically healthy Holstein calves. Hp and SAA are increased significantly after the 1st drugs administrations and they are remained at high levels to the 7th day. The concentrations of Hp and SAA in Iso group are higher than Dexa one. Stars indicate significant differences between two groups at similar days ($p<0.05$). Dexa Iso

isoflupredone induced a decreasing pattern in circulatory total protein, albumin and globulin (Figure 2). The inhibitory effect of glucocorticoids on protein synthesis is as a result of different mechanisms. First, is the inhibition of the transportation of amino acids into the muscle by glucocorticoids (Kostyoand Redmond, 1966) which could limit the protein synthesis. Second, is the inhibitionof the stimulatory action of insulin, insulin-like growth factor-I (IGF-I), and amino acids (in particular leucine) by glucocorticoids, on the phosphorylation of eIF4Ebinding protein 1 (4E-BP1) and

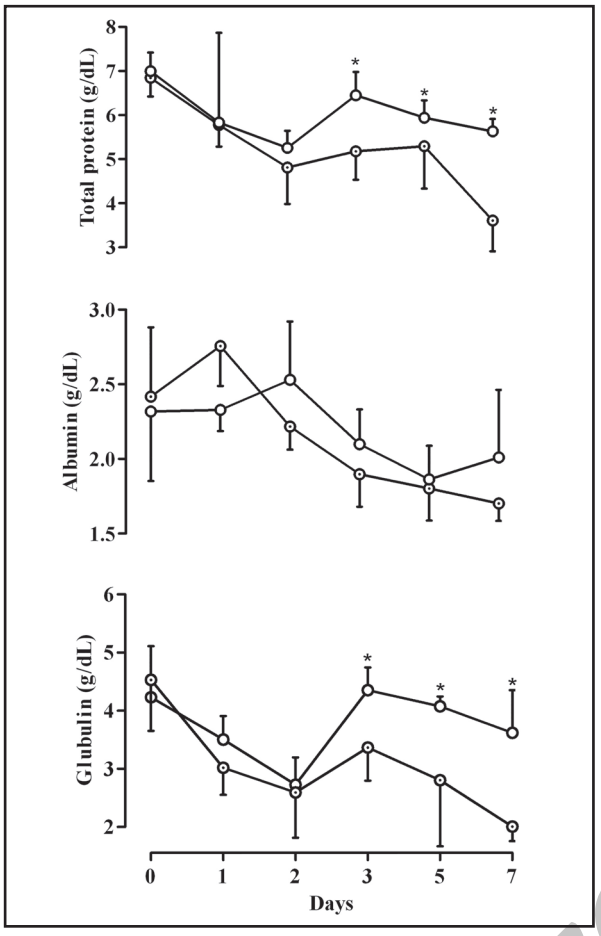

Figure 2. Effects of dexamethasone (1 mg/kg, intramuscularly, on two consecutive days) and isoflupredone (1 mg/kg, intramuscularly, on two consecutive days) on circulating total protein, albumin and glubulin concentrations in clinically healthy Holstein calves. The levels of protein profile are significantly decreased after the first dexamethasone and isoflupredone infusions. These decreasing patterns are remained to the final samplings. Circulating levels of these proteins in Iso group were lower than Dexa one, significantly. Stars indicate significant differences between two groups at similar days ($p<0.05$).

the ribosomal protein S6 kinase 1 (S6K1), two factors that play a key role in the protein synthesis machinery by controlling the initiation step of mRNA translation (Liu et al., 2004).

Many pathological conditions characterized by muscle atrophy (sepsis, cachexia, starvation, metabolic acidosis, severe insulinopenia, etc.) are associated with an increase in circulating glucocorticoids levels (Leckeret al., 1999), suggesting that these hormones could trigger the muscle atrophy due to their catabolic effects on muscular tissue and protein breakdown. In the case of sepsis, cachexia, starvation, and severe insulinopenia, adrenalectomy or treatment with aglucocorticoid receptor antagonist (RU-486) attenuate muscle atrophy, indicating that glucocorticoids are in part responsible for this muscle loss and protein breakdown (Schakman et al., 2008). In skeletal muscle, glucocorticoids result in a decrease in the rate of protein synthesis and an increase in the rate of protein breakdown (Lofberget al., 2002), thereby contributing to atrophy. Glucocorticoid-induced muscle atrophy results mainly from an increased protein breakdown in adult rats but mostly from depressed protein synthesis in the aged animals (Dardevetet al., 1998).

High doses of glucocorticoids acutely induce protein catabolism by increasing protein breakdown and oxidation in healthy adults (Garrel et al., 1995). Subjects with severe chronic glucocorticoids excess, such as in Cushing's syndrome, have a greater rate of protein oxidation than normal subjects, suggesting an ongoing protein loss. As the severity of glucocorticoids excess during therapeutic glucocorticoids usage is less than in Cushing's syndrome, it is uncertain whether an acute glucocorticoid-induced change in protein oxidation persists during chronic use (Aloia et al., 1974).

Short et al. (2004) reported that glucocorticoids exerted no acute effect on whole body protein breakdown, skeletal muscle fractional synthetic rate, limb amino acid kinetics and muscle mitochondrial function. However, whole body protein oxidation, which was significantly increased in this study, was not quantified. As protein oxidation results in irreversible loss of amino acids, increased protein oxidation is consistent with an acute catabolic effect of glucocorticoids. Therapeutic glucocorticoids acutely induce protein catabolism by increasing irreversible oxidative loss of amino acids. However, during the use of chronic glucocorticoid, the rate of protein oxidation is not significantly different from normal (Crawford et al., 2003).

Based on our findings, serum concentrations

of total protein, albumin and globulin in Iso group were significantly lower than Dexa one. It can be suggested that isoflupredone induces protein breakdown and oxidation more than dexamethasone in clinically healthy calves.

SAA and Hp, as well as other acute phase proteins, have been proposed as markers of oxidative stress in animals (Hickey et al., 2003; Pineiro et al., 2007). SAA is an apolipoprotein of high-density lipoprotein and considered as one of the major acute phase proteins in vertebrates. Determination and evaluation of SAA showed that this acute phase protein could be a valuable factor in the diagnosis of oxidative stress (Gruys et al., 1994). Hp is an α2-globulin synthesized in the liver and another major acute phase protein in numerous species of productive and companion animals. In ruminants, the level of circulating Hp is negligible in normal animals but increases over 100-fold with immune stimulation (Feldman et al., 2000). Furthermore, Hp is a clinically useful parameter for the evaluation of the occurrence and severity of inflammatory and oxidative situations in ruminants (Chalmeh et al., 2013).

In the present study, SAA and Hp as acute phase proteins experienced significant increase after administrations of dexamethasone and isoflupredone. Acute phase proteinsand their changes due tovarious inflammatory and non-inflammatory conditionshave been studied intensively in many animal species (Eckersall, 2000; Petersen et al., 2004; Murata et al., 2004; Murata, 2007). They usually experience increased concentrationsduring oxidative stress situations. Oxidation of proteins due to administrations of glucocorticoids can be considered as an oxidative stress status. It can be stated that the increasing patterns of both acute phase proteins in our study resulted from the oxidation of whole body proteins. Based on our findings, the concentrations of SAA and Hp in Iso group were significantly higher than Dexa one (Figure 1).Since the isoflupredone can induce protein oxidation and breakdown more than dexamethasone, it can be suggested that oxidative stress which results from the administration of isoflupredoneis greater than dexamthasone. Hence, the increasing pattern of acute phase proteins in Iso group was significantly higher than Dexa one.

Glucocorticoids induce catabolism in relation to dose, length of treatment and the status of nutrition. Catabolic activities and net protein losses are related to all these factors (McGrath and Goldspink, 1982; Horber and Haymond, 1990). Many human patients with long term glucocorticoids therapy are also at high risk of muscular breakdown, protein catabolism and low anabolic activities (Dardevet et al., 1998). Furthermore, glucocorticoids induce their catabolic effects due to several mechanisms such as increase in catabolic hormones (cortisol and catechols), decrease in anabolic hormones (human growth hormone and testosterone), elevating metabolic rate, increase in the conversion rate of amino acids to glucose through the liver gluconeogenesis and rapid skeletal muscle breakdown with the use of amino acid as an energy source (Liu et al., 2004; Burt et al., 2007; Schakman et al., 2008).

In conclusion, the results of the present study showed that isoflupredoneand dexamethasone can induce protein catabolism. Furthermore, there was an increase in the concentrations of acute phase proteins, which served as oxidative stress biomarkers, following both drugs administrations due to their oxidation effects on proteins. Finally, the effects of isoflupredone on the metabolism of proteins are significantly higher than dexamethasone in Holstein calves.

Conflict of interest statement: The authors have declared no conflicts of interest.

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References

1. Aloia, J.F., Roginsky, M., Ellis, K., Shukla, K., Cohn, S. (1974) Skeletal metabolism and body composition in Cushing's syndrome. J Clin Endocrinol Metab. 39: 981-985.
2. Beaufrere, B., Horber, F.F., Schwenk, W.F., Marsh, H.M., Matthews, D.,Gerich, J.E., Haymond, M.W. (1989) Glucocorticosteroids increase leucine oxidation and impair leucine balance in humans. Am J Physiol Endocrinol Metab. 257: E712-E721.
3. Burt, M.G., Gibney, J., Ho, K.K. (2007) Protein metabolism in glucocorticoid excess: study in Cushing's syndrome and the effect of treatment. Am J Physiol Endocrinol Metab. 292: E1426-1432.
4. Chalmeh, A., Badiei, K., Pourjafar,M., Nazifi, S. (2013) Anti-inflammatory effects of insulin regular and flunixin meglumine on endotoxemia experimentally induced by *Escherichia coli* serotype O55:B5 in an ovine model. Inflamm Res. 62:61-67.
5. Crawford, B.A., Liu, P.Y., Kean, M.T., Bleasel, J.F., Handelsman, D.J. (2003) Randomized placebo-controlled trial of androgen effects on muscle and bone in men requiring long-term systemic glucocorticoid treatment. J Clin Endocrinol Metab. 88: 3167-3176.
6. Dardevet, D., Sornet, C., Savary, I., Debras, E., Patureau-Mirand, P., Grizard, J. (1998) Glucocorticoid effects on insulin- and IGF-I-regulated muscle protein metabolism during aging. J Endocrinol. 156: 83-89.
7. Eckersall, P.D. (2000) Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. Rev Med Vet. 151: 577-584.
8. Feldman, B.F., Zinkl, J.G., Jain, N.C. (2000) Schalm's Veterinary Hematology. (1st ed.) Lippincott Williams and Wilkins. Philadelphia, USA.
9. Garrel, D.R., Moussali, R., De Oliveira, A., Lesiege, D., Lariviere, F. (1995) RU 486 prevents the acute effects of cortisol on glucose and leucine metabolism. J Clin Endocrinol Metab. 80: 379-385.
10. Gruys, E., Obwolo, M.J., Toussaint, M.J.M. (1994) Diagnostic significance ofthe major acute phase proteins in veterinary clinical chemistry: areview. Vet Bull. 64: 1009-1018.
11. Hickey, M.C., Drennan, M., Early, B. (2003) The effect of abrupt weaning of suckler calves on the plasma concentrations of cortisol, catecholamines, leukocytes, acute-phase proteins and in vitrointerferon-gamma production. J Anim Sci. 81: 2847-2855.
12. Horber, F.F., Haymond, M.W. (1990) Human growth hormone prevents the protein catabolic side effects of prednisone in humans. J Clin Invest. 86: 265-272.
13. Kostyo, J.L., Redmond, A.F. (1966) Role of protein synthesis in the inhibitory action of adrenal steroid hormones on amino acid transport by muscle. Endocrinology. 79: 531-540.
14. Lecker, S.H., Solomon, V., Mitch, W.E., Goldberg, A.L. (1999) Muscle protein breakdown and the critical role of the ubiquitin-proteasome pathway innormal and disease states. J Nutr. 129: 227S-237S.
15. Liu, Z., Li, G., Kimball, S.R., Jahn, L.A., Barrett, E.J. (2004) Glucocorticoids modulate amino acid-induced translation initiation in human skeletal muscle. Am J Physiol Endocrinol Metab. 287: E275-E281.
16. Lofberg, E., Gutierrez, A., Wernerman, J., Anderstam, B., Mitch, W.E., Price, S.R.,Bergstrom, J.,Alvestrand, A.(2002) Effects of high doses of glucocorticoids onfree amino acids, ribosomes and protein turnover in human muscle.Eur J Clin Investig. 32: 345-353.
17. McGrath, J.A., Goldspink, D.F. (1982) Glucocorticoid action on protein synthesis and protein breakdown in isolated skeletal muscles. Biochem J. 206: 641-645.
18. Murata, H., Shimada, N., Yoshioka, M. (2004) Current research on acute phase proteins in veterinary diagnosis: an overview. Vet J. 168: 28-40.
19. Murata, H. (2007) Stress and acute phase protein response: an inconspicuousbut essential

linkage. Vet J. 173: 473-474.
20. Petersen, H.H., Nielsen, J.P., Heegaard, P.M.H. (2004) Application of acute phase protein measurements in veterinary clinical chemistry. Vet Res. 35: 163-187.
21. Pineiro, M., Pineiro, C., Carpintero, R., Morales, J., Campbell, F.M., Eckersall, P.D., Toussaint, M.J., Lampreave, F. (2007) Characterization of the pig acute phase protein response to road transport. Vet J. 173: 669-674.
22. Radostits, O.M., Gay, C.C., Hinchcliff, K.W., Constable, P.D. (2007) Toxemia and endotoxemia. In: Veterinary Medicine: A Text Book of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. (10th ed.) Elsevier. Amsterdam, The Netherlands. p. 53-60.
23. Schakman, O., Gilson, H., Thissen, J.P. (2008) Mechanisms of glucocorticoid-induced myopathy. J Endocrinol. 197: 1-10.
24. Short, K.R., Nygren, J., Bigelow, M.L., Nair, K.S. (2004) Effect of short-term prednisone use on blood flow, muscle protein metabolism, and function. J Clin Endocrinol Metab. 89: 6198-6207.
25. Tomas, F.M., Munro, H.N., Young, V.R. (1979) Effect of glucocorticoid administration on the rate of muscle protein breakdown in vivo in rats, as measured by urinary excretion of N tau-methylhistidine. Biochem J. 178: 139-146.
26. Wajchenberg, B.L., Bosco, A., Marone, M.M., Levin, S., Rocha, M., Lerario, A.C., Nery, M., Goldman, J., Liberman, B. (1995) Estimation of body fat and lean tissue distribution by dual energy X-ray absorptiometry and abdominal body fat evaluation by computed tomography in Cushing's disease. J Clin Endocrinol Metab. 80: 2791-2794.

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استرس اکسیداتیو و کاتابولیسم پروتئینی متعاقب تجویز دگزامتازون و ایزوفلوپردون در گوساله‌های هلشتاین

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(دریافت مقاله: ۴ خرداد ماه ۱۳۹۴، پذیرش نهایی: ۱۰ شهریور ماه ۱۳۹۴)

چکیده

زمینه مطالعه: گلوکوکورتیکوئیدها منافع بسیاری در طب دام‌های بزرگ دارند اما در کنار منافعشان، زیان‌هایی متعاقب تجویز این داروها وجود دارد. در بین زیان‌ها، اختلال در متابولیسم پروتئینی یکی از عوارض جانبی گلوکوکورتیکوئیدهاست که در انسان و حیوانات آزمایشگاهی مورد بررسی واقع شده است. **هدف:** اطلاعاتی پیرامون اثرات گلوکوکورتیکوئیدها بر متابولیسم پروتئینی در دام‌های بزرگ موجود نیست. از این رو مطالعه حاضر به منظور بررسی متابولیسم پروتئینی متعاقب تجویز گلوکوکورتیکوئیدها در گوساله‌های هلشتاین انجام شد. **روش کار:** ۱۰ راس گوساله هلشتاین به ظاهر سالم (۶ تا ۸ ماهه) به دو گروه مساوی (هر گروه ۵ رأس) شامل دگزا و ایزو تقسیم شدند. دگزامتازون (۱mg/kg، عضلانی) و ایزوفلوپردون (۱mg/kg، عضلانی) به ترتیب به گروه‌های دگزا و ایزو در دو روز متوالی تجویز شد. نمونه‌های خون در روز صفر (اولین تجویز دارو)، ۱ (دومین تجویز دارو)، ۲، ۳، ۵ و ۷ از تمامی حیوانات مورد مطالعه اخذ شد. سرم‌ها به منظور بررسی پروتئین تام، آلبومین، گلوبولین، سرم آمیلوئید آ و هاپتوگلوبین مورد ارزیابی واقع شدند. **نتایج:** سرم آمیلوئید آ و هاپتوگلوبین به طور معنی‌داری پس از تجویز هر دو دارو افزایش یافتند. ایزوفلوپردون تولید سرم آمیلوئید آ و هاپتوگلوبین را بیش از دگزامتازون القا کرد ($p<۰/۰۵$). غلظت سرمی پروتئین تام، آلبومین و گلوبولین به طور معنی‌داری پس از تزریق دگزامتازون و ایزوفلوپردون کاهش یافت ($p<۰/۰۵$). سطح در گردش خون این پروتئین‌ها در گروه ایزو به طور معنی‌داری کمتر از گروه دگزا بود. **نتیجه‌گیری نهایی:** ایزوفلوپردون و دگزامتازون می‌توانند کاتابولیسم پروتئینی را القا کنند. علاوه بر این، غلظت سرم آمیلوئید آ و هاپتوگلوبین، به عنوان بیومارکرهای استرس اکسیداتیو، متعاقب تجویز هر دو دارو افزایش یافت که به واسطه تأثیر اکسیداسیونی آنها بر پروتئین‌ها بود. در نهایت، اثرات ایزوفلوپردون بر متابولیسم پروتئین‌ها در گوساله‌های هلشتاین به طور معنی‌داری بیش از دگزامتازون است.

واژه‌های کلیدی: استرس اکسیداتیو، کاتابولیسم پروتئینی، گلوکوکورتیکوئیدها، گوساله‌های هلشتاین

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