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Veterinary Faculty of the State University of Liège — Belgium

Effects of dexamethazone injections on performances in a pair of monozygotic cattle twins

By L. Istasse, V. de Haan, C. Van Eenaeme, B. Buts, P. Baldwin, M. Gielen, D. Demeyer and J. M. Bienfait

Introduction

Performance enhancers have been widely used in livestock production. They are either feed additives, anabolic agents or more recently β-agonists. Some of them are derived from drugs used in human or veterinary medicine. They increase meat production by improvement of live weight gain, feed conversion ratio or carcass quality. Glucocorticoids are generally considered as growth inhibiting substances (see review by Sharpe et al. 1986). Generally the data were obtained from "in vitro" experiments or experiments in which large doses often well over 1 mg/kg body weight were injected.

The present experiment was carried out to assess the effects of lower doses of dexamethazone on performances in a pair of monozygotic cattle twins.

Materials and methods

Animals

Two identical cattle twins obtained from a splitted embryo were used. They were from the Belgian While Blue breed — dual purpose type. They were maintained from birth in similar environmental conditions with a supplementary lighting of 400 lux from 06.00 h till 18.00 h. They were 14 months old at the beginning of the experiment. The fattening diet was made of 13% hay, 50% sugar beet pulp, 21% rolled barley, 15% soya bean meal and 1% mineral mixture. The crude protein content of the diet offered was 13%. Half of the ration was provided at 08.00 h and the other half at 16.00 h. Feed intakes were close to ad libitum by twice weekly adjustements.

Experimental design

The experiment was divided in 2 periods each of 28 days. During the first period, both animals were used as control. During the second period, one animal received 4 intramuscular injections of 12 mg Dexamethazone (Dexafort-Intervet) as a mixture of 5.28 mg phosphate and 10.68 phenylpropionate at a rate of one injection every 7 days. The other animal received 4 intramuscular injections of physiological saline.

Measurements

The amount of feed given was weighed out daily. The liveweight was recorded once weekly. Nitrogen balance was carried out on the last week of the first period and on week 3 of the second period. Blood samples were withdrawn twice a week before the morning feeding for

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evaluation of blood cells and for determination of glucose, urea, creatinine, free amino nitrogen, non esterified fatty acids and total cortisol. Blood samples were also taken every 20 minutes over a 24 hour period on the last day of the second period. Growth hormone was measured in all the samples while insulin and total cortisol were determined every 2 hours.

The liveweight of the animals and the weight of the warm and cold carcasses were recorded at the slaughterhouse. The 7 to 9 ribs were removed to be dissected in order to separate lean meat, fat and bones; the composition of the carcass was then estimated according to the method of MARTIN and TORREELE (1962). A sample of the longissimus dorsi was taken for chemical analyses and to assess meat quality. Samples from the liver and from the adrenal gland were immediately placed in 10% buffered formalin for histological evaluation.

Analytical methods

Kjeldahl nitrogen was estimated by block digestion and automated calorimetry using the Berthelot reaction. Plasma samples were analysed for glucose by o-toluidine, urea by the diacetyl monoxine method, creatinine by the Jaffe method and free amino nitrogen by the trinitrobenzene sulfonate method using a technicon autoanalyzer. Non esterified fatty acids were determined by capillary gas chromatography after a method described by MUELLER and BINZ (1982), cortisol, insulin and growth hormone were estimated by radio immuno assay respectively with a commercial kit procedure (Amersham, UK), by the method of MICHAUX et al. (1981) and the method of CLOSSET et al. (1986). Blood cells was measured in a coulter counter. Meat quality was assessed by estimating drip and cooking losses, loose water value, shear force, pH and sarcomere length (BUTS et al. 1986a). Myofibrillar protein fractions were extracted and separated by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) (BUTS et al. 1986b). The samples for histological evaluation were dehydrated in progressive alcohols, embedded in paraffin, sectioned in 5 μm slices and stained with hematoxylin-eosine. Liver samples were also examined after Pass coloration.

Results

Each period lasted for 28 days. The initial live weight of the 2 identical cattle twins on the beginning of period 1 (control period for both bulls) was 530 and 526 kg respectively. The total liveweight gain during this period was quite similar in both animals: 29 and 25 kg respectively. During period 2, the control bull gained 37 kg while the liveweight gain in the treated animals was much higher at 64 kg corresponding to 2.29 vs 1.32 kg/d. The pattern of live weight changes was quite similar between the 2 animals during period 1. By contrast, the live weight gain of the treated animal was improved directly after the first injection, remained sustained after the second and the third but tended to decrease after the fourth injection. Total feed intake was similar during period 1 and averaged 10.0 kg dry feed per day. During period 2, there was an increase in feed intake with both animals, the increase being higher with the treated bull. The feed conversion ratio was very low at 4.8 kg/kg in the treated bull vs 8.0 kg/kg for the control animal. The nitrogen retention did not differ in period 1 (55 g/d) while in period 2 there was a trend for a decrease in the treated animal. The final liveweight was 596 and 615 kg for the control and the treated bull respectively (Table 1). During the transport to the slaughterhouse, the weight loss was 27 and 22 kg corresponding to 4.5 and 3.6%. The weight of the warm carcass was greater (359 kg) in the dexamethazone treated bull than in the control animal (352 kg). The same trend was observed for the cold carcass. The killing-out percentage calculated from the weight of the cold carcass and the weight at slaughter was 59.1 and 58.0 in the control and the treated bull respectively. There were no differences in the proportion of lean meat or adipose tissue in the carcass.

Table 1. **Carcass traits and some characteristics of the longissimus dorsi in a control bull and its identical twin injected four times with 12 mg dexamethazone**

	Control bull	Injected bull
Warm carcass weight (kg)	352	359
Cold carcass weight (kg)	336	344
Killing-out percentage (%)	59.1	58.0
Boneless carcass		
Lean meat (%)	71.6	71.4
Adipose tissue (%)	28.4	28.6
Characteristics of the longissimus dorsi		
Drip losses (%)	10.8	6.7
Cooking losses (%)	30.9	24.8
Loose water value (%)	32.17	29.86
pH	5.79	5.60
Shear force (N)	26.40	29.20
Sarcomere length (μm)	1.86	1.92
Myofibrillar protein concentration[1]		
– titine	33.5	33.2
– filamine	1.0	1.6
– troponine T	0.6	2.3
– 30 kD	17.6	13.3
– 35.4 kD	5.2	15.0
Chemical composition		
– dry matter (%)	24.5	23.1
– crude protein (% of dry matter)	83.0	81.0
– Ether extract (% of dry matter)	6.1	12.5
– Ash (% of dry matter)	4.2	4.1

[1] μg Bovine Serum Albumine Equivalent/mg Myofibrillar Crude Protein

The characteristics of the longissimus dorsi are given in Table 1. The dexamethazone treatment resulted in a reduction in both drip and cooking losses. The water holding capacity was increased as indicated by the lower loose water value. There was a trend for an increase in the shear force. The main differences for myofibrillar protein composition were significantly higher concentrations of troponine T and 34.5 kilo Dalton (kD) components and corresponding lower concentrations of 30 kD components in the injected bull. The chemical composition of the longissimus dorsi of the treated animal was characterized by a lower dry matter content, a slightly lower crude protein content and a much higher ether extract content.

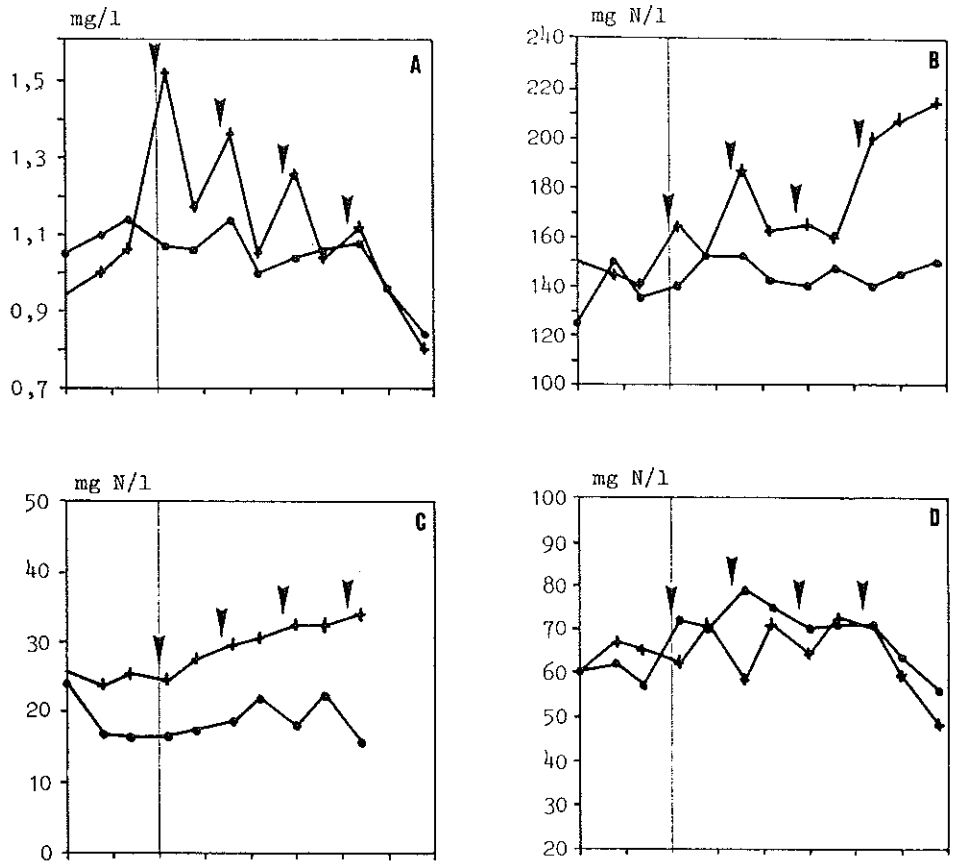
The effects of dexamethazone on the blood cells are given in Table 2. The injections did not change to a large extent the number of white and red cells. By contrast, dexamethazone reduced the proportion of lymphocytes and eosinophils and increased the proportion of neutrophils. The histological evaluation did not show any difference between treated and

Table 2. **Blood cells characteristics in a control bull and its identical twin injected four times with 12 mg dexamethazone**

	Control bull	Injected bull
White blood cell count (10^9/l)	11.3	12.2
Lymphocytes (%)	53.4	29.7
Neutrophils (%)	35.3	66.5
Eosinophils (%)	9.9	2.2
Monocytes (%)	0.8	1.3
Basophils (%)	0.5	0.1
Red blood cell count (10^{12}/l)	7.27	6.93

untreated animals in the adrenal gland and the liver. Many glycogen granules were found in the treated bull when the slices from the liver were stained with Pass.

The effects of dexamethazone injections on plasma samples taken twice weekly are given in Figure 1. In the treated bull, plasma glucose concentration (Figure 1A) was higher the day after each injection than 4 days later. One has to note that the extent of the increase was reduced with the consecutive injections. Urea concentration (Figure 1B) was also higher the day after the injections with the exception of the third. By contrast to the glucose pattern, urea concentration remained higher until the end of the experimental period. Creatinine concentration (Figure 1C) increased constantly in the treated bull while it did not show any particular pattern in the control animal. There was a trend for alpha amino nitrogen concentration to be lower in the treated animal (Figure 1D). Before treatment and during the first 3 weeks the concentration in non esterified fatty acids was similar for the 2 animals. The concentration decreased to a large extent in the treated animals during the final part of the experiment (Figure 1E). Although the concentration of total cortisol differed slightly between the 2 animals on day 0, total cortisol concentration decreased and remained low in the treated bull while it tended to increase in the control animal (Figure 1F).



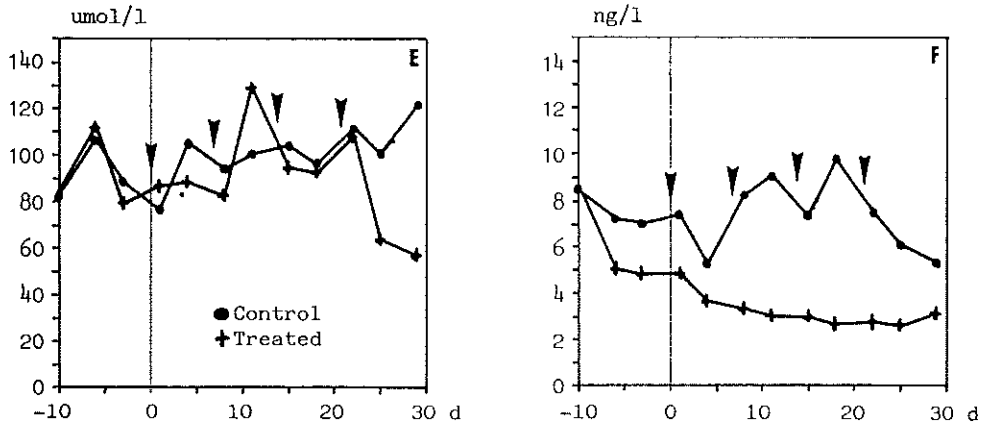


Fig. 1. Concentrations of glucose (A), urea (B), creatinine (C), alpha-amino-nitrogen (D), non esterified fatty acids (E), and total cortisol (F) in the plasma of a control bull (O) and its identical twin treated with dexamethazone (+). Arrows indicate the injection times

From the plasma samples taken over a 24 h period on the end of period 2 it appeared that insulin was higher (Figure 2A) and total cortisol lower (Figure 2C) in the treated bull than in the other animal. By contrast there were no differences in the pattern of growth hormone between the 2 animals (Figure 2B). It was of interest to note that no peaks were detected in the growth hormone curves.

Discussion

In the present experiment, one animal only was treated and the performances were compared with those of his identical twin. Dexamethazone obviously improved live weight gain to a large extent (2.29 vs 1.32 kg/day). The present improvement was much higher than the values reported by LAMBOT et al. (1982) (1.71 vs 1.14 kg/day) in bulls given trenbolone acetate and oestradiol benzoate in similar treatment conditions.

The increase in carcass weight was much lower since the cold carcass of the treated bull was only 8 kg heavier than that of the control. Such a difference suggested that part of the increase in weight occured at the gastro-intestinal tract level either as increase of gut content indicated by higher feed intake or increase in weight of glands and gastro-intestinal tract walls. Since there were no treatment effects on the composition of the carcass, the overall effect was an increase of about 5 kg of lean meat and 3 kg of adipose tissue. The present results therefore did not agree with the generally accepted concept that "glucocorticoids are growth inhibiting steroids" (SHARPE et al. 1986).

Meat composition was affected as indicated by the characteristics of the longissimus dorsi. There was a reduction in dry matter and crude protein content. Assuming that the effect of the treatment was similar in all the carcass muscles, the carcass of the treated animal would have contained 3 kg crude protein less than the control bull ($344 \times 0.714 \times 0.231 \times 0.81$ vs $336 \times 0.716 \times 0.245 \times 0.83$). Such results were in line with the reduction in net protein deposition indicated by a lower nitrogen balance and also suggested by higher urea concentration particularly the day after the injection. *"In vitro"* systems were used to assess the effects of dexamethazone on protein synthesis and protein degradation. The results are still conflicting. MC GRATH and GOLDSPINK (1982) found a reduction in protein synthesis and degradation. By contrast SKJAERLUND et al. (1988) did not find any effect

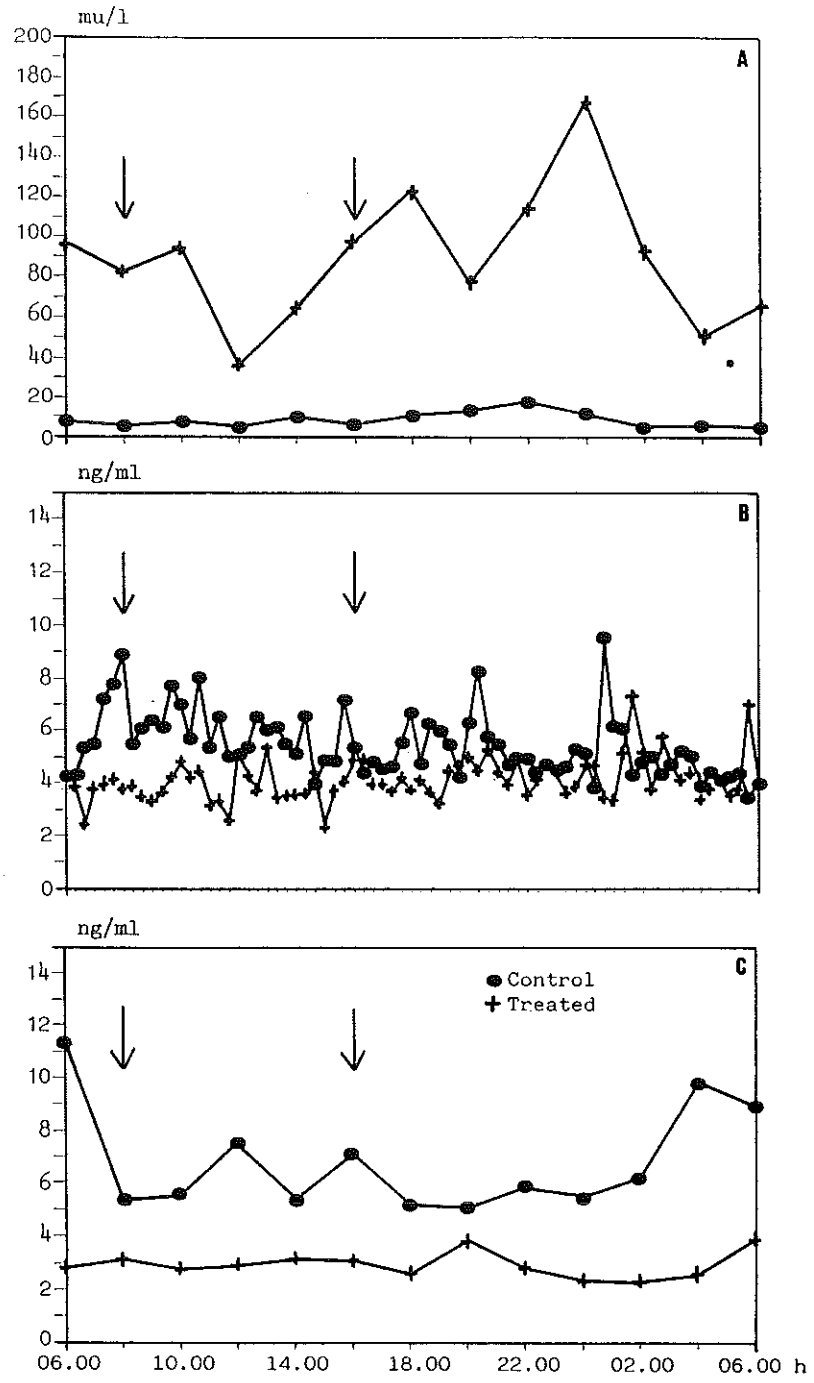


Fig. 2. Concentrations of insulin (A), growth hormone (B) and total cortisol (C) in the plasma samples taken over a 24 hours period in a control bull (○) and its identical twin treated with dexamethazone (+). Arrows indicate feeding times

on either protein synthesis or protein degradation. Our results on myofibrillar protein composition are in line with the drug inducing a depression of protein degradation. Troponin T concentration was higher after treatment, whereas 30 kD proportions were lower. Such results suggest a depression in post-mortal muscle tenderisation and protein fragmentation, normally, reflected in tougher meat (BUTS et al. 1987). No significant difference was however observed in shear force. Increased water retention may very well have a tenderizing effect, compensating for the toughening due to less maturation. Differences in the 34,5 kD proportion may be related to the difference in pH (BUTS et al., unpublished).

Dexamethazone treatment resulted also in a higher water retention in the longissimus dorsi indicated by a higher water holding capacity and a reduction of drip and cooking losses. Thyrostatic drugs were used some years ago to stimulate meat production. There was also an increase in the water retention but the meat was pale and soft with high water losses (VANSCHOUBROEK 1963). In the case of dexamethazone, water appeared to be well fixed to the tissues. The treatment increased also the ether extract content of the longissimus dorsi. This effect was opposite to the found with anabolic agents (LAMBOT et al. 1983; ISTASSE et al., 1988) and with β-agonists (BOUCQUÉ et al. 1987). Finally, it was of interest to note that, although the measurements in the present experiment tended to indicate a reduction in meat quality, the carcass of the treated bull would have been sold 6% more because of heavier carcass weight and higher unit price.

Plasma glucose rose immediately after the injection and dropped 3 days later; it was generally higher than in the control bull. Plasma free amino nitrogen tended to be lower in the treated animal. Such changes could be associated with a possible sparing effect of glucose for glycogen production in the liver as proposed by BAXTER and FORSHAM (1972) and indicated by the histological evaluation. Glycogen storage appeared to occur only in the liver not in muscles as suggested by the chemical composition of the longissimus dorsi. Glycogen amounted to 6.7% in the control bull and 2.4% in the treated animal assuming that glycogen content was the difference between dry matter and the sum of crude protein, ether extract and ash.

At hormonal level, dexamethazone treatment increased insulin concentration, did not change growth hormone and reduced total cortisol concentration. Although the histological evaluation did not reveal any difference between the 2 adrenal glands, the reduction of total cortisol could be considered as a negative feed back effect on the adrenal gland. According to TOUTAIN et al. (1982), the negative effect on the adrenal gland could last for a period longer than one month. TISCHLER (1981) emphasized the role of glucocorticoids: insulin ratio as determinant for protein deposition: the higher this ratio, the lower the protein content in muscles. The results of the present experiment did not support his hypothesis since the treated animal had a lower nitrogen retention and a lower total cortisol: insulin ratio. It seemed that the changes observed were rather due to the dexamethazone itself rather than to a lowering of the concentration in total cortisol. It should however, be noted that in the present study the doses used were relatively low since the treated bull received 4 times 12 mg corresponding to about 0.02 mg/kg body weight. The dosis used with laboratory animals were much higher. For example, KELLY and GOLDSPINK (1982) injected rats with 2.5 mg/kg body weight which was about 125 times higher than with the treated bull. The doses used were also lower than 30 mg suggested on the notice of the manufacturer.

Summary

Two growing fattening bulls, identical twins obtained from a splitted embryo, were given a fattening diet based on dried sugar beet pulp and containing 13% crude protein. During the first period of the experiment both animals were used as control. In the second period, one bull received 4 intramuscular injections of 12 mg dexamethazone at a rate of one injection a week. The other animals received physiological saline. The dexamethazone treatment increased the

live weight gain (2.3 vs 1.3 kg/d), reduced the feed conversion ratio (4.8 vs 8.0 kg/kg) and reduced nitrogen retention (50.0 vs 58.5 g/d). No changes were observed in the proportions of muscle and adipose tissue in the carcass but the composition of the longissimus dorsi was affected: reduction in dry matter and protein contents and increase in ether extract content. Furthermore, treatment increased water retention as reflected by a higher water holding capacity and a reduction of drip and cooking losses of the longissimus dorsi muscle. Myofibrillar proteins of the longissimus dorsi showed higher proportions of troponine T and corresponding lower amounts of 30 kD protein, suggesting a depression of post-mortal muscle protein degradation. Plasma glucose and urea concentrations were increased the day after the injections. At hormonal level, there was an increase in insulin, no change in growth hormone and a reduction in total cortisol in the treated bull.

Résumé

Effets d'injections de dexaméthazone sur les performances zootechniques d'une paire de taureaux jumeaux monozygotes en engraissement

L'expérience a été réalisée avec 1 paire de taurillons à l'engrais qui étaient des jumeaux vrais obtenus par division d'un embryon. Les animaux recevaient une ration à base de pulpes séchées contenant environ 13% de protéine brute. Au cours de la première période, les 2 animaux ont été considérés comme témoins. Dans la seconde période, un taureau a reçu 4 injections intramusculaires de 12 mg de dexaméthazone à raison d'une injection par semaine. L'autre animal a reçu du sérum physiologique. Le traitement à la dexaméthazone a augmenté le gain de poids (2,3 vs 1,3 kg/j), réduit l'indice de consommation (4,8 vs 8,0 kg/kg) et réduit la rétention azotée (50,00 vs 58,5 g/j). Il n'y a pas eu de modification au niveau de la carcasse en ce qui concerne les proportions de muscles ou de tissus conjonctivo-adipeux. En revanche, la composition du muscle longissimus dorsi a été changée: réduction de la teneur en matière sèche et augmentation de la teneur en graisse. En outre, le traitement a augmenté la rétention d'eau comme l'indique une meilleure capacité de rétention et la réduction des pertes d'eau après la congélation et la cuisson du muscle longissimus dorsi. Au niveau des protéines myofibrillaires du muscle longissimus dorsi, on a observé une proportion plus grande de troponine T et une proportion plus faible de protéines 30 kD, ce qui suggère une dépression de la dégradation des protéines musculaires post-mortem. Les concentrations en glucose et en urée dans le plasma sanguin ont été augmentées le jour suivant l'injection. Il y a eu également une augmentation en insuline, pas de changement en hormone de croissance et une réduction en cortisol total.

Zusammenfassung

Der Einfluß von Dexamethason-Injektionen auf die Wachstumsleistung von monozygoten Rinderzwillingen

Zwei monozygote Mastbullen erhielten eine Mastration auf der Basis von getrockneter Zuckerrübenpülpe mit 13% Rohprotein. Im ersten Versuchsabschnitt dienten beide Tiere als Kontrolle. Im zweiten Versuchsabschnitt erhielt ein Tier 4 intramuskuläre Injektionen von 12 mg Dexamethason in wöchentlichem Abstand. Das andere Tier erhielt Injektionen aus physiologischer Kochsalzlösung. Die Behandlung mit Dexamethason führte zu höheren Gewichtszunahmen (2,3 gegenüber 1,3 kg/Tag), zu niedrigerer Futterverwertung (4,8 gegenüber 8,0 kg/kg) und niedrigerem Stickstoffansatz (50,0 gegenüber 58,5 g/Tag). Es konnten keine Veränderungen der Verteilung von Muskel- und Fettgewebe beobachtet werden. Lediglich die Zusammensetzung des M. longissimus dorsi war unterschiedlich, und zwar konnten eine Abnahme des Trockensubstanz- und Proteingehaltes, eine Zunahme des Fettgehaltes, eine erhöhte Wassereinlagerung durch größeres Wasserhaltevermögen und ein geringerer Kochverlust nach Dexamethason-Injektionen gefunden werden. Die myofibrillären Proteine des M. longissimus dorsi wiesen einen höheren Anteil an Troponin T und entsprechend einen niedrigeren Anteil an 30 kD Protein auf, was auf einen geringeren post mortalen Abbau an Muskelproteinen hinweist. Die Plasma-Glucose- und Harnstoffgehalte waren am Tag nach der Dexamethason-Injektion erhöht. Das Gleiche trifft für die Insulinkonzentration im Plasma zu. Beim Wachstumshormon wurden keine Unterschiede beobachtet, beim Cortisol wurde nach Dexamethason-Behandlung eine Reduzierung beobachtet.

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Authors' address: L. ISTASSE, V. DE HAAN, C. VAN EENAEME, P. BALDWIN, M. GIELEN and J. M. BIENFAIT, Faculté de Médecine Vétérinaire de l'Université de Liège, 45, rue des Vétérinaires – 1070 Brussels – Belgium; B. BUTS and D. DEMEYER, Faculteit van Landbouwwetenschappen, Proefhoevestraat, 10–12 – 9230 Melle Belgium