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Live changes in muscle glycogen concentration of steers due to feeding and fasting as determined through serial biopsies of the *Longissimus dorsi* muscle

Ariel Apaoblaza[1], and Carmen Gallo[1*]

Insufficient glycogen content in bovine muscle at slaughter produces meat with high final pH (> 5.8) which is undesirable. The objective of this study was to determine through biopsies the live changes in muscle glycogen concentration (MGC) of housed steers fed on hay or hay plus energy concentrate for 30 d and then determine the effect of food deprivation (fasting) for 24 h on the same variable. Ten steers of similar age, weight, and phenotypic characteristics were housed in individual pens and randomly assigned to two feeding treatments: *ad libitum* hay only (H, n = 5) and *ad libitum* hay plus flaked corn (*Zea mays* L.) (HC, n = 5). Biopsies (B) were taken from the *Longissimus dorsi* muscle at four occasions: the day before the start of experiment (B_0), after 15 d (B_1), after 30 d (B_2), and fasted for 24 h after B_2 (B_3). Before each biopsy, steers were sedated with xylazine (0.03 mL kg^{-1}) and lidocaine was applied locally; samples were frozen in liquid nitrogen to determine MGC. Results showed a significant ($P < 0.05$) effect of diet treatment (HC > H) and also of time ($B_2 > B_1$) on MGC; the decrease in MGC of steers due to fasting (B_2 *vs*. B_3) was not significant ($P > 0.05$). It was concluded that muscle biopsies allowed to detect a difference in the increase of MGC in steers fed an energy supplementation compared to steers fed hay only, and that fasting for 24 h tended to reduce MGC in both groups.

Key words: Cattle, muscle biopsies, muscle glycogen.

INTRODUCTION

Insufficient glycogen content in bovine muscle at slaughter decreases the availability of glucose that serves as a substrate for the action of the glycolytic enzymes, producing less lactic acid in the *postmortem* muscle; therefore, the resulting meat presents a high pH (> 5.8), high water retention capacity and an unattractive dark color, a problem known as dark cutting or DFD (dark, firm, and dry) (McVeigh and Tarrant, 1982). Fasting, transportation, herding and social regrouping of unknown bovines, as well as many other stress triggers occurring during stages prior to slaughter can lower muscle glycogen concentration in cattle (Gallo, 2004; Ferguson and Warner, 2008). The darker color of meat, its increased water retention capacity, and high pH have a negative effect on meat quality, its trade in the domestic market and especially for the export market, because the high pH decreases shelf life of meat due to a higher predisposition to the attack by microorganisms (Hofmann, 1988).

It has been reported that the energy supply for at least 2 wk prior to transport of cattle to slaughter increases muscle glycogen concentration (Immonen et al., 2000) and can mitigate the effects of chronic stress on pH (Knee et al., 2007; Gallo et al., 2013). Measuring muscle glycogen concentration in cattle by using muscle biopsies allows to determine variations of glycogen in the same animal over time (Pethick et al., 1994; Immonen et al., 2000); for example, if an animal receives different levels of energy intake through food, it can be determined if this contribution increases its muscle glycogen concentration; if these animals are subsequently subjected to different stressors, it is possible to measure the incidence that these have on the muscle glycogen concentration and to assess the risk involved by each one of them. It has been reported that physical exercise or environmental stressors reduce muscle glycogen in steers from 77 to 50 mmol kg^{-1} (35%), while the recovery of glycogen concentration develops at a rate of only 3 mmol kg^{-1} d^{-1} (Crouse et al., 1984). McVeigh and Tarrant (1982) found that glycogen recovery after a treatment with adrenalin depended on the energy supplied previously; with a barley-based diet the glycogen speed recovery was 7.6 mmol kg^{-1} d^{-1}, whereas a hay-based diet showed a recovery speed of 6.1 mmol kg^{-1} d^{-1}, and fasted animals reported only 1.5 mmol kg^{-1} d^{-1}. Same authors concluded that the recovery rate of muscle glycogen is influenced by the type of diet and that a low recovery rate may be caused by a low amount of available glucose, especially in fasted animals. Immonen et al. (2000) reported that the decrease in muscle glycogen concentration in cattle treated with adrenaline depends on previous feeding of animals; they found that cattle

[1]Universidad Austral de Chile, Facultad de Ciencias Veterinarias, Casilla 567 Valdivia, Chile.
[*]Corresponding author (cgallo@uach.cl).
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that had previously been fed energy food had a muscle glycogen decrease of 8 mmol kg^{-1}, while the decrease was 46 mmol kg^{-1} in cattle under a low energy input.

In Chile, there have been several studies on the high pH problem in cattle (Hargreaves et al., 2003; Gallo, 2004; Amtmann et al., 2006). Some of them have reported the effect of energy supplementation on pH and *postmortem* muscle glycogen in steers (Gallo et al., 2013), others have determined the effects of different transport times and fasting on the same variable (Gallo and Lizondo, 2000; Gallo et al., 2003, Herrera and Gallo, 2009). However, it has not been demonstrated yet the extent of increase or decrease of *in vivo* muscle glycogen caused by each of these factors in particular, which is possible to achieve through serial biopsies. Since the high pH is an important problem in Chile (Hargreaves et al., 2003; Gallo, 2004) and causes significant economic losses to the meat industry (Vidal et al., 2009; Leyva-García et al., 2012), the objective of this study was to determine through biopsies the live changes in the concentration of muscle glycogen of housed steers fed on hay alone or hay plus an energy concentrate for 30 d and then determine the effect of food deprivation (fasting) for 24 h on the same variable.

MATERIALS AND METHODS

Ten steers, approximately 1-yr old, were used. The animals were bought from a livestock market for the experiment and presented similar phenotypic characteristics and body condition; they were 'Friesian' × 'Red Angus' ('Clavel') with an average weight of 341 ± 18.7 kg. The steers, previously fed with pasture only, were housed individually in pens of approximately 9 m^2; they were weighed and allocated in blocks to two treatments according to weight. One treatment consisted of *ad libitum* hay-based diet only (H) and the other was *ad libitum* hay plus an amount of steam flaked corn (HC); the latter was equal to 1% of their live weight, for 30 d. The flaked corn (88% DM, 1.12% ash, 6% crude protein, and 2.65 Mcal kg^{-1}) was given in a single daily ration, and hay (88.2% DM, 6.9% ash, 11.9% crude protein, 1.87 Mcal kg^{-1} gross energy, and 50.3% NDF) was offered three times a day. Steers were weighed the day before the start of the experiment (B_0), at 15 d (B_1), and at 30 d (B_2) to determine the daily weight gain; total DM consumed (DM kg^{-1}) and energy intake per day (Mcal kg^{-1} d^{-1}) were determined.

Muscle biopsies from all the steers were obtained at B_0, B_1, B_2, and 24 h after food deprivation after B_2 (B_3). Biopsies were obtained by sedating each steer with xylazine 0.03 mL kg^{-1} body weight, and then 3 mL subcutaneous and 3 mL intramuscular lidocaine were applied on the biopsy site. Samples of about 1 g muscle tissue were obtained from *Longissimus dorsi* muscle at the level of L1 and L2, as this location is easy to find behind the last rib, according to the protocol established by Pethick et al. (1994). Samples were identified, placed in cryovials, and immediately frozen in liquid nitrogen at -80 °C until analysis. The method for determining muscle glycogen concentration (MGC) was based on that described by Chan and Exton (1976), which involves purification and enzymatic digestion of samples. Samples were first grounded in liquid nitrogen and 100 mg tissue were mixed with 400 µL 30% KOH and incubated for 15 min at 100 °C. Filter paper (cellulose chromatography paper Whatman 31ET) was cut in pieces of 2 × 2 cm, and a known volume of each sample was poured on the paper (50 µL). At the same time 15, 30, and 75 µL standard sample (glycogen solution 0.8 mg mL^{-1}) and 15 µL distilled water were added in duplicate on filter papers, each paper was then placed in a glass tube and left to dry. Subsequently, 5 mL of 66% ethanol (-20 °C) were added and papers were dried in an oven at 40 °C. A volume of 1 mL of amyloglucosidase solution 0.5 mg mL^{-1} was added in 400 mM sodium acetate buffer pH 4.8 and the solution was incubated 2 h at 37 °C. A sample for the determination of glucose (Glucose PAP kit) was taken from each tube. After enzymatic oxidation in presence of glucose oxidase, the hydrogen peroxide formed reacts under the catalysis of peroxidase with phenol and 4-aminophenazone forming a red-violet complex. Calibration curves were performed for the calculations of glucose and glycogen, which is expressed in mmol kg^{-1}.

The statistical analyses were carried out using CORR, GLM, and MIXED procedures of SAS (SAS Institute, 2011). Pearson correlations between MGC and average daily weight gain for B_1 (period B_0-B_1) and for B_2 (period B_1-B_2) and between MGC and metabolizable energy intake (only B_2) were estimated within each diet treatment. For time B_0 a one way ANOVA was used to evaluate the differences between treatments (HC *vs*. H). A repeated measures design was used to detect differences between treatments (HC *vs*. H) and between measurements in time B_1 and B_2. In the last period (B_3), two way ANOVA were applied on MGC and muscle glycogen change between B_2 and B_3. Shapiro-Wilk test for normality was applied on the residual of MIXED and GLM procedures.

RESULTS AND DISCUSSION

The use of muscle biopsies allowed to observe changes in MGC produced in the same animal at different times and under different feeding treatments. To date, no studies have been carried out in Chile using muscle biopsies to determine live MGC and the MGC values that had been previously reported came from *postmortem* muscle (Gallo and Lizondo, 2000; Mencarini, 2002; Hargreaves et al., 2003). Although muscle biopsies were relatively simple to perform, the procedure seems useful only as a research technique and would not apply in commercial practice because it causes some minor damage to the tissue and also it would leave some residues, which would affect quality of the carcass and meat in the invaded zone, when

performing the procedure shortly before sending animals to slaughter.

Table 1 shows that supplementation of steers with flaked corn as used in this study during 30 d did not cause significant changes in terms of daily weight gain due to diet treatment or time. MGC was similar at B_0, however there was a significant effect on MGC due to treatment (HC > H, $P < 0.05$) and time ($B_2 > B_1$) (Table 2). No interaction between both factors was found. This different response in MGC would be caused by the metabolizable energy intake of HC steers, which was higher than that of H steers (Table 1). These results are similar to those of Immonen et al. (2000), who reported that increasing 2.5 times energy provided in the diet produces an increase from 14 to 49 mmol kg^{-1} in MGC. Correlation found between MGC and metabolizable energy intake ($r = 0.61$ in HC and $r = -0.11$ in H at B_2) and between MGC and weight gain ($r = -0.57$ and 0.57 in HC, and $r = 0.25$ and -0.69 in H, at B_1 and B_2 respectively), were not significant ($r = 0$ with $P > 0.05$).

The muscle biopsies to *L. dorsi* muscle in this study showed high variability in MGC between individuals (Table 2), registering a maximum concentration of 82.72 mmol kg^{-1} and a minimum of 9.76 mmol kg^{-1}. In general, these samples obtained from field biopsies, without transportation, fasting nor *antemortem* handling, allowed to observe an average MGC of 59.46 and 49.44 mmol kg^{-1}, respectively, which are both higher (Table 2) than those previously found in steers immediately *postmortem* at the slaughterhouse. Gallo and Lizondo (2000) found in *L. dorsi* of steers immediately after slaughter (30 min), a mean MGC of 23.6 mmol kg^{-1} after 3-h transport and 17.6 mmol kg^{-1} after 16-h transport; in this study it was also observed that MGC decreased when animals spent more time in lairage prior to slaughter. Results of the present study also confirm that fasting for 24 h reduces muscular glycogen (Table 2).

Daly et al. (1999) demonstrated that providing steers with more energy in their diet (corn based) caused a 20% increase in MGC compared to animals fed pasture only. Mencarini (2002) found in *L. dorsi* of steers that had a pH > 5.8 at 24 h *postmortem*, a MGC of 7.36 mmol kg^{-1} at slaughter while those with pH < 5.8 had 36.5 mmol kg^{-1}. Gallo et al. (2013) found a mean of 30.6 mmol kg^{-1} of *postmortem* glycogen in *Semimembranosus* muscle.

After being subjected to food deprivation for 24 h (B_3), steers from both treatments tended to decrease their MGC ($P > 0.05$). These results demonstrate that steers had to resort to using their energy reserve due to being deprived of food; additionally, food deprivation itself can also be a stressor. Pethick et al. (1994) found a decrease of 20 mmol kg^{-1} of muscle glycogen in animals fasted for 18 h at the slaughterhouse and this decrease reached 30% to 40% depending on the muscle. Probably due to the high variability between individuals and the low number of animals used in this study no significant changes were found (Table 2). However it should be noted that there was a 22% decrease in MGC in HC steers whereas there was a 27% decrease in those fed hay only, which could indicate a certain protective effect of the increased energy intake when facing deprivation of food and/or stress. This is consistent with previous reports of Gallo et al. (2013), who showed that in steers on pasture, energy supplementation with flaked corn for 4 wk prior to slaughter, could reduce the presentation of high pH and *postmortem* dark cutting and also reduced variability of MGC in these animals when compared to non-supplemented animals, although MGC (measured *postmortem*) was not significantly different from control steers fed pasture only. In this sense, McVeigh and Tarrant (1982) also found that after a treatment with adrenaline (simulated stress) MGC decreased by 25%, 27%, 38% in animals previously fed barley, hay, and fasted, respectively. Moreover, Immonen et al. (2000) observed in animals with and without prior energy supply, a decrease in MGC of 8 and 40 mmol kg^{-1}, respectively, when treated with adrenaline.

An important aspect to consider is that a change in feeding and housing can lead to an initial decrease in MGC, due to the stress associated with these changes (Table 2). The initial muscle glycogen decrease observed between samples B_0 and B_1 had also been recorded by Knee et al. (2007), who found that moving cattle from pasture grazing to being housed caused a decrease of 30% MGC the first week of assay. Both the change in diet and the stress of changing from pasture to housing system, and in this case probably the very act of taking muscle biopsies, could explain the initial decrease of MGC in

Table 1. Means and standard deviations (SD) of initial live weights, weight gains at 15 d (B_1) and at 30 d (B_2) and daily metabolizable energy (ME) consumed by steers fed hay (H) or hay plus concentrate (HC).

	HC (Mean ± SD)	H (Mean ± SD)
Initial live weight, kg	339.2 ± 17.3	343.2 ± 21.8
B_1 Weight gain, kg d^{-1}	1.08 ± 1.01	0.75 ± 0.36
B_2 Weight gain, kg d^{-1}	0.89 ± 0.96	0.47 ± 0.67
B_2 ME intake, Mcal kg^{-1} d^{-1}	507.6 ± 28.9	432.4 ± 7.2

Table 2. Arithmetic and least squares means and standard deviations (SD) of muscle glycogen concentrations in *Longissimus dorsi* biopsies of steers fed hay (H) or hay plus concentrate (HC).

	HC		H	
	Glycogen	SD	Glycogen	SD
	mmol kg^{-1}		mmol kg^{-1}	
B_0 (initial)[1]	39.25A	15.23	42.92A	25.45
B_1 (15 d)[1]	34.37	10.40	21.33	13.13
B_2 (30 d)[1]	59.46	6.73	49.44	10.40
B_3 (fasting)[1]	46.44A	9.20	35.97A	9.60
Treatment[2]	46.91A		35.77B	
B_1[(2)]	27.85a			
B_2[(2)]	54.44b			

[1]Arithmetic means.
[2]Least squares means obtained by repeated measures analysis.
Different capital letters mean significant differences between treatments ($P < 0.05$).
Different lower-case letters mean significant differences between periods ($P < 0.05$).

steers of both treatments. This is an important factor to consider when planning a strategy for supplementation of steers before sending them to slaughter, because it must consider a period of adaptation of animals to the new diet (and/or environment) before achieving an increase of muscle glycogen that can in turn protect from the stress prior to slaughter and reduce high pH problems. Further studies should be carried out with a larger number of animals and up to slaughter, in order to test how pre-slaughter operations, like transport and fasting in a different environment (slaughterhouse), affect MGC and how this is related to carcass pH.

CONCLUSIONS

Even though results are preliminary due to the low number of animals used, muscle biopsies allowed detecting a difference in the increase of muscle glycogen concentration (MGC) in steers fed an energy supplementation compared to steers fed hay only, and that fasting for 24 h tended to reduce MGC in both groups.

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