THE EFFECT OF SUPPLEMENTING BEEF CATTLE WITH CRUDE GLYCEROL ON THE PH OF THE *M. LONGISSIMUS THORACIS*

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Abstract – The use of crude glycerol added to drinking water was trialed in a beef cattle feedlot in an attempt to reduce pH of the *m. longissimus thoracis* (LT), minimising the incidence of dark cutting. Cattle were offered crude glycerol (a by-product of ethanol production from canola) in their drinking water at 5% concentration for two days prior to leaving the property. The pH of the LT and the amount of water consumed was analysed using a general linear model in SAS with fixed effects for treatment group, lot number and sex. Glycerol supplementation increased pH (P<0.05) resulting in a 2.7% increase in the incidence of dark cutting. Water intake was reduced (P<0.001) in cattle supplemented with glycerol. Hence using crude glycerol supplementation in drinking water was not a suitable treatment for reducing the incidence of dark cutting.

Key Words – dark cutting, Meat Standards Australia, glycogen.

I. INTRODUCTION

Meat Standards Australia (MSA) sets grading thresholds to exclude dark cutting beef with an AUSmeat colour score greater than 3 and/or a pH greater than 5.7 [1]. Carcasses not meeting MSA meat colour and pH specifications remained the greatest reason for non-compliance during the 2013 to 2014 financial year at 5.63% [2].

Dark cutting is largely caused by low glycogen concentration in the muscle at slaughter. Muscle glycogen at slaughter is a function of muscle glycogen stored on farm minus that lost due to the stresses imposed during the pre-slaughter period. If muscle glycogen is low at slaughter, lactate production is restricted, resulting in an elevated ultimate pH which leads to dark cutting beef, which is rejected by consumers. Meat with a pH greater than 5.7 has reduced value due to its dark colour, variable tenderness and reduced shelf life due to the more neutral pH allowing microbial growth [3].

There is a clear relationship between muscle glycogen concentration and metabolisable energy intake [4]. Feeding high energy grain diets during the pre-slaughter period has been found to significantly increase muscle glycogen levels at slaughter [5]. Pethick *et al.* [4] found that animals destined for slaughter should be on a high plane of nutrition to increase muscle glycogen prior to slaughter and help reduce the problem of dark cutting meat. Thus nutritional measures to increase muscle glycogen could be a cost effective management strategy for producers to reduce dark cutting [5].

Glycerol is the principal by-product of biodiesel production and can be used as a feed additive in ruminant diets [6]. In its purest form, glycerol is a colourless and odourless liquid, however different batches of glycerol vary in colour and taste, with most batches being light brown in colour with a strong odour [7]. These differences in colour and taste can be explained by differences in compounds from grains used for the production of biodiesel [7]. Ash and methanol residues present in glycerol can give some batches a strong taste, which requires acclimatisation for animals when being used as a feed additive [6].

Glycerol has been used as a valuable component of animal feed supplements and can be mixed into feed or water [8]. In some cases, feed ingredients such as cereals have been substituted with glycerol to reduce feed costs, although in this case glycerol can only be successfully supplemented in feedlot diets at levels up to 8% [7]. Concentrations greater than 8% have been found to have detrimental effects on feed and water intake, digestibility, and carcass quality [7]. Using glycerol in feed at levels greater than 8% can also present diet mixing and feed flow problems [7] due to its viscosity and stickiness.

Glycerol could be used as an effective feed supplement to increase glycogen storage in ruminants and therefore reduce dark cutting. One previous study in cattle used water-soluble glycerol as an energy supplement added to drinking water prior to slaughter [9] This lead to increased water intake, and reduced pH in the *M. longissimus thoracis* (LT) at 24 hours post slaughter [9].

However in this case the glycerol had been purified and was thus colourless and odourless, as opposed to the stronger tasting, brown coloured glycerol typically sourced as a by-product from biodiesel production.

The aim of this study was to investigate the effects of adding crude glycerol biodiesel to the drinking water of cattle in a feedlot on the pH of their LT. It was hypothesized that cattle supplemented with 5% crude glycerol in their drinking water for 48 hours prior to leaving the property will have a lower pH in their LT at 24 hours post slaughter.

II. MATERIALS AND METHODS

A. Animals

Three hundred cattle of different age, breed and sex were obtained from sale yards in southern Western Australia. These animals were fed at a commercial feedlot for between 12 and 37 days and then slaughtered at a commercial abattoir. There were 6 different lots of cattle that were purchased, fed, then slaughtered. All cattle were weighed upon entry and exit to the feedlot.

B. Diet

The animals were offered an ad libitum mixed grain ration in self feeders and ad libitum meadow hay. The experimental ration consisted of 31.5% barley, 24.2% fibre pellets, 13.6% lupin, 11.6% malt combings, 7.8% pollard, 3.9% amipro minerals, 2.9% canola meal, 1.5% glycerol and 3% buffers. The metabolisable energy and crude protein content were 10.95 MJ/kg and 13.75% on a dry matter (DM) basis. The ration contained 88% DM content.

C. *Glycerol supplementation*

Two days before transport to the abattoir, animals from the feedlot pen were weighed and then randomly distributed into control and glycerol treatment groups. The control and glycerol treatment groups were kept in separate outdoor feedlot pens for the final 48 hours before dispatch. These groups consisted of between 20 to 34 animals in each treatment group. The glycerol treatment group was supplemented with glycerol in their water at 5% for 48 hours prior to transport to the abattoir. The control group received normal water for the equivalent 48 hour period. Both the glycerol treatment and control groups received the same *ad libitum* grain ration and *ad libitum* hay. Water intakes were measured for both the treatment and control groups.

D. Slaughter Measurements

After 48 hours on their water treatments, the animals were transported 15 minutes to the abattoir and maintained in lairage for 12 hours before slaughter. During lairage all animals were maintained off feed and given access to clean fresh drinking water.

Following slaughter carcass measurements were recorded. These measurements included pH of the LT, meat colour of the LT, sex, carcass weight, rib fat depth, fat colour, ossification, marbling and hump height (data for effect of carcass traits on carcass pH notshown). They were taken by accredited graders according to the MSA and AUSmeat protocols.

E. Statistical analysis

The pH of the LT and the quantity of water consumed were analysed in SAS using a general linear model [10]. The fixed effects were treatment (glycerol or control), slaughter group (1 to 6) and sex, and their interactions. Covariates were incorporated as linear and curve linear terms, and included post treatment weight, carcass weight, ossification, fat colour and marbling. Fixed effect interactions and covariates were removed from the model if not significant ($P>0.05$).

III. RESULTS AND DISCUSSION

The pH of the LT was higher in the glycerol treated group ($P<0.05$) than the control group, rejecting the initial hypothesis. The average pH of the glycerol treated group was 5.62 ± 0.001 which was 0.03 pH units higher than the control group at 5.59 ± 0.001. The proportion of dark cutting carcasses in the glycerol treated group was 9.7% compared to the control group which had 7% dark cutting carcasses. This translates to an extra 4 of 148 cattle reaching a pH>5.7 in the glycerol treatment group.

Treatment also had a significant impact on water intake ($P<0.001$). The control group consumed an average of 54.2L/head $\pm$ 0.71 which was 19.7% higher than the glycerol treatment group which consumed 45.3L/head $\pm$ 0.71 over the 48 hour period. When included in the statistical model, post treatment weight did not affect water intake.

The increased pH and reduced water intake in this trial contradicts the results of Gardner *et al.*[9]. This is probably due to the use of purified glycerol in the experiment by Gardner *et al.* [9], compared with glycerol from biodiesel production used in the current experiment. This is likely to have had reduced palatability, leading to reduced water intake. Pure glycerol is clear, odourless and sweet tasting compared to the bitter brown crude glycerol [11] used in the current experiment. Furthermore, the cattle in the treatment group were only given a 24 hour adjustment period to the addition of glycerol in their drinking water. This may have been improved if more time for acclimation had been allowed.

The reduced water intake for glycerol treated cattle is suspected to be the cause of higher pH of the LT at 24 hours post slaughter. Jones *et al.* [12] found steers held for 48 hours in lairage without water had elevated pH and darker meat colour. As time off water increased and dehydration increased there was a trend for increased pH and darker meat colour. It can therefore be concluded that cattle kept for 24 hours or longer without water before slaughter will produce meat with increased pH [12]. Future work is needed in the beef industry to determine whether the use of pure glycerol in drinking water before slaughter is an effective method of reducing dark cutting.

The composition of crude glycerol varies depending on the parent feedstuff and the production process and it is believed to be the reason for reduced water intake in the glycerol treatment group. Glycerol is a very valuable product when refined to a chemically pure substance, however this purification process is generally not economically feasible for small to medium sized biodiesel plants [13]. The purity of glycerol has been suggested as a determining factor for reduced intake when added to ruminant diets [14]. Impurities in biodiesel derived glycerol have been found to have negative effects on feed intake [15]. The impurities in crude glycerol include catalysts, salts, residual methanol, methyl esters, soaps, free fatty acids, fats and oils, commonly called matter organic non-glycerine or MONG. Hansen *et al.* [7] found the chemical composition of crude glycerol to vary considerably between samples. Glycerol content was found to range from 38% to 96%, with some samples also containing up to 29% ash content and 14% methanol [7]. The impurities such as ash and methanol found in crude glycerol are suspected to be the cause of the reduced palatability of crude glycerol compared with the sweet tasting pure glycerol. The eleven samples of glycerol from seven Australian biodiesel manufactures analysed by Hansen *et al.* [7] were found to also range in colour from clear to nearly black. The quantity of MONG between samples in the study by Hansen et al ranged from 14 to 29%, indicating that some crude glycerol can contain high percentages of impurities which have bitter characteristics. The crude glycerol used in this study was light brown in colour. These differences in colour, MONG percentage, glycerol concentration and taste between samples may be explained by differences in compounds from feedstuffs used for biodiesel production.

Even when crude glycerol is put in feed, Donkin *et al.* [17] found feed intake was significantly decreased at a concentration of 15% glycerol on a dry matter basis in lactating Holstein dairy cows. Feed intakes were reduced for the first 7 days of the trial while the cows adapted to the taste of the glycerol in the diet, but no difference in feed intake was detected for the subsequent 7 weeks of the trial [16]. This suggests that recovery of feed intake can be achieved even with an inclusion rate of 15% glycerol if glycerol is gradually introduced into the diet. Although the variable composition of crude glycerol can present issues with palatability, there does not appear to be any detrimental impact of feeding glycerol at up to 15% of the total feed ration dry matter. However, it should be noted that approximately 7 days is required to adapt the rumen to glycerol when introducing it to ruminant diets. Donkin *et al.* [16] showed that the use of pure food grade glycerol eliminates detrimental effects on feed intake that occur when using biodiesel derived glycerol as a feed additive [16]. Groesbeck [15] and Hansen et al. [7] both agreed that glycerol percentages above 6% in the ration also impeded the use of mixing equipment because the feed would form a sticky aggregate on the walls of the mixers.

IV. CONCLUSION

The results of this experiment indicate that addition of 5% crude glycerol to drinking water during the pre-slaughter period increases muscle pH. Cattle also had reduced water intake when given water with 5% glycerol. The reduced intake was probably caused by the unpalatable taste of crude glycerol from biodiesel production. Cattle supplemented with glycerol had an increased proportion of dark, firm and dry carcasses. Future work needs to be done to determine whether pure or less bitter glycerol could be useful in reducing dark cutting.

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