Effects of crude glycerin supplementation on performance and meat quality of Holstein bulls fed high-concentrate diets

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Effects of crude glycerin supplementation on performance and meat quality of Holstein bulls fed high-concentrate diets[1]

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ABSTRACT: Forty-eight bulls (335 ± 8.6 kg of initial BW) were randomly assigned to 4 glycerin levels (0, 4, 8, and 12% of concentrate DM) with the objective of evaluating the effects of glycerin supplementation on performance, ruminal fermentation, metabolism, and carcass and meat quality in Holstein bulls fed high-concentrate diets. Concentrates were formulated to be isonitrogenous and isocaloric (assuming a glycerin ME content of 3.47 Mcal/kg of DM). Concentrate and straw were fed for ad libitum intake. Bull BW and feed consumption were recorded monthly. Additionally, rumen and blood samples were collected every month. Bulls were slaughtered after 91 d of study (460 ± 11 kg of final BW). Hot carcass weight, carcass backfat, and conformation were recorded. The area, Warner-Bratzler shear force, and intramuscular fat content of LM were determined. Glycerin level did not affect daily concentrate intake (6.89 ± 0.34 kg/d of DM), straw intake (1.38 ± 0.069 kg/d of DM), total DMI (8.27 ± 0.32 kg/d of DM), ADG (1.36 ± 0.087 kg/d), or G:F (0.17 ± 0.009). Similarly, rumen molar proportions of propionic, acetic, and butyric acids, and rumen liquid osmolality were unaffected by treatment. However, a decreased rumen pH ($P < 0.05$), and greater rumen total VFA concentration ($P = 0.09$), serum insulin concentration ($P < 0.05$), and insulin to glucose ratio ($P < 0.05$) were observed in bulls fed 8% glycerin in concentrate compared with those receiving 0, 4, or 12%. No changes were observed in carcass and meat quality. The ME content of glycerin (86% glycerol) can be assumed to be 3.47 Mcal/kg of DM in Holstein bulls fed high-concentrate diets. In addition, feeding concentrate containing up to 12.1% of glycerin does not lead to detrimental effects on performance, ruminal fermentation, metabolism, and carcass and meat quality variables.

Key words: beef, glycerol, meat, rumen

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INTRODUCTION

Glycerin (rich in glycerol) is a by-product from the biodiesel industry (Dasari et al., 2005). The recent increase in biodiesel production has resulted in an increase of available quantities of glycerin generated from transesterification of vegetable oils (Crandall, 2004). A potential application for glycerin is as a gluconeogenic substrate for ruminants (Chung et al., 2007). Glycerol can be converted to glucose in the liver of cattle and can provide energy for cellular metabolism (Goff and Horst, 2001). Glycerol enters the gluconeogenic pathway at the level of dihydroxyacetone phosphate and 3-phosphoglyceraldehyde (Leng, 1970; Krehbiel, 2008). Several authors (DeFrain et al., 2004; Chung et al., 2007) have supplemented relatively large doses of glycerol to prevent ketosis in lactating cattle without observing effects on milk production or milk composition.

Glycerin has been used as a gluconeogenic supplement for short-term consumption. However, glycerin could be included in ruminant rations as an energetic feed ingredient and substitute for other feed ingredients such as cereals, and, in turn, reduce feeding costs. To our knowledge, there are no studies that evaluate the effects of glycerin supplementation to finishing bulls fed high-concentrate diets on performance, rumen fermentation, metabolism, and carcass and meat quality. The aim of this study was to determine the effects of feeding different concentrate glycerin amounts, as an energetic ingredient, on performance, ruminal fermentation, me-

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tabolism, and carcass and meat quality from Holstein bulls fed high-concentrate diets.

MATERIALS AND METHODS

Animals were managed following the principles and specific guidelines of the IRTA Animal Care Committee.

Animals, Housing, and Diets

Forty-eight Holstein bulls were used in a complete randomized design. Animals were weighed and distributed in 48 individual pens (1.5 × 3 m). After an adaptation period of 21 d with the animals consuming a control concentrate (0% glycerin), bulls were weighed on 2 consecutive days and started the study with an average initial BW of 335 ± 8.6 kg and age of 9.0 ± 0.42 mo. Then, bulls were randomly assigned to 1 of 4 concentrates containing: 0, 4, 8, or 12% glycerin on a DM basis (Table 1). These inclusion levels were chosen after a preliminary experiment conducted to assess pellet quality based on the level of glycerin inclusion (2, 5, 10, 15, and 20%). An attempt was made to include up to 20% glycerin, but pellet quality was moderately and severely reduced at 15 and 20% inclusion levels, respectively. The glycerin was produced in a soy-diesel facility (Loiret and Haentjens, Barcelona, Spain) and contained 85.7% glycerol, 8.6% water, 5.5% salt, and 0.09% methanol. Glycerin fed in the current study was used as an energetic ingredient; therefore, to obtain 4 isoenergetic concentrates, the increase in glycerin content was counterbalanced, mainly by a decrease in cereal grain content (Table 1). The ME content of glycerin was calculated based on the hypothesis that 1 mol of glycerol would be fermented to 0.5 mol of propionic acid, thus providing 0.367 Mcal of ME per mol (Baldwin, 1968). The remaining 50% was assumed to escape ruminal fermentation and reach the intestine with an energy content of 4.3 Mcal/kg of DM (NRC, 2001). Therefore, assuming a 95% digestibility and 100% metabolizability, the glycerol escaping ruminal degradation would supply about 0.376 Mcal of ME per mol (1 kg of glycerol = 10.87 mol). Based on these assumptions, estimated glycerol ME was 4.03 Mcal of ME/kg of DM, and in consequence the estimated glycerin (86% purity) ME was 3.47 Mcal/kg of DM. All concentrates were also formulated to be isonitrogenous. Furthermore, as glycerin content increased, the inclusion of sodium chloride was reduced to maintain the same salt concentrations in all 4 concentrates (Table 1). Bulls were fed concentrate and barley straw (3.5% CP, 1.6% EE, 70.9% NDF, and 6.1% ash; DM basis) in 2 separate troughs (0.6 m × 1.2 × 0.3 m), both for ad libitum intake, until 91 d of the experiment when they reached a target final BW of approximately 460 kg. Bulls were housed at Cooperativa Agraria de Guissona experimental station (Guissona, Spain).

Measurements and Sample Collection

Bull BW and concentrate and straw consumptions were recorded monthly. Also, rumenocentesis was performed monthly during 2 consecutive days (24 bulls/d). Rumenocentesis was conducted with a 14-cm, 14-gauge needle inserted into the ventral sac of the rumen approximately 15 to 20 cm caudal-ventral to the costocondral junction of the last rib. Rumen liquid pH was measured immediately with a portable pH meter (model 507, Crisson Instruments SA, Barcelona, Spain). Following Jounay (1982), 4 mL of ruminal liquid were mixed with 1 mL of a solution containing 0.2% (wt/wt) mercuric chloride, 2% (wt/wt) orthophosphoric acid, and 0.2% (wt/wt) 4-methylvaleric acid (internal standard) in distilled water, and stored at −20°C until subsequent VFA analyses. Additionally, 10 mL of rumen liquid was collected and stored at −20°C for subsequent osmolality analyses as described by Andersen et al. (1994). Blood samples were taken monthly by jugular venipuncture. One blood sample (10 mL) was harvested without additives (BD Vacutainer, Franklin Lakes, NJ) for insulin concentration analysis, whereas a second blood sample (5 mL) was harvested with 5 mg of sodium fluoride and 4 mg of potassium oxalate (BD Vacutainer) for subsequent glucose determination. All blood samples were centrifuged at 1,500 × *g* at 4°C for 15 min, and plasma and serum were stored at −20°C until further analysis.

Bulls were transported to the slaughterhouse after 91 d of study with a final BW of 460 ± 10.9 kg. Truck stocking density was 0.82 ± 0.23 animals/m^2, and transport distance was less than 1 km. At the slaughterhouse, animals were housed in 3 different pens, forming groups of 16 bulls (4 animals from each treatment) with 0.25 animals/m^2, for approximately 3 h before slaughter. Immediately following slaughter, HCW was recorded, and carcass backfat and conformation were graded according to the EU classification system into 1.2.3.4.5 (EU Regulation 1208/81) and into (S)EUROP categories (EU Regulation 1208/81, 1026/91), respectively. The conformation class designated by the letter **E** (excellent) describes carcasses with all profiles convex to super-convex and with exceptional muscle development, whereas the conformation classified as **U** (very good) describes carcasses with profiles on the whole convex and with very good muscle development. The carcasses classified as **R** (good) present profiles on the whole straight and good muscle development. Carcasses classified as **O** (fair) present profiles straight to concave and with average muscle development, whereas carcasses classified as **P** (poor) present all profiles concave to very concave with poor muscle development. In addition, the degree of fat cover describes the amount of fat on the outside of the carcass and in the thoracic cavity. Whereas the class of fat cover that classifies as 1 (low) describes none to low fat cover, the class of fat cover classified as 5 (very high) describes an entire carcass covered with fat and with heavy fat deposits in

Table 1. Ingredient and nutrient composition of the experimental concentrates

	Dietary glycerin, %			
Item	0	4	8	12
Ingredient, % of DM				
Corn grain meal	35.9	36.0	36.0	36.0
Barley grain	32.7	24.5	15.5	9.1
Glycerin[1]	—	4.4	8.7	12.1
Corn gluten feed	0.9	1.0	4.5	4.5
Beet pulp	9.1	9.0	9.0	9.0
Wheat straw	1.3	0.9	0.9	1.3
Soybean hulls	7.5	10.8	12.2	13.5
Soybean meal	7.8	8.9	9.1	10.3
Calcium soaps of palm fatty acids	0.4	0.9	0.9	1.0
Palm oil	2.2	1.6	1.4	1.4
Calcium carbonate	0.89	0.89	0.89	1.07
Dicalcium phosphate	0.33	0.36	0.35	0.35
Vitamin premix[2]	0.20	0.20	0.20	0.20
Sepiolite	0.18	0.18	0.18	0.18
Salt	0.60	0.39	0.18	—
Nutrient				
ME,[3] Mcal/kg	3.19	3.22	3.23	3.23
CP, % of DM	11.9	11.6	11.9	12.5
Ether extract, % of DM	4.7	4.2	4.8	4.7
NDF, % of DM	20.0	20.4	19.4	19.4
Ash, % of DM	4.3	4.5	4.7	4.8

[1]Contained 85.7% glycerol, 8.6% water, 5.5% salt, and 0.09% methanol.
[2]Every kilogram contained 5,084 IU of vitamin A, and 1,016 IU of vitamin D_3, and 50,850 IU of vitamin E.
[3]Calculated with an estimated ME for glycerol of 3.47 Mcal/kg of DM.

the thoracic cavity. Dressing percentage was calculated from HCW. After 24 h of carcass chilling, a sample of LM from the 6th to the 9th ribs was dissected and analyzed immediately for LM area by artificial vision (Pomar et al., 2001). The LM from the 6th to the 9th ribs was dissected, and 200-g samples were stored at −20°C until intramuscular fat content and Warner-Bratzler shear force (**WBSF**) determinations.

Chemical Analyses

Feed samples were analyzed for DM (24 h at 103°C), ash (4 h at 550°C), CP by the Kjeldahl method (AOAC, 1995), NDF according to Van Soest et al. (1991) using sodium sulfite and α-amylase, and fat by Soxhlet with a previous acid hydrolysis (AOAC, 1995). Rumen VFA concentration was analyzed with a polyethylene glycol terephthalic acid-treated capillary column (25 m × 0.25 mm ID, 0.25 μm film thickness, BP21, SGE, Europe Ltd., Barcelona, Spain) using GLC (Carlo Erba Instruments chromatograph, CE 5300 HT, Milano, Italy). Rumen liquid osmolality was determined with a vapor pressure osmometer (Vapro Model 5520, Wescor Inc., Logan, UT). Plasma glucose concentration was determined following the hexokinase/G-6-DH enzymatic method (Burrin and Price, 1985), and serum insulin concentration was determined using ELISA (kit 10–1131–01, Mercodia, Uppsala, Sweden).

Intramuscular fat content of LM was predicted using near-infrared transmission spectroscopy spectrum in the region 850 to 1,050 nm (Infratec 1265 Meat Analyzer, Tecator AB, Uppsala, Sweden). The LM sample was homogenized before near-infrared transmission analysis using a food processor (Robot coupe Blixer3, Montceau Les Mines, France). Intramuscular fat content was expressed as grams of fat/100 g of muscle. Meat samples obtained for WBSF measurements were wrapped in aluminum foil and cooked to an internal temperature of 71°C in a convection oven preheated to 200°C. Sample internal temperature was monitored with a data logger and a thermocouple probe inserted horizontally at the steak midpoint. The maximum shear force was determined from 5 replicates (1 cm^2 cross-section, 3-cm long) with fiber direction parallel to the longest dimension of the strip and perpendicular to the direction of the blade (Honikel, 1998), using a texture analyzer (Alliance RT/5 MTS Systems Corp., Eden Prairie, Minneapolis, MN) equipped with a Warner-Bratzler blade.

Statistical Analyses

All data were normally distributed, with the exception of serum insulin concentration that was transformed to a log-scale to achieve a normal distribution before statistical analysis. The values presented herein correspond to nontransformed means of the raw data, but SEM and *P*-values correspond to the ANOVA of log-transformed data.

Performance, rumen fermentation, and metabolism data were analyzed using a mixed-effects model with repeated measures (SAS Inst. Inc., Cary, NC). The model included initial BW as a covariate, glycerin level,

Table 2. Intake and performance of Holstein bulls fed high-concentrate diets containing different glycerin content

	Dietary glycerin, %					
Item	0	4	8	12	SEM	*P*-value[1]
Initial BW, kg	336	335	334	333	8.6	0.99
Final BW, kg	456	468	463	454	10.9	0.67
ADG, kg/d	1.31	1.43	1.40	1.29	0.087	0.71
Concentrate intake, kg of DM/d	6.80	6.84	7.15	6.78	0.34	0.85
Straw intake, kg of DM/d	1.38	1.35	1.39	1.41	0.069	0.94
Total DMI, kg/d	8.18	8.19	8.53	8.19	0.32	0.83
G:F	0.16	0.17	0.17	0.16	0.009	0.21

[1]Effect of glycerin level.

time, and the interaction between glycerin level and time, as fixed effects, and animal as a random effect. Time was considered a repeated factor, and for each analyzed variable, animal nested within glycerin level (the error term) was subjected to a compound symmetry variance-covariance structure. To analyze rumen fermentation data, the sampling hour within day entered the model as a covariate to account for the difference in time elapsed between sampling of the first and last animal (about 4 h). Carcass and meat quality characteristics were analyzed as described above but without the time effect (as there were no repeated measures), and final BW was used as a covariate. A chi-square-test was conducted to evaluate the effects of glycerin content on carcass classification data (categorical variables).

RESULTS AND DISCUSSION

Although concentrates were formulated to be isonitrogeneous, the CP content of the 4% glycerin treatment was 2.5% less than expected (11.6% instead of 11.9%), and the CP content of the 12% glycerin treatment was 5.0% greater than expected (12.5% instead of 11.9%).

Intake and Animal Performance

Average daily concentrate intake (6.89 ± 0.345 kg/d of DM), total straw intake (1.38 ± 0.069 kg/d of DM), and total DMI (8.27 ± 0.324 kg/d of DM) were not affected by glycerin content (Table 2). Ogborn (2006) reported that 5% glycerol increased DMI in prepartum dairy cattle, but 3.3% glycerol in the total ration or 500 mL/d of glycerol drench tended to reduce DMI after calving. To our knowledge, there are no studies that report DMI of bulls consuming a concentrate with glycerin content near 12% of DM. Supporting the present results, some studies conducted with lactating cattle fed high-forage diets (DeFrain et al., 2004; Chung et al., 2007) have reported no negative effects on feed intake when supplementing diets with glycerin at inclusion rates similar to the present study. The concentrate to straw consumption ratio (83:17) was similar among treatments. In contrast to a previous study (Pyatt et al., 2007) where the replacement of 10% of corn by crude glycerin in a concentrate fed to Angus steers improved ADG and feed conversion, in the current study glycerin level did not affect ADG (1.36 ± 0.087 kg/d) or feed efficiency (0.17 ± 0.009 kg/d). These results provide evidence that the assumption that concentrates were isocaloric was correct. It could also be concluded that glycerin can be used as an energetic ingredient that can effectively substitute cereals in the diets of finishing Holstein bulls and that the estimated glycerin ME content was 3.47 Mcal/kg of DM. Present results also indicate that some potentially negative glycerin components such as salt (5.5%) and methanol (0.09%) might not exert detrimental effects on DMI and animal growth when glycerin is included in the diets of Holstein bulls up to about 800 g/d.

Ruminal Fermentation

Rumen liquid osmolality was not affected by glycerin level (Table 3). Average rumen pH was less ($P < 0.01$) in bulls fed 8% glycerin than in those fed no glycerin, 4% glycerin, or 12% glycerin. The decreased rumen pH observed with the 8% glycerin treatment was probably due to the numerically greater daily concentrate intake obtained with this glycerin level compared with the rest of treatments. Consequently, total rumen VFA concentration tended ($P = 0.09$) to be greater in bulls fed 8% glycerin than in the animals receiving the other treatments (Table 3). However, the decreased pH and the greater VFA concentration observed in animals consuming the 8% glycerin concentrate had no impact on animal performance and health. In contrast, no differences were detected in rumen molar proportions of propionate, acetate, and butyrate, nor in the acetate to propionate ratio (Table 3). In disagreement to these results, previous studies (DeFrain et al., 2004; Trabue et al., 2007) have reported that animals supplemented with glycerol had greater total rumen VFA, greater rumen molar proportions of propionate, and a decreased ratio of acetate to propionate than unsupplemented animals. Linke et al. (2004) found that the administration of 1 kg of glycerol as a dietary supplement, as

Table 3. Rumen fermentation variables of Holstein bulls fed high-concentrate diets containing different glycerin content

	Dietary glycerin, %					
Item	0	4	8	12	SEM	*P*-value[1]
pH	6.07[a]	6.06[a]	5.68[b]	6.08[a]	0.087	0.01
Osmolality, mmol/kg	398	407	424	399	27.2	0.90
Total VFA, m*M*	139	146	149	132	5.0	0.09
Individual VFA, mol/100 mol						
Acetate	54.6	54.1	50.9	53.3	1.53	0.11
Propionate	35.6	34.3	38.6	35.4	1.46	0.38
Butyrate	9.8	11.6	10.5	11.3	0.63	0.47
Acetate:propionate ratio	1.53	1.57	1.32	1.50	0.13	0.21

[a,b]Within rows, means not bearing a common superscript letter differ ($P < 0.05$).
[1]Effect of glycerin level.

an oral drench, and via rumen tube increased rumen propionate.

Animal Metabolism

Plasma glucose concentration was not affected by glycerin content (0.796 ± 0.021 g/L; Table 4). Serum insulin concentration tended ($P = 0.06$) to increase linearly from 0 to 91 d of study (from 1.08 to 1.22 μg/L). It has been previously reported that serum insulin concentrations may increase with age (Martin et al., 1979) and carcass fatness (Trenkle and Topel, 1978). Serum insulin concentration was greater ($P < 0.05$) in bulls that received the 8% glycerin treatment (1.37 μg/L) than in those that received the no glycerin (0.94 μg/L) or 12% glycerin (0.98 μg/L) treatments. This fact was probably due to the numerically greater daily concentrate intakes and total rumen VFA concentrations in bulls on the 8% glycerin treatment compared with the other treatments. The insulin to glucose ratio was greater ($P < 0.05$) in bulls fed 8% glycerin (1.66 ± 0.14 μg/g) than in those fed no glycerin (1.19 ± 0.14 μg/g) or 12% glycerin (1.22 ± 0.14 μg/g). In agreement with the results of this study, Ogborn (2006) reported that a 500-mL oral bolus of crude glycerin significantly decreased plasma NEFA concentrations with no overall significant effects on plasma glucose or insulin in dairy cattle 5 d after calving. But, in contrast to the present results, Parker et al. (2007) reported greater blood glucose concentrations in animals supplemented with 642 g of pure glycerol 48 h before slaughter than in unsupplemented animals, and Goff and Horst (2001) reported an increase in plasma glucose when administering glycerol via an esophageal pump. Probably, the increase in blood glucose concentrations following glycerin supplementation depends on the physiological state of the animal and its energy balance.

Carcass and Meat Quality Characteristics

Carcass and meat quality characteristics are presented in Table 5. Dietary treatments did not affect HCW (244.2 ± 2.7 kg), dressing percentage (52.6 ± 0.51%), backfat (58% classified as 3), and conformation (63% classified as O). Mach et al. (2008) reported similar carcass quality data when feeding Holstein bulls high-concentrate diets to a final BW similar to that of the current study. In addition, the area (39.8 ± 2.3 cm^2) and intramuscular fat content (3.82 ± 0.45%) of LM were not affected by treatments. In the present study, it was hypothesized that glycerin supplementation would reduce the acetate to propionate ratio in the rumen, mainly resulting from an increase in rumen molar proportions of propionate, which is a glucose precursor. In addition, glycerol can be converted to glucose in the liver of cattle. Thus, it was expected that glucose supply would increase in bulls supplemented with glycerol, fostering a rise in blood insulin concentrations and lipogenesis. In fact, bulls receiving the 8% glycerin treatment had the numerically greatest blood insulin concen-

Table 4. Plasma glucose and serum insulin concentration of Holstein bulls fed high-concentrate diets containing different glycerin content

	Dietary glycerin, %					*P*- value[1]		
Item	0	4	8	12	SEM	G	T	G × T
Insulin, μg/L	0.94[b]	1.19[ab]	1.37[a]	0.98[b]	0.12	0.05	0.11	0.06
Glucose, g/L	0.798	0.769	0.821	0.797	0.021	0.36	0.40	0.54
Insulin:glucose, μg/g	1.19[b]	1.56[ab]	1.66[a]	1.22[b]	0.14	0.03	0.001	0.13

[a,b]Within rows, means not bearing a common superscript differ ($P < 0.05$).
[1]G = effect of glycerin content, T = effect of time.

Table 5. Carcass and meat quality of LM from Holstein bulls fed high-concentrate diets containing different glycerin content

	Dietary glycerin, %					
Item	0	4	8	12	SEM	*P*-value[1]
HCW, kg	240.6	246.3	245.6	244.4	2.72	0.46
Dressing percentage, %	52.3	53.1	52.9	52.5	0.51	0.66
Backfat classification,[2] %						
1	0.0	0.0	0.0	8.3	1.25	0.65
2	33.3	50.0	33.3	41.7	1.25	0.65
3	66.7	50.0	66.7	50.0	1.25	0.65
Conformation classification,[3] %						
O	58.3	75.0	58.3	58.3	1.50	0.77
P	41.7	25.0	41.7	41.7	1.50	0.77
LM						
Area, cm^2	38.2	40.2	40.5	40.2	2.31	0.86
Fat content, %	3.65	3.83	4.10	3.73	0.45	0.89
Warner-Bratzler shear force, kg	4.17	4.01	3.81	3.79	0.36	0.87

[1]Effect of glycerin content.
[2]1 = low to 5 = very high.
[3]O = fair; P = poor.

tration and also the numerically greatest intramuscular fat content. Some studies (Purchas et al., 2002) have associated tenderness with intramuscular fat content. However, the different glycerin content used in the current study had no effect on WBSF measurements of LM (3.92 ± 0.36 kg). Nevertheless, the obtained WBSF results (<4.0 kg) ensure a tenderness that should result in high consumer acceptance (Miller et al., 2001).

In conclusion, glycerin (86% glycerol) could be assigned an ME estimate of 3.47 Mcal/kg of DM when fed to Holstein bulls receiving high-concentrate diets. In addition, feeding concentrate containing up to 12.1% of glycerin does not lead to detrimental effects on performance, ruminal fermentation, metabolism, animal health, and carcass and meat quality variables, and thus it could be effectively used as an alternative energy source to substitute for cereals in the diet.

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