

# Serum metabolite concentrations and enzyme activities in finishing bull calves fed different types of high-grain diets

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## Abstract

Between the ages of 23 and 35 weeks, various serum metabolites and enzymes were monitored in three 10-animal groups of double-muscled Belgian Blue bull calves maintained in a feedlot in Galicia (NW Spain) on high-grain finishing diets that mainly differed in whether the grain used was predominantly maize (group M), predominantly barley (group B), or a mixture of maize and barley in approximately equal proportions (group MB). The parameters determined were glucose, non-esterified fatty acids (NEFA), total serum protein concentration (TSP), albumin, serum urea nitrogen (SUN), creatinine, aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT). Throughout the study period, all these parameters remained within the physiological ranges for beef under intensive conditions, and no animal ever showed clinical symptoms of ruminal alterations; indicating that none of these high-grain diets were detrimental to animal health. Although average serum NEFA, creatinine, albumin, AST and GGT levels all differed among groups, only AST can be considered as possible age-independent marker of grain-type-related metabolic alterations, since the other parameters all showed significant time × group interaction. In terms of this parameter, animals fed a MB diet behaved similarly to those fed a B diet. The absence of between-group differences in blood glucose level may reflect a genetic characteristic of this double-muscled breed. Our results, in conjunction with the best productive results obtained in animals fed de B-diet, aim us to suggest that the risk of an acidogenic diet would depend strongly on the nutritional management (in terms of crude protein (CP) and quality of straw in the ration) and not only the type of grain.

**Keywords:** cattle, double muscling, high-grain diets, metabolites

## Zusammenfassung

### **Serum-Metaboliten-Konzentrationen und Enzymaktivitäten in mit verschiedenen Futtermittelarten mit hohem Getreideanteil gefütterten Mastbullenkälbern**

Zwischen dem Alter von 23 und 35 Wochen wurde ein Monitoring durchgeführt der verschiedenen Serum-Metaboliten und Enzyme mit drei Gruppen zu jeweils 10 Weißblauen Belgier-Bullenkälbern mit starker Muskelfülle, die in einer Mastparzelle in Galizien (NW

Spanien) mit Mastfuttermittelarten mit hohem Getreideanteil gehalten wurden, die sich hauptsächlich darin unterschieden, dass das verwendete Getreide überwiegend aus Mais (Gruppe M), überwiegend aus Gerste (Gruppe B) oder aus einem Gemisch von Mais und Gerste zu ungefähr gleichen Teilen (Gruppe MB) bestand. Die nachgewiesenen Parameter waren Glukose, nicht veresterte Fettsäuren (NEFA), Serum-Gesamtproteinkonzentration (TSP), Albumin, Serum-Harnstoffstickstoff (SUN), Creatinin, Aspartataminotransferase (ASTZ) und  $\gamma$ -Glutamyltransferase (GGT). Während des Untersuchungszeitraums blieben diese Parameter durchwegs innerhalb des für Rindfleisch unter intensiven Haltungsbedingungen physiologischen Bereichs, und kein Tier zeigte zu irgendeiner Zeit klinische Symptome einer Rumen-Veränderung, so dass keines dieser Futtermittel mit hohem Getreideanteil der tierischen Gesundheit abträglich war. Obwohl die durchschnittlichen Konzentrationen von NEFA, Creatinin, Albumin, AST und GGT im Serum in den Gruppen alle unterschiedlich waren, kann nur AST als möglicher altersunabhängiger Marker von getreidebedingten Stoffwechseleränderungen betrachtet werden, da die anderen Parameter alle eine signifikante Wechselwirkung zwischen Zeit und Gruppe zeigten. Im Hinblick auf diese Parameter verhielten sich Tiere, an die ein MB-Futtermittel verfüttert wurde, wie diejenigen, an die ein B-Futtermittel verfüttert wurde. Das Fehlen von Intergruppenunterschieden hinsichtlich des Blut-Glukosespiegels könnte ein genetisches Merkmal dieser Rasse mit starker Muskelfülle widerspiegeln. Unsere Ergebnisse, zusammen mit den besten, mit Tieren, denen das B-Futter verfüttert wurde, erhaltenen Nutzergebnissen veranlassen uns zu dem Vorschlag, dass das Risiko einer azidogenen Fütterung stark vom Fütterungsmanagement (hinsichtlich Roheiweiß (CP) und Qualitätsstroh in der Ration) und nicht nur vom Getreidetyp abhängt.

**Schlüsselwörter:** Rind, Muskelfülle, Futtermittel mit hohem Getreideanteil, Metaboliten

## Introduction

The optimization of cereal grain utilization while maintaining normal rumen function and animal health continues to be a major challenge for the feedlot industry. Although it is widely recognized that the starch contained in small cereal grains (barley and wheat) is degraded more rapidly in the rumen than the starch in maize, little is known about whether different grains have different effects on metabolism.

The clinical chemistry profile is a valuable diagnostic tool that can be used to evaluate the internal balance. When used in conjunction with the physical examination the chemistry panel may be useful for establishing initial baseline parameters, formulating a problem or rule out list, planning nutritional options and monitoring the response to it (Russell & Roussel 2007). In fact, in previous studies, plasma metabolite concentrations and the activities of certain enzymes involved in energy metabolism have been shown to be useful indicators of changes in the metabolic status of beef cattle and other animals (Brown *et al.* 2000a, Arai *et al.* 2003, Mori *et al.* 2007).

In a previous paper (Castillo *et al.* 2009) we described how the acid-base status and L-lactate levels of finishing bull calves was influenced by grain mix in diets with total grain contents of 45-60%. We showed that serum L-lactate levels were almost invariably higher in calves fed a diet with barley as the main component than in calves fed a maize-based diet. Following to last article, the work described here report the influence of the same diets on other serum

metabolic parameters, namely glucose, non-esterified fatty acids (NEFA), urea nitrogen (SUN), creatinine, total protein concentration (TSP), albumin, aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT), looking for metabolic indicators connected with the nutritional protocol received by the animals.

This study was carried out in a commercial feedlot farm using animals destined for market, and covered the entire 80-day finishing period employed in the authors' region, Galicia (NW Spain). Several studies have generally focused on short-term effects (<5 days), but in the present study, we considered longer-term effects on these parameters. Furthermore, of course, it is of interest to know whether these easily measured indicators of systemic metabolic status are affected to different extents, if at all, by different high-grain diets used under commercial conditions. As far as we know no studies of such health variables have been carried out in which diet, as the independent variable, has been manipulated taking into account the nutritional protocols used in commercial feedlot farms, or in which health variables have been evaluated jointly with production variables.

## Materials and methods

### *Animals, feeding management and experimental design*

We studied 30 double-musled Belgian Blue steers that had been brought to the commercial study farm (Coren SCL, Ourense, NW Spain) at an age of 3-5 weeks. Upon arrival, each animal was weighed, ear-tagged, and vaccinated against clostridial infections (with 4 mL of Toxipra-S7, from Hipra, Girona, Spain) and against infectious bovine rhinotracheitis, parainfluenza-3 virus, bovine respiratory syncytial virus and bovine viral diarrhoea (with 2 mL of Cattlemaster-4, from Pfizer, Madrid, Spain); booster doses were given 5 weeks later. The animals were also treated for endo- and ectoparasites with the recommended dose of ivermectin (Ivomec, from Merial Laboratorios, S.A., Barcelona, Spain), and were given an injection (0.2 mL per 10 kg) of a preparation containing vitamins A, D<sub>3</sub>, and E (Hipravit-AD<sub>3</sub>E-Forte, from Hipra, S.A., Girona, Spain). After medication, the calves were housed in pens with straw bedding in a sheltered, unheated barn.

Until age 14 weeks the calves were weaned to grain-based diets with a combination of milk replacer (1 L per 20 kg bodyweight) combined with a starter diet formulated on the farm, made up of maize (25% DM basis), wheat (10%), barley (25%), soybean meal (44% CP; 21%), soybean hulls (3%), wheat bran (8%), palm oil (98% bypass fat; 1%), barley sprouts (2%), sweet whey powder (3%) and vitamin-mineral premix (see footnote to Table 1; 2%), with water and straw *ad libitum* (Bacha 1999). They were then allotted randomly to one of three 10-calf groups that were fed different high-grain diets during a 60-day growing period (age 14-23 weeks). Group M was fed maize as the main cereal grain and sodium bicarbonate as diet buffer; group B was fed barley as the main cereal grain; and group MB was fed equal proportions of maize and barley (Table 1). Finally, at age 23 weeks, the three groups were placed for 80 days on the finishing diets detailed at the right of Table 1.

All grains were ground in a hammer mill to a particle size less than 5 mm. All calves had free access to feed, water and barley straw, which was chopped to a length of 7-8 cm. Fresh feed was provided once a day at 08:00 h. Throughout the study, the animals were cared for and managed in accordance with official Spanish guidelines on animal care.

Table 1  
Ingredients and chemical composition of the concentrates fed during the growing and finishing periods

Ingredient, %DM	Growing Groups			Finishing Groups		
	M	MB	B	M	MB	B
Barley	14.5	27.0	32.6	15.8	32.9	30.5
Rye	-	-	5.0	6.0	-	6.0
Wheat	-	6.0	10.0	-	-	10.0
Maize	30.0	25.0	10.0	30.0	27.5	10.0
Molasses	3.0	3.3	2.5	2.5	3.3	2.5
Sunflower meal	4.0	-	-	-	-	-
Palm oil (98% bypass)	2.0	1.9	1.8	0.5	1.6	2.0
Palm kernel oil	-	-	-	4.0	4.0	4.0
Soybean meal, 44% CP	14.3	16.5	15.1	13.5	12.9	9.6
DDGS	-	-	7.0	-	-	8.0
Barley sprouts	2.0	2.0	-	-	-	-
Corn gluten feed	10.0	14.0	10.0	10.0	14.0	10.0
Wheat bran	9.0	-	-	5.3	-	4.2
Soybean hulls	8.0	1.5	3.2	10.0	1.6	1.1
Sodium bicarbonate	0.6	-	-	0.3	-	-
Vitamin/mineral premix <sup>a</sup>	3.2	2.8	2.8	2.1	2.2	2.1
Chemical composition, %DM						
CP	16.6	16.5	16.6	15.0	15.0	15.5
CF	7.6	4.6	5.0	7.3	5.0	5.0
NDF	21.3	17.5	19.0	20.8	19.3	21.6
ADF	10.8	6.0	6.6	11.1	6.8	7.2
EE	4.9	4.0	4.1	3.5	4.1	4.7
NFC	50.9	56.3	54.5	57.2	56.6	53.1
Ash	6.3	5.7	5.8	3.4	5.0	5.1

M: maize-based diet, B: barley-based diet, MB: equal mixture of maize and barley, DDGS: maize distillers' dried grain with solubles, CP: crude protein, CF: crude fibre, NDF: neutral detergent fibre, ADF: acid detergent fibre, EE: ether extract content, NFC: non-fibre carbohydrates calculated as  $100 - (CP + ash + NDF + EE)$  <sup>a</sup>Vitamin and mineral premix containing (per kg DM premix): 10 000 IU vitamin A, 2 000 IU vitamin D, 10 IU vitamin E, 0.4 mg Co, 16 mg Cu, 25 mg Fe, 2 mg I, 110 mg Mn, 0.3 mg Se, and 120 mg Zn

### Measurements and analysis

At the beginning of the growing and finishing periods, samples of concentrate were analysed in the farm laboratory. Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined by the method of Van Soest *et al.* (1991), crude fibre (CF) as per European Union Directive 92/89/EEC, ether extract (EE) as per EU Directive 98/64/EEC, starch as per EU Directive 99/79/EEC, and ashes as per EU Directive 81/680/EEC. Crude protein (CP) was determined using the French standard method (Dumas; N $\times$ 6.25: Association Française de Normalisation, NF V18-120).

Before blood sampling, the calves were examined for clinical signs of metabolic acidosis, i.e. for changes in posture or behaviour, signs of dehydration or shock (position of the eyeballs, temperatures of the oral cavity and extremities), diarrhoea, and altered palpebral reflex (Lorenz 2004).

Blood samples were collected between 09:00 and 11:00 h (hence after feed delivery) on day 0 (the last day before beginning of the finishing diet) and on days 3, 7, 22, 50 and 80.

Samples were taken by jugular venous puncture and collected in Vacutainer tubes without EDTA, and were allowed to clot at room temperature for 3 h before centrifugation at 2000g for 20 min and removal of the supernatant, which was stored at  $-20^{\circ}\text{C}$  until analysis. Serum NEFA were assayed with kits supplied by Randox Laboratories Ltd. (UK) and Spinreact SA, (Girona, Spain), respectively. All other metabolic parameters were measured using Gernon diagnostic kits from RAL S.A. (Barcelona, Spain). In all cases, appropriate controls were used. Within-run and within-day coefficients of variation were less than the limits of 5 % and 10 %, respectively that were recommended by Lumsden (2000).

### Statistical analysis

Non-normal distributions were tested for using the Shapiro-Wilk test. Analyses of variance (ANOVAs) were performed with group (M, MB or B) as fixed effect factor and time as repeated-measures factor, and with the time $\times$ group interaction included in the model. All statistical analyses were performed using the package SPSS version 12.1 (SPSS Inc 2004). The criterion for statistical significance was  $P \leq 0.05$ .

## Results and discussion

### Physical examination

Animal performance is reported elsewhere (Castillo *et al.* 2009, Table 2). Briefly, the three groups did not differ with respect their final weights in the slaughterhouse although best productive performance was afforded by the barley-based diet (group B gained significantly more weight and had a lower daily intake than either group M or group MB during the finishing period).

Table 2  
Productive performance (means $\pm$ SEMs) of finishing-stage bull calves (Castillo *et al.* 2009)

Variable	Group			P
	M	MB	B	
Initial weight, kg	222.0 $\pm$ 3.0 <sup>b</sup>	232.0 $\pm$ 4.4 <sup>c</sup>	208.0 $\pm$ 3.4 <sup>a</sup>	<0.001
Final weight, kg	405.0 $\pm$ 5.5	420.0 $\pm$ 2.7	416.0 $\pm$ 4.1	>0.1
Average daily gain, kg	1.5 $\pm$ 0.04 <sup>a</sup>	1.5 $\pm$ 0.05 <sup>a</sup>	1.7 $\pm$ 0.03 <sup>b</sup>	0.019
Daily intake, kg/d	7.9	8.1	7.5	-
Feed:gain ratio	5.3	5.4	4.4	-

M: maize-based diet, B: barley-based diet, MB: equal mixture of maize and barley, Significance P of variation among groups; within each row, means with the same superscript letter do not differ significantly at the 5% level.

As expected in view of the relatively moderate grain contents of all three diets, at no time during the study did any of the animals develop clinical signs of acidosis (changes in posture or behaviour, altered palpebral reflex, or signs of dehydration such as anomalous mouth or limb temperature or eyeball position; Lorenz 2004) or of other disorders related with high grain consumption (variations in feed intake, in the frequency and amplitude of ruminal contractions or decrease in venous blood pH; Brown *et al.* 2000a, 2000b). The health of the animals may also have been favoured by the amount of CP in these diets, which was greater than the recommended 12-13 % (Bailey & Duff 2005) and may have acted as a pH buffer

(Brown *et al.* 1998, Castillo *et al.* 2008). Additionally, the forage fibre source employed, barley straw, contains more long-fibre NDF than traditional silage forage, and may thus be more effective in promoting chewing activity and saliva secretion (Krause *et al.* 1998).

### *Serum parameters*

The group means and standard errors of each parameter studied on each sampling date are listed in Tables 3 and 4 together with the corresponding ANOVA results. Taking the study group as a whole, all these parameters except AST showed significant variation among sampling dates. In particular, serum glucose tended to fall with increasing calf age in group B and, after the first week, in group M, while total serum protein increased with age in groups B and MB, the latter especially. A similar though much more pronounced fall in serum glucose in 2 to 12-week-old dairy calves was attributed by Khan *et al.* (2007) to the use of a step-down weaning procedure.

The trend in total serum protein is attributable to the fact that microbial protein synthesis in the rumen increases with age (Devant *et al.* 2000, Kaneko *et al.* 2008). All the other parameters except glucose, SUN and total serum protein were also significantly affected by group, i.e. by cereal type (Figure 1). Averaging over time points, group M animals had higher AST activities and lower NEFA, creatinine, albumin and GGT levels than the others, while group B had the highest GGT activities and creatinine and albumin levels. Although the higher lactate levels of group B were not associated with clinical signs of acidosis (Castillo *et al.* 2009), it may nonetheless be significant that this group also had the highest levels of GGT, a sensitive indicator of hepatobiliary disorders (Latimer *et al.* 2003, Russell & Roussel 2007), though all values were below the pathological range and the reverse between-group trend was shown by AST, a nonspecific marker of acute liver damage that can be elevated in acidotic animals (Latimer *et al.* 2003, Mori *et al.* 2007). Serum creatinine is an index of body muscle mass and protein catabolism (Latimer *et al.* 2003, Kaneko *et al.* 2008), and the fact that it was highest in group B is in keeping with reports that a barley-based diet can be more efficient than a corn-based diet (Boss & Bowman 1996, Surber & Bowman 1998, Bengoechea *et al.* 2005, Castillo *et al.* 2009). Differences in NEFA values – although within physiological ranges – can be attributed to the different ether extract content of the ration in this period (Table 1) as in line with the observations made by Yambayamba *et al.* (1996).

It is noteworthy that there were no significant differences in serum glucose between the three groups of animals, since Tiffany & Spears (2005) reported plasma glucose to be lower in Angus steers fed barley-based diets than in those fed maize-based diets, which they attributed to the lower ruminal propionate levels of barley-fed steers limiting postabsorptive metabolism and gluconeogenesis. However, glucose metabolism depends on the endocrine actions of insulin and glucagon (Huntington 1997), which in turn depend on breed (Pareek *et al.* 2007); the insensitivity of serum glucose to group in our study is in keeping with the results of a study of double-musled Belgian Blue bulls in which serum glucose remained stable even after energy supply restriction (Fiems *et al.* 2007).

Although several parameters studied showed between-group differences in values averaged across time points, as discussed above, only AST did not exhibit significant group  $\times$  time interaction. Thus AST can be considered as possible marker of grain-type-related metabolic alteration throughout the finishing period of these bull calves.

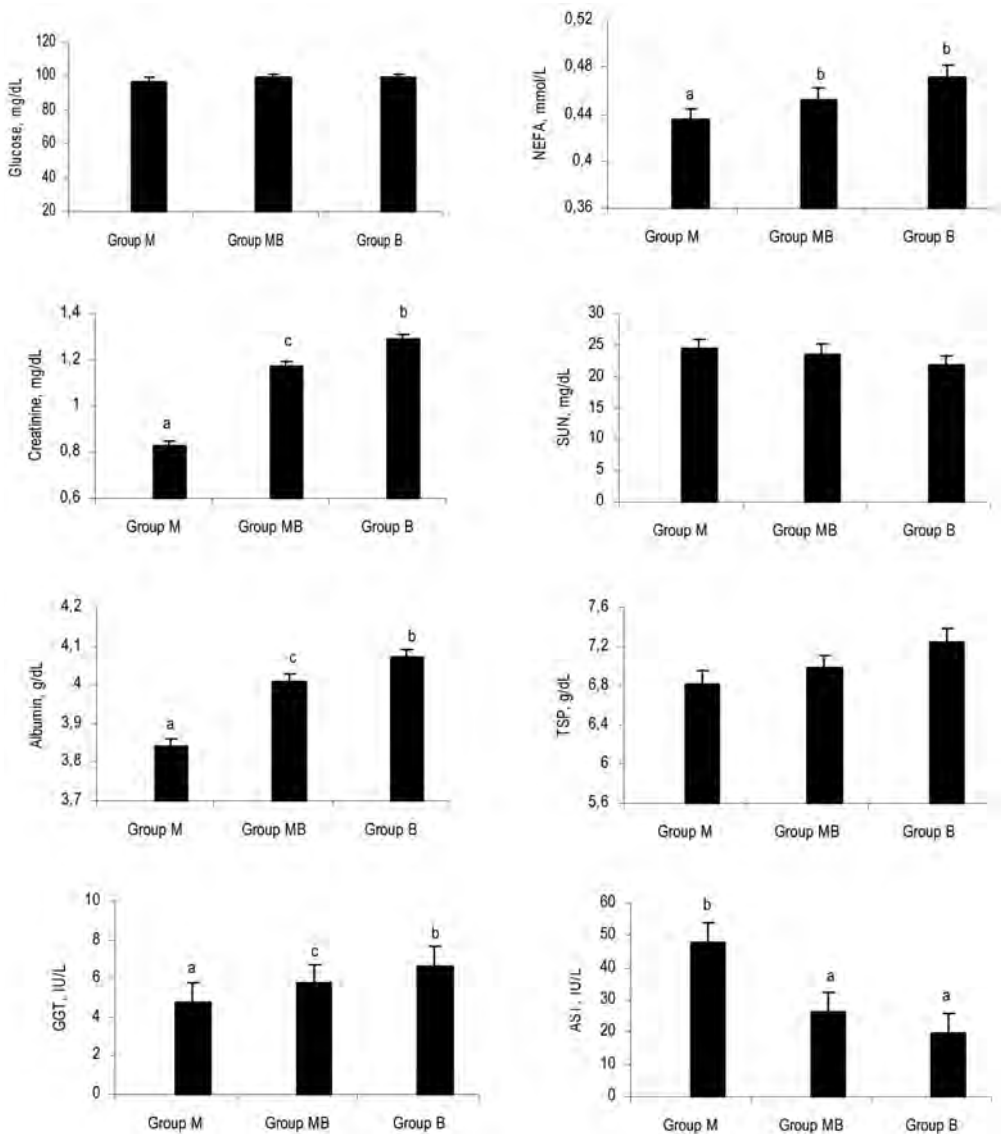


Figure 1 Mean serum glucose, NEFA, urea nitrogen, creatinine, protein, albumin, AST and GGT values in the study groups (bars show pooled SEs). Values labelled with different letters differ significantly ( $P < 0.05$ ).

In conclusion, under the conditions of this study none of the diets employed was associated with either clinical signs of acidosis or pathological alterations in the metabolic parameters studied, possibly because of the high CP content of these diets and the high long-fibre NDF content of the forage, barley straw. This result in conjunction with the best productive results obtained in animals fed de B-diet, aim us to suggest that the risk of an acidogenic diet would depend strongly on the nutritional management (in terms of CP and quality of straw in the ration) and not only the type of grain.



Although average serum NEFA, creatinine, albumin, AST and GGT levels all differed among groups, only AST can be considered as possible age-independent marker of grain-type-related metabolic alterations, since the other parameters all showed significant time  $\times$  group interaction.

Table 3  
Mean ( $\pm$ SE) of blood metabolites in Belgian Blue calves fed different high grain diets<sup>1</sup> during the finishing period

Item and day	Treatment			<i>P</i>		
	M	MB	B	Time	Group	Time $\times$ Group
Serum glucose, mg/dL				0.007	0.38	0.41
Day 0	95.4 $\pm$ 3.81	96.6 $\pm$ 4.54	102.2 $\pm$ 3.83			
Day 3	101.3 $\pm$ 3.61	101.4 $\pm$ 1.03	96.8 $\pm$ 7.81			
Day 7	101.2 $\pm$ 3.04	101.6 $\pm$ 2.11	94.6 $\pm$ 2.87			
Day 22	97.7 $\pm$ 1.35	97.8 $\pm$ 2.06	94.0 $\pm$ 7.46			
Day 50	96.6 $\pm$ 0.93	103.5 $\pm$ 3.32	90.4 $\pm$ 2.62			
Day 80	88.6 $\pm$ 3.23	93.2 $\pm$ 3.34	88.0 $\pm$ 3.77			
Serum NEFA, mmol/L				<0.001	0.025	<0.001
Day 0	0.45 $\pm$ 0.01	0.56 $\pm$ 0.07	0.46 $\pm$ 0.03			
Day 3	0.41 $\pm$ 0.01	0.41 $\pm$ 0.01	0.43 $\pm$ 0.02			
Day 7	0.36 $\pm$ 0.03	0.42 $\pm$ 0.01	0.45 $\pm$ 0.01			
Day 22	0.41 $\pm$ 0.03	0.43 $\pm$ 0.02	0.51 $\pm$ 0.01			
Day 50	0.39 $\pm$ 0.02	0.49 $\pm$ 0.01	0.49 $\pm$ 0.01			
Day 80	0.59 $\pm$ 0.05	0.41 $\pm$ 0.01	0.50 $\pm$ 0.01			
SUN, mg/dL				0.001	0.48	<0.001
Day 0	26.2 $\pm$ 1.24	24.4 $\pm$ 2.52	26.6 $\pm$ 0.68			
Day 3	22.1 $\pm$ 0.91	19.2 $\pm$ 2.33	23.6 $\pm$ 1.21			
Day 7	22.2 $\pm$ 0.86	24.6 $\pm$ 2.89	21.2 $\pm$ 1.43			
Day 22	24.0 $\pm$ 1.30	23.2 $\pm$ 3.18	18.8 $\pm$ 1.46			
Day 50	26.0 $\pm$ 2.05	26.7 $\pm$ 3.05	18.6 $\pm$ 1.43			
Day 80	25.8 $\pm$ 1.36	23.6 $\pm$ 2.11	21.6 $\pm$ 1.03			
Serum creatinine, mg/dL				<0.001	<0.001	<0.001
Day 0	0.9 $\pm$ 0.04	1.0 $\pm$ 0.03	1.3 $\pm$ 0.02			
Day 3	0.7 $\pm$ 0.02	1.2 $\pm$ 0.01	1.2 $\pm$ 0.03			
Day 7	0.9 $\pm$ 0.03	1.3 $\pm$ 0.06	1.4 $\pm$ 0.03			
Day 22	0.9 $\pm$ 0.02	1.1 $\pm$ 0.03	1.2 $\pm$ 0.03			
Day 50	0.9 $\pm$ 0.05	1.3 $\pm$ 0.04	1.4 $\pm$ 0.03			
Day 80	0.6 $\pm$ 0.07	1.0 $\pm$ 0.02	1.3 $\pm$ 0.05			
TSP, g/dL				<0.001	0.09	0.10
Day 0	6.9 $\pm$ 0.13	6.5 $\pm$ 0.25	6.8 $\pm$ 0.12			
Day 3	6.3 $\pm$ 0.35	6.5 $\pm$ 0.12	7.0 $\pm$ 0.11			
Day 7	6.8 $\pm$ 0.06	6.8 $\pm$ 0.30	6.7 $\pm$ 0.18			
Day 22	6.7 $\pm$ 0.20	6.9 $\pm$ 0.24	7.3 $\pm$ 0.10			
Day 50	7.3 $\pm$ 0.12	7.3 $\pm$ 0.30	7.9 $\pm$ 0.17			
Day 80	7.0 $\pm$ 0.15	7.7 $\pm$ 0.30	7.6 $\pm$ 0.31			
Albumin, g/dL				<0.001	<0.001	<0.001
Day 0	3.8 $\pm$ 0.02	3.9 $\pm$ 0.01	4.1 $\pm$ 0.01			
Day 3	3.6 $\pm$ 0.03	3.8 $\pm$ 0.04	4.1 $\pm$ 0.02			
Day 7	4.0 $\pm$ 0.05	4.0 $\pm$ 0.03	4.0 $\pm$ 0.02			
Day 22	3.8 $\pm$ 0.04	4.1 $\pm$ 0.02	4.0 $\pm$ 0.03			
Day 50	3.9 $\pm$ 0.15	4.0 $\pm$ 0.18	4.1 $\pm$ 0.32			
Day 80	3.8 $\pm$ 0.02	4.1 $\pm$ 0.04	4.0 $\pm$ 0.02			

M: maize-based diet, B: barley-based diet, MB: equal mixture of maize and barley



Table 4  
Mean ( $\pm$ SE) of blood serum enzymes in Belgian Blue calves fed different high grain diets during the growing and finishing periods

Item and day	Treatment			Time	P	
	M	MB	B		Group	Time $\times$ Group
Serum AST, IU/L				0.255	0.006	0.081
Day 0	59.1 $\pm$ 13.3	27.7 $\pm$ 1.1	17.8 $\pm$ 2.4			
Day 3	54.0 $\pm$ 16.3	22.8 $\pm$ 2.6	12.4 $\pm$ 1.0			
Day 7	49.8 $\pm$ 13.9	23.7 $\pm$ 1.4	14.0 $\pm$ 2.6			
Day 22	41.3 $\pm$ 8.8	27.7 $\pm$ 5.0	15.9 $\pm$ 2.1			
Day 50	46.8 $\pm$ 11.8	24.4 $\pm$ 3.1	12.0 $\pm$ 1.2			
Day 80	35.4 $\pm$ 3.7	30.6 $\pm$ 3.5	18.3 $\pm$ 2.3			
Serum GGT, IU/L				<0.001	<0.001	<0.001
Day 0	4.5 $\pm$ 0.20	6.4 $\pm$ 0.19	5.8 $\pm$ 0.37			
Day 3	4.2 $\pm$ 0.37	7.1 $\pm$ 0.45	7.4 $\pm$ 0.42			
Day 7	6.2 $\pm$ 0.20	5.9 $\pm$ 0.40	8.0 $\pm$ 0.66			
Day 22	3.9 $\pm$ 0.19	5.0 $\pm$ 0.51	6.1 $\pm$ 0.54			
Day 50	5.0 $\pm$ 0.32	4.9 $\pm$ 0.24	7.4 $\pm$ 0.60			
Day 80	5.0 $\pm$ 0.27	5.1 $\pm$ 0.37	5.3 $\pm$ 0.44			

M: maize-based diet, B: barley-based diet, MB: equal mixture of maize and barley

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