Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1806-2636
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v37i2.26446

Performance of beef cattle bulls in feed lots and fed on diets containing enzymatic complex

Leonardo Guimarães de Oliveira[1*], Reginaldo Nassar Ferreira[2], João Teodoro Padua[3], Cirano José Ulhoa[2], Cristine Dos Santos Settimi Cysneiros[2] and Emmanuel Arnhold[3]

*[1]Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, Av. Esperança, s/n, 74690-900, Goiânia, Goiás, Brazil. [2]Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, Goiás, Brazil. [3]Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, Goiânia, Goiás, Brazil. *Author for correspondence. E-mail: leonardogovet@hotmail.com*

ABSTRACT. Current paper evaluates the performance of confined beef cattle supplemented with amylolytic enzyme complex produced by fungus *Aspergillus awamori* and a commercial product containing multienzyme complex, yeast and MOS. Treatments comprised control (basal diet composed of 16% Mombasa grass silage, 66% ground corn, 3% vitamin nuclear mineral and 15% cottonseed meal), amylase treatment (control diet with the addition of 48.7 saccharifying units kg^{-1} diet) and compound treatment (control diet with the addition of enzymatic complex composed of 83.2 saccharifying units, 8.8 fibrolytic units, 0.05 g of mannan oligosaccharides and 0.2 g of inactivated yeast Kg^{-1} of the dry matter diet). The addition of products did not significantly increase daily weight gain, intake, feed conversion and carcass yield of cattle. There was no difference between *in vitro* digestibility of dry matter (IVDMD) in the diets. The percentage of residual fecal starch was not influenced by exogenous amylolytic enzymes of amylase and compound treatments. The tested products were not able to improve animal performance.

Keywords: starch, biotechnology, feedlot, ruminant.

Desempenho de bovinos não castrados confinados e alimentados com dietas contendo complexo enzimático

RESUMO. Este trabalho foi realizado para avaliar o desempenho de bovinos não castrados confinados e suplementados com um complexo enzimático amilolítico produzido pelo fungo *Aspergillus awamori* e com um produto contendo um complexo multienzimático, leveduras e mananoligossacarídeos. Os tratamentos foram: controle (dieta composta por 16% de silagem de capim mombaça, 66% de milho triturado, 3% de núcleo mineral vitamínico e 15% de torta de algodão), amilase (dieta controle com adição de 48, 7 unidades sacarificantes kg^{-1} da dieta) e composto (dieta controle com adição de 83,1 unidades sacarificantes, 8,8 unidades fibrolíticas, 0,05 g de mananoligossacarídeos e 0,2 g de leveduras inativas kg^{-1} de matéria seca da dieta). Os tratamentos não alteraram o ganho de peso diário, consumo voluntário, conversão alimentar e rendimento de carcaça dos bovinos confinados. Não houve diferença entre as digestibilidade *in vitro* da matéria seca (DIVMS) das dietas. A porcentagem de amido residual fecal não foi influenciada pela suplementação com enzimas amilolíticas exógenas dos tratamentos amilase e composto. Os avaliados não foram capazes de melhorar o desempenho dos animais.

Palavras-chave: amido, biotecnologia, confinamento, ruminantes.

Introduction

Several diets may provide low rates of rumen degradation due to the impairment of enzyme activities produced by rumen microorganisms on the substrate. The amount of enzymes and substrate and the interaction between the two are relevant for hydrolysis. Since the amount of substrate and its availability in the rumen are not restricting, the quantity of available enzyme in the rumen may limit the degradation process (Dehority & Tirabasso, 1998).

Enzyme supplementation may be an alternative to increase animal efficiency and reduce diet costs by greater amounts of available nutrients. Exogenous amylolytic and fibrolytic enzymes are currently being studied to increase animal efficiency (Beauchemin et al., 2003; Bowman et al., 2002; Gencoglu et al., 2010; Martins et al., 2006a, b, c; Martins et al., 2007; Martins et al., 2008). A positive aspect that causes the use of exogenous enzymes in the diet of ruminants is that they function within a wide temperature range in the rumen (Colombatto et al., 2003).

Current research evaluated the performance of bulls fiished in feedlot and supplemented with amylolytic complex produced by the fungus *A. awamori*, coupled to a compound based on

amylolytic and fibrolytic enzymes, yeasts and mannan oligosaccharids.

Material and methods

The fungus *A. awamori*, employed for the production of the amylolytic complex, was isolated from a soil sample. Petri plates with MEX medium containing [malt extract 3.0% (p v^{-1}) and agar 2.0% (p v^{-1})], autoclaved at 120ºC for 20 minutes were used. The culture was kept for 10 days at 30ºC and stored at 4ºC. The amylolytic enzymatic complex was produced by removing three 5mm-culture discs from the Petri plate and the spores of the microorganism were inoculated in 1.0 L-flasks with 250 mL of the induction medium (ground maize 10 g L^{-1}; yeast extract 10 g L^{-1}; $CaCl_2.2H_2O$ 0.1 g L^{-1}; magnesium sulfate 0.5 g L^{-1}; ferrous sulfate 0.1 L^{-1}; KH_2PO_4 0.2 g L^{-1}). The maize used as carbon source for the production of the multienzymatic complex was ground in a Willey mill with a 1mm diameter mesh. The flasks were incubated in a rotation shaker (Controlled Environment Incubator Shaker, Brunswick Scientific Co. Inc., U.S.A) at 30°C and 180 rpm. After 72 hours culture, the amylolytic enzymatic complex in the water solution was filtered in a vacuum and aliquots were collected, centrifuged at 4000 rpm for 10 minutes for the enzymatic assay by the saccharifying method (Miller, 1959), and later given to the animals.

Animal performance assay was performed at a commercial feedlot in Rondonópolis, Mato Grosso State, Brazil, with 63 Nellore bulls finished in feedlot, average 24 years old and weighing 418 kg live weight, contemporary, and from the same cattle ranch Fazenda Santa Luzia in the same municipality.

Animals were given the helminth killer ivermectin 1% (Merial®) and vaccinated against chlostridiose, tetanus and botulism with vaccine (Vencofarma®). So that averages of diet intake could be obtained, the animals were divided into nine lots of seven animals each. Each lot was randomly housed in an experimental pen measuring 150 m^2, with a 15 meter trough for feed. Water was given *ad libitum*.

The animals received diet and water *ad libitum* during the whole period and thus accustomed to the experimental diet during 19 days prior to the experimental period, followed by 47 days for assessment. The treatments comprised:

- Control treatment (Control): 21 animals divided into three pens, fed on experimental diet without the addition of enzymes;

- Amylase treatment (Amylase): 21 animals, divided into three pens, fed on experimental diet with 48.7 saccharifying units kg^{-1} of dry matter;

- Compound treatment (Compound): 21 animals divided into three pens, fed on 83.1 saccharifying units kg^{-1} of dry matter of the diet, 8.8 fibrolytic units kg^{-1} of dry matter of the diet, 0.05 g manna oligosaccharids kg^{-1} of dry matter of diet; 0.2 g of inactive yeasts kg^{-1} of dry matter of the diet.

The experimental diet was composed of 16% mombaça grass silage; 66% cracked corn, 15% cottonseed meal and 3% vitamin mineral nucleus. Table 1 shows rates of the chemical analyses.

Table 1. Chemical composition of the basal experimental diet (% of dry matter).

Nutrients	Diets		
	Control	Amylase	Compound
Dry matter (%)	61.63	60.27	60.41
Crude protein	12.63	13.07	12.60
Ether extract	3.89	3.80	3.43
Mineral matter	3.46	3.82	3.36
Acid detergent fiber	13.33	13.60	13.52
Neural detergent fiber	28.31	28.88	27.99
Starch	33.69	32.58	33.42
Non-fibrous carbohydrates	51.71	50.42	52.63
Total digestible nutrients	78.19	78.00	78.39

The solution with amylolytic compound in the Amylase treatment was diluted in 3.5 liters of water at room temperature five minutes prior to the start of the daily diet mixture, sprayed on the cracked corn, which was then mixed with the other ingredients.

In compound treatment, powder 105 g of the product with the multienzyme complex was first mixed in 20 kg of cracked corn and then mixed with the other ingredients. Mixer (Realmix 8000 Realmaq®) was used to mix and distribute the diet. Prior to the addition of ingredients of each diet, the mixer was completely emptied and cleaned so that it would not be contaminated by the previously diet.

The diets of each treatment were prepared daily and provided once a day in the morning. The ingredients of each diet were weighed separately in a Digi-Star® scale, calibrated by Inmetro (0.1%) and mixed during 5 minutes in the mixer. The diet was then given to the animals of each treatment.

Prior to the above, the diet of the previous day was removed from the feed trough and weighed to calculate intake and the quantity that had to be provided on that day to each lot. Daily amount was based on diet intake of the previous day with 10% surplus.

Diet samples were collected weekly during the experimental period and frozen for chemical analysis and to determine the *in vitro* degradability of the dry matter (IVDDM). Samples for each treatment were mixed and homogenized at the end of the experiment to prepare a compound sample of each

treatment; they were then dried in a forced air oven at 55ºC for 72 hours. Further, samples were ground in a Willey mill with a 1 mm sieve for laboratory analysis.

The animals were slaughtered, following humane protocols, at the end of the experimental period at an abattoir (Minerva Abattoir), with federal health inspection, at the same municipality, some 5 km from the feedlot ranch. The hot half-carcasses were weighed to calculate carcass dressing.

The animals were weighed at the start and finishing of feedlot on an electronic balance Beckhuser® idBECK 2.0, calibrated by Inmetro (0.1%), within the management pen. This event was preceded by a 16-hour feed and water fast.

Technique described by Tilley and Terry (1963) was used in the IVDDM assay. Samples with 0.5 g ground meal from each treatment were conditioned in nylon filter bags (F57 Ankom®). Fourteen bags were placed in each jar at equal amounts, at incubation periods of 0, 6, 12, 24, 48 and 72 hours; procedure included two bags per incubation period and two empty sealed bags for weight correction. Four incubation replications were performed, totaling 56 bags per treatment.

The bags were added to the jars in a decreasing time order but removed together. The white sealed empty bag was used to calculate the correction factor. After removed from the jars, all the bags were washed in running water for 10 min. till the water was transparent once more. They were dried in a forced air oven at 55ºC for 72 hours to determine residual dry matter.

Contents of the rumen used to determine IVDDM was manually collected from a Holstein *vs.* Zebu calf, with average weight 370 kg. The animal was kept in a 12 m^2 pen with water *ad libitum* and meal comprising 89% Tifton hay, 10% ground corn and 1% mineral mixture, for 14 days prior to the collecting the rumen liquid.

Analyses of mineral matter (MM), dry matter (DM), crude protein (CP), ether extract (EE) of total diet provided and of remains were performed according to AOAC (2005). NDF and ADF were calculated following methodology by Van Soest et al. (1991).

Fecal pH and fecal residual starch were calculated by collecting feces samples of all animals from the floor immediately after defecation, once a day, between 1 and 3 p.m., for 5 days during the experimental period. Care was taken so that no contamination of samples occurred with the soil. Immediately after collection of samples, 50 g of the sample were diluted in 50 mL of deionized water. pH was measured daily by a manually calibrated potency meter

Approximately 200 g of each sample were frozen and maintained in a freezer at -25ºC to determine fecal residual starch.

Starch concentration and fecal residual starch were calculated by the enzyme method in triplicate, proposed by Bach-Knudsen and Glitso (1997). The collected feces samples were dried in a oven at 105ºC for 72 hours and then ground in a Willey mill with a 0.5 mm sieve. Starch percentage was determined by 0.1 g of the diet sample and 0.2 g of the dry feces sample.

Statistical design was totally randomized, with three seven-animal lots for each experimental treatment. Each treatment comprised 21 animals equally divided into three pens. Each corral was an experimental unit for the variables dry matter intake, fecal starch residual feed conversion, percentage of diet starch and fecal pH. The animals were the experimental units for the other variables.

Data on weight gain, dry matter intake, feed conversion and carcass dressing underwent analysis of variance and means were compared by tukey's test ($p > 0.05$) with statistic program R.

The mathematical model was:

$$Y_{ij} = \mu + S_j + e_{ij};$$

in which Yij is the observation on the 5^{th} animal, belonging to the j^{th} supplemented group;

μ = general average of the characteristic;

Si = effect of the supplementation type;

Eij = residual effect.

IVDDM data were adjusted to the model by Ørskov and McDonald (1979) to determine the degradability curve of DM to obtain degradability parameters. Results were analyzed by the test for model identity (Regazzi, 2003), with statistical program R.

Results and discussion

Treatments failed to alter ($p < 0.05$) average daily gain, voluntary intake, feed conversion and carcass yield of bulls finished in feedlot (Table 2).

There are several variation sources that affect responses to exogenous responses, such as its stability and activity in the rumen environment (Beauchemin et al., 2003; Martins et al., 2006c; Martins et al., 2008). Nozière et al. (2014) did not report any positive effects on animal performance with enzyme supplementation higher than doses in current experiment, even though the inclusion of

exogenous amylolytic enzymes increased by 8% the degradability of starch in the rumen without any increase in starch digestibility in the entire digestive tract.

Table 2. Data of mean weight of animals at the start of confinement (MWI), mean weight of animals finished in feedlot (PMF), daily weight gain (DWG), intake of dry matter (IDM), intake of dry matter as a ratio of live-weight (IDM LW), feed conversion (FC) and carcass yield (CY).

Parameters	Treatments			MSE†	CV‡
	Control	Amylase	Compound		
MWI (kg)	417.42*	418.52	419.00	4.70	5.15
PMF (kg)	486.52	487.38	486.42	6.56	6.17
DWG (kg)	1.47	1.47	1.44	0.07	20.68
IDM (kg)	11.15	11.48	10.83	0.13	3.23
IDM LW (%)	2.47	2.51	2.39	0.04	3.02
FC	7.62	7.78	7.56	0.21	5.91
CY (%)	55.60	56.22	55.06	0.52	4.3

*Means on the same line preceded by similar letters do not differ by tukey's test ($p < 0.05$). †Mean standard error. ‡Coefficient of variation.

In an experiment with beef cattle in feedlot with the inclusion of 580 amylolytic units of amylase per kg of dry matter of the diet, J. M. Tricarico et al. (2007) registered an increase in voluntary intake of DM and weight gain in the first 28 days in feedlot (14%). The above failed to occur in current experiment perhaps due to the smaller quantity of enzymes provided to animals.

Although employing a low amount of enzymes, Rojo-Rubio et al. (2005) evaluated the starch efficiency of sorghum grain and reported a 13.9% improvement in the weight gain of lambs when given 4.2 amylolytic unities per kg of sorghum in diet produced from the bacterium *B. licheniformis*. This fact shows that there are differences in results using different microorganisms for the production of the enzyme complex.

Non-linear responses on animal performance were reported by Tricarico et al. (2005) and Tricarico et al. (2007). The authors reported a quadratic response on animal performance when receiving exogenous enzymes and attributed improvement in performance to a greater intestinal digestibility of the starch, favored by amylolytic enzymes when passing intact through the abomasum.

There was no difference ($p > 0.05$) for the different treatments in IVDDM (Table 3; Figure 1), which together with intake data, corroborated with the fact that animals failed to show any difference ($p > 0.05$) in weight gain and feed conversion.

The amount of exogenous enzymes added to the diet was not sufficient to increase degradation of dry matter in the rumen. Besides its high quantities of enzymes, its stableness and its activity in the rumen environment were relevant aspects for the increase of diet degradation in the rumen (Beauchemin et al., 2003). Similar results were also reported by Tricarico et al. (2005) and Hristov et al. (2008) who did not evidence any difference in the digestibility of dry matter in the diet of animals treated with the enzymatic complex.

Rojo-Rubio et al. (2001) showed a 64.3% increase after 12 hours of incubation with regard to the *in vitro* degradability of starch treated with amylolytic enzymes of *B. licheniformis.* In fact, the microorganism used for the production of enzymes affects ruminal activity.

When Hristov et al. (2000) supplemented calves with polysaccharides, they did not register any difference in diet digestibility and in the dry matter intake, probably due to the hydrolysis of exogenous enzymes by the proteases in the liquid of the rumen and by the rate increase of passage through the rumen (Martins et al., 2006b). Soluble enzymes are taken to the neighboring compartments and the enzyme concentration in the rumen diminishes.

Fine grinding of maize improves total digestibility and decreases starch fecal excretion in bulls due to the increase of the grain surface subjected to enzymatic and microbial attack. It causes a greater ruminal digestion of starch with greater benefits in the diet (Yang et al., 2001; Zinn et al., 2002).

Table 3. *In vitro* degradability of dry matter of experimental data

Parameters	Treatments			MSE†	CV‡ (%)
	Control	Amylase	Compound		
Fraction a	21.45*	22.23	20.67	1.22	11.48
Fraction b	59.01	62.95	61.38	3.50	11.48
Kd	3.35	2.87	3.56	0.49	30.14
DE (kp = 2%)	57.64	59.12	58.31	1.04	3.58
DE (kp = 5%)	44.53	45.03	44.92	1.07	4.79
DE (kp = 8%)	38.42	38.74	38.64	1.05	5.46
Lag Time (hours)	1.02	1.38	0.70	0.32	61.86
FI (%)	19.54	14.81	17.94	3.60	41.33
DP (%)	80.45	85.18	82.05	3.60	8.73

Soluble fraction % (Fraction a); potentially degradable fraction (Fraction b); degradation rate of fraction b (Kd) in % per hour; Effective Degradability (DE) according to passage rates (kp) 2, 5 and 8% per hour; non-degradable fraction (FI); Potential Degradability (DP). *Data did not show statistically any difference in the identity of model test. †Mean standard error. ‡Coefficient of variation.

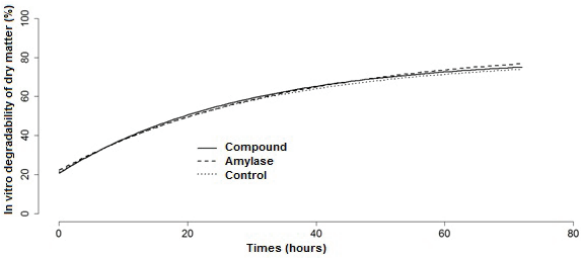

Figure 1. IVDDM curve of experimental diets.

The percentage of residual fecal starch was not influenced by supplementation with exogenous amylolytic enzymes of the treatments amylase and compound (Table 4).

Table 4. Residual fecal starch (RFS) and fecal pH.

	Treatments			MSE†	CV‡ (%)
	Control	Amylase	Compound		
RFS (%)	14.09	15.06	14.32	33.59	39.94
Fecal pH	5.61*ab	5.50b	5.71a	0.02	2.53

*Means on the same line preceded by similar letters do not differ by tukey's test ($p<0.05$). †Mean standard error. ‡Coefficient of variation.

The excretion of starch by the feces is also affected by the type of cereal and by the way grain is processed. In fact, the manner feed is processed may influence the rate and place of starch digestion (Huntington, 1997). Prolamin, a protein in the maize grain that wraps starch granules, is another factor that may jeopardize starch granules in cereals (Chandrashekar & Mazhar, 1999). The protein occurs in the endosperm (30 - 60% of total grain protein) and envelops and turns impervious starch granules in a hydrophobic matrix, making difficult the access of microorganisms and their enzymes in the granule (Nascimento et al., 2009).

Amylase treatment caused a lower pH when compared to compound treatment, possibly due to inactive yeasts in its composition. Yeasts in the diet remove the oxygen in the viable cellulolytic bacteria which use lactic acid and a pH decrease of the ruminal contents occurs (Wallace, 2004). Fecal pH has an inversely proportional behavior to rumen degradation and fecal excretion of starch, as reported by Barajas and Zinn (1998) and Depenbusch et al. (2008). Therefore, in diets with high grain inclusion, a greater quantity of starch does not undergo rumen fermentation and more starch passes through the small intestine. Starch in the intestine will be fermented by bacteria in the intestine with the production of a higher amount of fatty acids within the short chain and, consequently, a decrease in fecal pH will occur. A greater quantity of starch goes through without being digested by the gastrointestinal reaction and excreted by the feces.

Conclusion

The enzymatic complexes evaluated in current assay were not capable of improving the performance of bull confined in finishing lots.

Acknowledgements

The authors would like to thank Nutripura Nutrição Animal Ltda for the help given in current assay.

References

AOAC. Association Official Analytical Chemists. (2005). *Official methods of analysis*. (18th ed.). Gaitherburg, Maryland, USA: .

Bach-Knudsen, K. E. & Glitso, V. (1997). Methods for analysis of dietary fibre-advantage and limitations. *Journal of Animal and Feed Sciences, 6*(2), 185-206.

Barajas, R. & Zinn, R. A. (1998). The feeding value of dry-rolled and steam-flaked corn in finishing diets for feedlot cattle: influence of protein supplementation. *Journal of Animal Science, 76*(7), 1744-1752.

Beauchemin, K. A., Colombatto, D., Morgavi, D. P. & Yang, W. Z. (2003). Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. *Journal of Animal Science, 81*(E.Suppll. 2), E37-E47.

Bowman, G. R., Beauchemin, K. A. & Shelford, J. A. (2002). The proportion of the diet to which fibrolytic enzymes are added affects nutrient digestion by lactating dairy cows. *Journal of Dairy Science, 85*(12), 3420-3429.

Chandrashekar, A. & Mazhar, H. (1999). The biochemical basis and implications of grain strength in sorghum and maize. *Journal of Cereal Science, 30*(3), 193-207.

Colombatto, D., Morgavi, D. P., Furtado, A. F. & Beauchemin, K. A. (2003). Screening of exogenous enzymes for ruminant diets: Relationship between biochemical characteristics and in vitro ruminal degradation. *Journal of Animal Science, 81*(10), 2628-2638.

Dehority, B. A. & Tirabasso, P. A. (1998). Effect of ruminal cellulolytic bacterial concentrations on in situ digestion of forage cellulose. *Journal of Animal Science, 76*(11), 2905-2911.

Depenbusch, B. E., Nagaraja, T. G., Sargeant, J. M., Drouillard, J. S., Loe, E. R. & Corrigan, M. E. (2008). Influence of processed grains on fecal pH, starch concentration, and shedding of O157 in feedlot cattle. *Journal of Animal Science, 86*(3), 632-639.

Gencoglu, H., Shaver, R. D., Steinberg, W., Ensink, J., Ferraretto, L. F., Bertics, S. J., ... Akins, M. S. (2010). Effect of feeding a reduced-starch diet with or without amylase addition on lactation performance in dairy cows. *Journal of Dairy Science, 93*(2), 723-732.

Hristov, A. N., Basel, C. E., Melgar, A., Foley, A. E., Ropp, J. K., Hunt, C. W. & Tricarico, J. M. (2008). Effect of exogenous polysaccharide-degrading enzyme preparations on ruminal fermentation and digestibility

of nutrients in dairy cows. *Animal Feed Science and Technology, 145*(1), 182-193.

Hristov, A. N., McAllister, T. A. & Cheng, K. J. (2000). Intraruminal supplementation with increasing levels of exogenous polysaccharide-degrading enzymes: effects on nutrient digestion in cattle fed a barley grain diet. *Journal of Animal Science, 78*(2), 477-487.

Huntington, G. B. (1997). Starch utilization by ruminants: from basics to the bunk. *Journal of Animal Science, 75*(3), 852-867.

Martins, A. S., Vieira, P. D. F., Berchielli, T. T., Prado, I. N. & Moletta, J. L. (2006a). Intake and apparent total tract digestibility in cattle supplemented with fibrolytic enzymes. *Revista Brasileira de Zootecnia, 35*(5), 2118-2124.

Martins, A. S., Vieira, P. F., Berchielli, T. T., Prado, I. N., Canesin, R. C. & Setti, M. C. (2006b). Taxa de passagem e parâmetros ruminais em bovinos suplementados com enzimas fibrolíticas. *Revista Brasileira de Zootecnia, 35*(3), 1186-1193.

Martins, A. S., Vieira, P. V., Berchielli, T. T., Prado, I. N. & Garcia, J. A. S. (2006c). Eficiência de síntese microbiana e atividade enzimática em bovinos submetidos à suplementação com enzimas fibrolíticas. *Revista Brasileira de Zootecnia, 35*(3), 1194-1200

Martins, A. S., Vieira, P. F., Berchielli, T. T. & Prado, I. N. (2008). Degradação ruminal da silagem de milho e da palha de arroz utilizando enzimas fibrolíticas exógenas. *Acta Scientiarum. Animal Sciences, 30*(4), 435-442.

Martins, A. S., Vieira, P. F., Berchielli, T. T., Prado, I. N. & Paula, M. C. (2007). Degradabilidade *in situ* e observações microscópicas de volumosos em bovinos suplementados com enzimas fibrolíticas exógenas. *Revista Brasileira de Zootecnia, 36*(6), 1927-1936.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*(3), 426-428.

Nascimento, M. L., Paulino, M. F., Detmann, E., Valadares Filho, S. C., Porto, M. O. & Sales, M. F. L. (2009). Fontes de energia em suplementos múltiplos para recria de novilhos mestiços em pastejo durante o período de transição seca/águas: desempenho produtivo e características nutricionais. *Revista Brasileira de Zootecnia, 38*(6), 1133-1141.

Nozière, P., Steinberg, W., Silberberg, M. & Morgavi, D. P. (2014). Amylase addition increases starch ruminal digestion in first-lactation cows fed high and low starch diets. *Journal of Dairy Science, 97*(4), 2319-2328.

Ørskov, E. R. & McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *The Journal of Agricultural Science, 92*(2), 499-503.

Regazzi, A. J. (2003). Teste para verificar a igualdade de parâmetros e a identidade de modelos de regressão não-linear. *Revista Ceres, 50*(287), 9-26.

Rojo-Rubio, R., Martínez, G. D. M. & Galván, M. M. C. (2001). Uso de la amilasa termoestable de *Bacillus licheniformis* en la digestibilidad in vitro del almidón de sorgo y maíz. *Agrociencia, 35*(4), 423-427.

Rojo-Rubio, R., Mendoza, G. D., González, S. S., Landois, L., Barcena, R. & Crosby, M. M. (2005). Effects of exogenous amylases from *Bacillus licheniformis* and *Aspergillus niger* on ruminal starch digestion and lamb performance. *Animal Feed Science and Technology, 123*, 655-665.

Tilley, J. M. A. & Terry, R. A. (1963). A two□stage technique for the in vitro digestion of forage crops. *Grass and Forage Science, 18*(2), 104-111.

Tricarico, J. M., Abney, M. D., Galyean, M. L., Rivera, J. D., Hanson, K. C., McLeod, K. R. & Harmon, D. L. (2007). Effects of a dietary *Aspergillus oryzae* extract containing □-amylase activity on performance and carcass characteristics of finishing beef cattle. *Journal of Animal Science, 85*(3), 802-811.

Tricarico, J. M., Johnston, J. D., Dawson, K. A., Hanson, K. C., McLeod, K. R. & Harmon, D. L. (2005). The effects of an *Aspergillus oryzae* extract containing alpha-amylase activity on ruminal fermentation and milk production in lactating Holstein cows. *Animal Science, 81*(3), 365-374.

Van Soest, P. J., Robertson, J. B. & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74*(10), 3583-3597.

Wallace, R. J. (2004). Antimicrobial properties of plant secondary metabolites. *Proceedings of the Nutrition Society, 63*(4), 621-629.

Yang, W. Z., Beauchemin, K. A. & Rode, L. M. (2001). Effects of grain processing, forage to concentrate ratio, and forage particle size on rumen pH and digestion by dairy cows. *Journal of Dairy Science, 84*(10), 2203-2216.

Zinn, R. A., Owens, F. N. & Ware, R. A. (2002). Flaking corn: processing mechanics, quality standards, and impacts on energy availability and performance of feedlot cattle. *Journal of Animal Science, 80*(5), 1145-1156.

Received on January 30, 2015.
Accepted on March 24, 2015.