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Apparent digestibility of a dietetic feed claimed for horse clinical nutrition

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Abstract

The management of horses in the post-surgical phase involves many aspects concerning stress reactions and the recovery of the intestinal functions in the case of surgical treatments of colics, enteritis and dismicrobial problems. During this period it is necessary to keep horses in quiet conditions and, if possible, without loss of muscular tonus. At the same time, it is necessary to fight against the loss of minerals, vitamins and electrolytes that occurs during illness. Recently the industries producing dietetic feeds are more and more involved in the research of special formulae concerning mixed feeds for the recovery of horses from the post-surgical phase. Rough materials generally characterized by high digestibility together with probiotics are often employed to satisfy these exigence. In fact they promote the development of enterophil bacteria in opposition to pathogens with a consequent re-establishment of healthy physiological functions. The apparent digestibilities of meadow hay or meadow hay plus dietetic feed rich in probiotics, inulin, minerals and vitamins were determined by means of three *in vivo* digestibility trials, each performed on 6 adult healthy horses weighting about 450 kg over a 6 days faeces total collection period with a previous 14 days adaptation period. The dietetic feed was tested at two different amounts: 0.5 and 0.8 kg/q live weight as fed basis (75:25 and 62:38 on DM basis, respectively). These increasing amounts were studied with the aim of verifying if the digestibility of the dietetic feed in suggested amounts by the producer were optimal for the claimed in clinical nutrition. Faeces and feed dried samples were analysed to determine dry matter, organic matter, crude protein, crude fibre, fibrous fractions and gross energy. The results showed that the addition of the dietetic feed to the hay improves the CP digestibility of the 75:25 and 62:38 rations, while the crude fibre and fibrous fractions digestibilities of 62:38 ration were statistically lower than those of hay alone. These results could mainly be ascribed to the decreasing digestibility of the fiber with the increasing of the inclusion of the dietetic feed.

Key words: *horse, apparent digestibility, probiotics, clinical nutrition, stress.*

Introduction

In horses the prolonged housing together with lack of moving in the post-surgical phase involves many aspects concerning different stress reactions. Specially in horses went on surgical treatments of intestinal pathologies a long recovery period of the normal intestinal functions could be observed mainly in short-tempered animals. The most common consequences are colic, enteritis, microbial disorders or systemic inflammation pathologies such as lameness (Garner *et al.*, 1978; Miraglia *et al.*, 1999a; Reeves *et al.*, 1996). The feeding management of these horses is a very delicate question because they are subjected to forced hunger, to frequent changes in feeding, to a restricted ingestion of forages and, in the recovery phase or to the ingestion of concentrated feeds rich in energy. During this period it is necessary to keep horses in quiet conditions and, if possible, without loss of muscular tonus. At the same time, it is necessary to fight against the loss of minerals, vitamins and electrolytes that occurs during illness. To prevent these problems a correct feeding strategy based on a deep understanding of the mechanisms involved in the onset of digestive disturbances is needed. In the large intestine the limited peristalsis causes a considerable water re-absorption: consequently the intestinal transit becomes very hard and painful with possible large intestine colic. This situation is often linked to changes of the hindgut bacteria population because the high production of lactate is accompanied to an increased production of Gram+ bacteria and a decreased production of Gram- bacteria. Besides, in these conditions it is possible to have the release of vasoactive amines and of toxins that go into the blood through the damaged intestinal wall because it has became permeable; this is a possible cause of lameness (Yoder *et al.*, 1997). The use of mixed feeds in equine nutrition can determine considerable changes of the intestinal ecosystem and consequent changes in the diet digestibility (de Frombelle *et al.*, 2001). In fact the increase of amylolitic population associated to the increase of lactate and to the hindgut acidosis determines the reduction cellulolytic bacteria and a reduction of fibre digestibility (NDF and ADF) (Kerrour *et al.*, 1997; Drogoul *et al.*, 2001). In recent times, different commercial mixed feeds have been proposed to prevent digestive disorders in particular for sport horses that suffer of high stress conditions. But many factors, related to the feedstuff itself or to external conditions, can influence the digestive functions of horses (Orton *et al.*, 1985; Bergero *et al.*,1998). Recent experimentations demonstrated a reduction of fibre digestibility when the fibre source comes from by-products such as citrus pulp and apple by-products (Peiretti *et al.*, 2003; Miraglia *et al.*, 2004; Miraglia *et al.*, 2006); consequently, the use of mixed feeds rich in "dietetic fibre" or simply "high fibre concentrates" has to be deeply evaluated before their administration, mainly in the case of horses affected by digestive disturbances. In other cases, it has been demonstrated that the use of high digestible cereals (mainly those heated processed) reduces the fibre digestibility (Sarkijarvi and Saastamoinen, 2006; Julliand *et al.*, 2006) when the amount is high. This phenomenon could be linked to the increasing fraction of starch resistant to the enzymatic attach (cereals rich in amylase) (Bailoni *et al.*, 2006) and to the variations that could appear amongst the microbial population and the intestinal environment depending on the quantity and on the fermentation of starch flowing into the hindgut ecosystem. If the overloading of the capacity for starch digestion in the small intestine occurs, it is likely that the normal microbial activity in the large intestine would be compromised (Potter *et al.*, 1992). The consequence could be a reduction of the digestibility of the different fibre components of the whole ration. Recently the industries producing dietetic feeds are more and more involved in the research of special formulae concerning mixed feeds (Martin-Rosset *et al.*, 2006): every year feed industry works out many formulations of compounds feeds. Some of them are claimed for clinical nutrition for the recovery of horses from the post-surgical phase; thus, the nature and the proportion of the feed ingredients may vary considerably. Rough materials generally characterized by high

digestibility together with probiotics are often employed to satisfy these exigence. In fact probiotic promote the development of enterophil bacteria in opposition to pathogens with a consequent re-establishment of healthy physiological functions. More recently the use of mixed feeds including fructooligosaccharide (FOS) evidenced that these substances improve the structure of the mucous membrane of the gut and influences the production of Volatile Fatty Acids (VFA) (Swanson *et al.*, 2002; Tsukahara *et al.*, 2003). Some experimentations carried out on horses evidenced a positive effect of FOS on cecum-colic bacteria (Bergero, 1998). Other experimentations showed that FOS administration to horses can reduce the total anaerobic bacteria populations, lactobacillus and streptococcus with a decrease bacteria using lactate: this condition should prevent abnormal fermentations linked to the D-lactate increase (Respondek *et al.*, 2006). Other studies showed that FOS reduce considerably the yeast counting with a reduction of coliform bacteria, an increase of lactobacillus and an increase of their ratio with the number of *E. coli* (Pellegrini *et al.*, 1999). Nevertheless, even though there is literature available concerning starch utilisation, on one side, and the use of pre-probiotic administered as supplement in specific premix on the other, no data available exist in literature concerning the *in vivo* digestibility of mixed feeds including probiotic such as FOS or other else. Many of these experimentations refer to microbiological trials and not to the utilisation of the nutrient parameters by the horse. Besides, data concerning *in vivo* digestibility trials of simple processed cereals or of mixed feeds rich in cereals differ between them because of treatments, kind of cereal and experimental procedures; in fact the experimental procedures refer to "*ingesta-egesta*" total faeces collection (Bergero *et al.*, 1998; Miraglia *et al.*, 2004 - 2006; Peiretti *et al.*, 2003), chromium-mordanted (Sarkijarvi and Saastamoinen, 2006), acid insoluble ashes (Bergero *et al.*, 2005 - 2006; Casini *et al.*, 2002; Miraglia *et al.*, 1999b; Peiretti *et al.*, 2003), etc.. An experimental mixed feed rich in high digestible cereals, probiotics, inulin, minerals and vitamins previously tested in small amounts in the post-surgical phase of horses in the period of housing in the clinic was administered to healthy horses to assess the apparent digestibility of rations including increasing levels of it with the aim to suggest it in the diets of horses after discharging from the clinic. These trials represent the first step of the experimentation consisting in testing the mixed feed on healthy horses to have a standard reference values of nutrients utilisation and before using it in clinical nutrition.

Material and Methods

Three *in vivo* digestibility trials have been performed on 6 healthy standardbred horses, weighting 458 +/- 35 and 453 +/-31 kg respectively at the beginning and at the end of the experimental period; these horses were aged between 4 and 8 years. The horses were fed, in all the trials, at a feeding level close to maintenance. According to the standard experimental procedures of the *in vivo* digestibility trials (Martin-Rosset *et al.*, 1984) in the first trial the horses received hay (first cut meadow hay) as the only feedstuff (100%); in the two following trials the same hay was given together with an experimental dietetic feed (C), tested at two different amounts: 0.5 and 0.8 kg/q live weight as fed basis corresponding respectively to 75:25 F:C ratio (R_1) and 62:38 F:C ratio (R_2) on DM basis. These increasing amounts were suggested by the producer to verify if they were optimal for the claimed in clinical nutrition both for the administration in the post-surgical phase and in the period of convalescence before beginning the normal physical activity. The components of the mixed feed (Recovery ACME®) were: flaked barley, soybean (meal solv. extd.), flaked corn, dextrose, sugar cane molasses, soybean hulls, diosmectite, yeast (*Saccharomyces cerevisiae*), and FOS. The vitamin and mineral supplementation was constituted by a commercial premix rich in oligoelements produced by the same industry (Osteparon ACME ®). The experimental period

lasted 20 days for each trial: 14 days of adaptation to the diet and 6 days of total faeces collection, according to Martin-Rosset *et al.* (1984), using a suitable device (Horse Diaper) that allows the complete recovery of the faeces (and the urines, if required, in separate bags) and avoids the forced reclusion of the horses in digestibility stalls thus allowing the horses to walk and roam around freely. The horses were individually stabled in boxes with free access to water. Cumulative samples were prepared by mixing daily samples and were stocked for following analysis. The faecal and hay cumulative samples were dried in a forced-draft oven at 65°C for at least 48 hours till constant weight. The mixed feed were taken for immediate determination of the dry matter (DM) content at 105°C. All the dried samples were ground in a mill to pass a 1 mm screen. The samples were analysed to determine crude protein (CP), crude fibre (CF), and ash, according to AOAC (2000). The neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest *et al.* (1991). The gross energy (GE) was measured with an adiabatic calorimeter bomb (IKA C7000, Staufen, Germany). The nitrogen-free extract plus ether extract (NFE + EE) and organic matter (OM) were calculated for each material on the basis of the previous data. The cellulose and hemicellulose were calculated from the NDF, ADF and ADL. The apparent digestibility coefficients of hay and rations were then calculated for DM, OM, GE, CP, CF, NDF and ADF. The digestibility of hay and of rations were determined according to Martin-Rosset *et al.*, 1984:

d = (Ingested amounts – Excreted amounts)/Ingested amounts % .

The digestibility of the mixed feed was determined according to Martin-Rosset *et al.*, 1984:

$dC = (dR - f\,x\,dF)\colon c$

dC = mixed feed digestibility

dR = ration digestibility

dF = hay digestibility (previously determined)

f = percentage of forage (hay) in the ration

c = percentage of the mixed feed in the ration

The digestibility data of the rations were processed using one-way analysis of variance (ANOVA), with the kind of ration being the only comparison factor, using the SPSS statistics package (v 11.5, SPSS Inc., Chicago, IL, USA). The groups were compared using the Duncan Test (Norusis, 1992).

Results and Discussion

Table 1 shows the chemical composition of feeds used in the different trials. The apparent digestibility coefficients are summarized in Table 2. The digestibility coefficients of the hay (H) are in agreement with previous experimentations carried out on similar forages produced in Mediterranean areas (Bergero *et al.*, 2005; Miraglia *et al.*, 1995; Peiretti *et al.*, 2006). The comparison between the hay digestibility (1st trial) and the digestibilities of R_1 and R_2 rations shows that the digestibility coefficients of R_1 and R_2 rations are higher for CP ($P<0.05$); this is in agreement with Miraglia *et al.* (2006), that showed that CP digestibility coefficients increase with increasing the amounts of concentrates in the ration. The NDF, ADF and CF digestibility coefficients of R_2 ration are statistically lower ($P<0.10$) than those of the H ration. This means that the higher amount of the mixed feed in R_2 determined a negative effect on the digestibility of fibrous fractions. The calculated digestibility coefficients of the mixed feed (C) are higher in the 2nd trial than in the 3rd trial for DM, OM, GE and CP; the NDF, ADF and CF digestibility coefficients are negative in both cases and that confirm the previous researches from Julliand *et al.* (2006) concerning the decreasing digestibility of the fibre fractions with the excess of the inclusion of the dietetic feed. Julliand *et al.* (2001) and de Fonbelle *et al.* (2001) observed an increase in the horse gut of total anaerobic bacteria,

lactobacillus and streptococcus in rations constituted by a concentrate percentage higher then 50%. In these cases there is a reduction of the molar percentage of acetate that determines a reduction of the [(acetate+butyrrate)/propionate] ratio. The changes of the microbial activity determine a cecum-colic acidosis and a consequent reduction of the intestinal cellulolytic bacteria. This is the possible cause of the digestibility decrease of CF, NDF and ADF of the diet. A balanced diet should be necessary to compensate the abnormal fermentations consequences such as a gas increase in the small intestine and a limited peristalsis. It is not possible to confirm the preliminary hypothesis concerning the considerable improving of diet digestibility when this kind of mixed feeds is introduced in the diet. This means that it is necessary to verify the role of FOS on diet digestibility when they are included in the mixed feeds. The introduction of diosmectite and yeast (*Saccharomyces cerevisiae*) doesn't seem to improve the digestive utilisation. But there is no available literature concerning it and, consequently, it has not been possible to compare the present results with similar experimentations.

Conclusions

The use of mixed feeds in equine nutrition can determine considerable changes of the intestinal ecosystem and consequent changes in the diet digestibility. For this reason in recent times different commercial mixed feeds have been proposed as dietetic feed and/or to prevent digestive disorders in particular for sport horses that suffer of high stress conditions. But many factors, related to the feedstuff itself or to external conditions, can influence the digestive functions of horses and a spreading use of these feeds could result in negative effects. More data are needed, however, to assess the effect of these products on the utilisation of the whole ration. The feeding management of horses affected by digestive disorders or coming from surgical treatments is a very delicate question that needs a deeper knowledge concerning the hindgut bacteria population and the effects of the mixed feeds on the intestinal ecosystem. Recently the industries producing dietetic feeds are more and more involved in the research of special formulae concerning mixed feeds claimed for clinical nutrition. But the questions that arise from the use of these feeds concern their cost (they are very expensive) and the certainty of their beneficial effects; besides, it is important to know which is the best way of administration of pre-probiotic: the inclusion in the feed or their distribution as a premix supplement not included in the mixed feed.

References

AOAC, 2000. Official Methods of Analysis of the AOAC. AOAC, Arlington, VA.

Bailoni L., Mantovani R., Pagnin G., Schiavon S., 2006. Effects of physical treatments on the resistant starch content and in vitro organic matter digestibility of different cereals in horses. Livest. Sci. 100 (1), 14-17.

Bergero D., 1998. Ruolo dei frutto-oligosaccaridi nel cavallo. Riv. Zoot. Vet. 26 (1), 31-37.

Bergero D., Miraglia N., Polidori M., 1998. Digeribilità di mangimi composti per cavalli sportivi. Atti LV Congresso S.I.S.Vet., Silvi Marina (TE), Italia, 467-468.

Bergero D., Meineri G., Miraglia N., Peiretti P.G., 2005. Apparent digestibility of hays in horses determined by total collection of faeces and using internal marker methods. Journal of Food, Agriculture & Environment, 3(1), 199-202.

Bergero D., Préfontaine C., Miraglia N., Peiretti P.G., 2006. A comparison between 2N and 4N HCl methods for digestibility trials in horses. Book of abstracts 10th Congress of the European Society of veterinary and Comparative Nutrition, Nantes, France, 220.

Casini L., Magni L., Liponi G.B., Gatta D., 2002. Valutazione della digeribilità di due razioni contenenti amidi sottoposti a diverso trattamento in purosangue in attività. In: proceedings 4th Convegno "Nuove acquisizioni in materia di Ippologia", Campobasso, Italy, 171-176.
de Frombelle A., Julliand V., Drogoul C, Jacotot E., 2001. Feeding and microbial disorders in horses: 1 - effects of an abrupt incorporation of two levels of barley in a hay diet on microbial profile and activities. J. Equine Vet. Sci. 21 (9), 439-445.
Drogoul C., de Frombelle A., Julliand V., 2001. Feeding and microbial disorders in horses: 2 - effect of three hay:grain ratios on digesta passage rate and digestibility in ponies. J. Equine Vet. Sci. 21 (10), 487-491.
Garner H.E., Moore J.N., Johnson J.H., Clark L., Amend J.F., Tritschler L.G., Coffman J.R., 1978. Changes in the caecal flora associated with the onset of laminitis. Equine Vet. J. 10, 249-252.
Julliand V., de Fombelle A., Drogoul C., Jacotot M., 2001. Feeding and microbial disorders in horses: 3 - effects of three hay:grain ratios on digesta passage rate and digestibility in ponies. J. Equine Vet. Sci. 21 (11), 543–546.
Julliand V., de Fombelle A., Varloud M., 2006. Starch digestion in horses: the impact of feed processing. Livest. Sci. 100 (1), 44-52.
Kerrour M., Raskin P., Minet V., Mayombo A.P., Istasse L., 1997. Different grass silage and cereals proportions in exercided horses. Proc. 48th EAAP Annual Meeting, Vienna, Austria, 389.
Martin-Rosset W., Andrieu J., Vermorel M., Dulphy J.P., 1984. Valeur nutritive des aliments pour le cheval. In: Jarrige, R., Martin-Rosset, W. (Eds.) Le Cheval. Reproduction, Seléction, Alimentation, Exploitation, Inra, Paris, pp. 209-238.
Martin-Rosset W., Andrieu J., Vermorel M., Jestin M., 2006. Routine methods for predicting the net energy and protein values of concentrates for horses in the UFC and MADC systems. Livest. Sci. 100 (1), 53-69.
Miraglia N., Corino C., Polidori M., 1995. In vivo digestibility of mediterranean forages employed in Italy in horse feeding. 46th EAAP Meeting, Prague, Czech Republic, 338.
Miraglia N., Bergero D., Polidori M., Gagliardi D., Tarantola M., 1999a. Stima della digeribilità della razione in un caso di enterite cronica nel cavallo. In: proceedings "Nuove acquisizioni in materia di alimentazione, allevamento e allenamento del cavallo sportivo", Campobasso, Italy, 15-19.
Miraglia N., Bergero D., Bassano B., Tarantola M., Ladetto G., 1999b. Studies of apparent digestibility in horses and the use of internal markers. Livest. Prod. Sci. 60, 21-25.
Miraglia N., Polidori M., Peiretti P.G., Bergero D., 2004. Digeribilità *in vivo* di due mangimi composti ricchi in fibra in razioni per cavalli. In: proceedings 6th Convegno "Nuove acquisizioni in materia di Ippologia", Campobasso, Italy, 135-140.
Miraglia N., Bergero D., Polidori M., Peiretti P.G., Ladetto G., 2006. The effects of a new fibre-rich concentrate on the digestibility of horse rations. Livest. Sci. 100 (1), 10-13.
Norusis M.J., 1992. Statistical Package for the Social Sciences, Mc Graw-Hill, New York, USA.
Orton R.K., Hume I.D., Leng R.A., 1985. Effects of exercise and level of dietary protein on digestive functions in horses. Equine Vet. J., 17, 386-390.
Peiretti P.G., Miraglia N., Meineri G., Polidori M., Bergero D., 2003. Stima della digeribilità di mangimi concentrati nel cavallo: un confronto fra metodologie diverse. In: proceedings 5th Convegno "Nuove acquisizioni in materia di Ippologia", Grugliasco, Italy, 49-57.
Peiretti P.G.,Meineri G., Miraglia N., Mucciarelli M., Bergero D., 2006. Intake and apparent dogestibility of hay or hay plus concentrate diets determined in horses by the total collection of faeces and n-alkanes as internal markers. Liv. Sci., 100, 189-194.

Pellegrini L., Miliani A., Bergero D., 1999. Frutto-oligosaccaridi sulla microflora intestinale del cavallo sportivo: nota pratica. Riv. Zoot. Vet. 27 (2), 49-51.
Potter G.D., Arnold F.F., Householder D.D., Hansen D.H., Brown K.M., 1992. Digestion of starch in the small or large intestine of the equine. Pferdeheilkunde, 3/4, 107-111.
Respondek F., Goachet A.G., Julliand V., 2006. Dietary supplementaion of scFOS prevent biochemical disturbances occurring in the hindgut following an abrupt diet change. Proc. 10th ESVCN Congress, Nantes, France, 134.
Reeves M.J. , Salman M.D., Smith G., 1996. Risk factors for equine acute abdominal disease (colic): results from a multi-center case-control study. Prev. Vet. Med. 26, 285-301.
Sarkijarvi S., Saastamoinen M., 2006. Feeding value of various processed oat grains in equine diets. Livest. Sci. 100 (1), 3-9.
Swanson K., Grieshop C., Flickinger E., Bauer L., Chow J., Wolf B., Garleb A., Fahey G.C., 2002. Fructooligosaccharides and Lactobacillus acidophilus modify gut microbial populations, total tract digestibilities and fecal protein catabolite in healty adults dogs. J. Nutr. 132 (suppl.), 3721S-3731S.
Tsukahara T., Iwasaki Y., Nakayama K., Ushida K., 2003. Stimulation of Butyrate production in the large intestine of weaning piglets by dietary fructooligosaccharides and its influence on the histological variables of the large intestinal mucosa. J. Nutr. Sci. Vitaminol. 49, 414-421.
Van Soest P.J., Robertson J.B., Lewis B.A., 1991. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.
Yoder M.J., Miller E., Rook J., Shelle J.E., Ullrey D.E., 1997. Fiber level and form: Effects on digestibility, digesta flow and incidence of gastrointestinal disorders. Proc. 15th Equine Nutr. Physiol. Symp., Fort Worth, Texas, 122–127.

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Table 1. Chemical composition (% on DM) of hay (H) and of the mixed feed (C)

	H 1st trial	H 2nd trial	C 2nd trial	H 3rd trial	C 3rd trial
Dry Matter	92.9	90.5	92.4	90.6	92.5
Organic Matter	93.2	93.6	91.4	92.0	91.5
Ashes	6.8	6.4	8.6	8.0	8.5
Drude protein	7.4	7.3	18.8	7.4	19.9
Crude fibre	35.5	36.9	5.3	36.0	5.1
NDF[1]	69.2	71.4	16.9	66.7	18.1
ADF[2]	45.8	46.4	8.1	46.4	9.1
ADL[3]	5.8	9.9	1.1	7.2	1.2
Cellulose[4]	40.0	36.5	7.1	39.2	7.9
Emicellulose[5]	23.4	25.0	8.7	20.3	9.0
Nitrogen-free extract	48.7	47.7	65.7	46.8	64.9
Ether Extract	1.7	1.8	1.6	1.7	1.5
GE (MJ/kg)	18.5	18.9	17.3	17.7	17.7

[1] Neutral Detergent fibre
[2] Acid Detergen fibre
[3] Lignine
[4] Cellulose: ADF-ADL
[5] Emicellulose: NDF-ADF

Table 2. Digestibility coefficients (mean values ± S.E.) of hay (H), rations (R_1 and R_2) and mixed feed (C).

	H 1st trial	R_1 2nd trial	R_2 3rd trial	Diet effect	C 2nd trial	C 3rd trial
Dry Matter	44.6±2.1	45.8±5.6	42.6±6.3	NS	49.6±17.2	39.3±13.6
Organic Matter	45.2±2.1	47.0±5.8	43.0±6.8	NS	52.4±17.6	39.5±14.8
Crude Protein	37.6±3.4^{a}	53.8±4.3^{b}	59.1±2.7^{b}	0.002	102.4±9.3	94.1±3.1
GE	43.6±2.2	46.0±5.7	39.6±6.7	NS	53.1±17.3	33.1±14.4
Crude Fibre	36.6±3.2^{a}	12.0±12.1^{ab}	-6.8±17.1^{b}	0.071	-62.0±40.3	-77.7±40.1
NDF	40.6±2.5^{a}	30.1±8.7^{ab}	7.7±12.8^{b}	0.059	-1.5±28.0	-45.9±30.1
ADF	40.0±2.8^{a}	23.5±10.1^{ab}	3.9±14.2^{b}	0.072	-25.9±33.4	-54.9±33.1

^{a,b,c} Means in the same row with unlike superscripts differ ($P<0.05$ for CP and $P<0.10$ for CF, NDF and ADF)
R_1: ration 2nd trial (hay:concentrate = 75:25 on DM basis)
R_2: ration 3rd trial (hay:concentrate = 62:38 on DM basis)