IN SITU EVALUATION OF THE RUMINAL STABILITY OF DIFFERENT CHOLINE PRODUCTS

ELEK, PÉTER — HUSVÉTH, FERENC

SUMMARY

The objective of the experiment was to measure the ruminal stability of different choline sources (Procol-25®, Norcol-25®, Sintocol-25®, Reashure® as rumen-protected products and choline chloride as a non-stabilized compound). Three rumen-canulated ewes were used to assess the ruminal degradation of different choline products. Triplicates of samples were incubated for 0, 2, 4, 8, 16, 24 and 48 hours; the washing phase was changed to tender dipping of the samples into cold water to minimize the loss of choline. The ruminal degradation of unprotected choline chloride calculated at 12%/hour ruminal passage rate was the highest; 88.92%. The rumen protected products Procol-25®, Norcol-25® and Sintocol-25® were degraded significantly ($P<0.05$) slower (69.42 and 69.51 and 83.29%, respectively) as compared to the unprotected form, but considerable differences could be detected among them as well. The by-pass product called Reashure® showed the lowest disappearance from the rumen, only 13.85% of its choline content was degraded. The modified *in situ* method seems to be a quick and cost-effective way of studying the ruminal stability of different choline sources.

ÖSSZEFOGLALÁS

Elek, P. – Husvéth, F.: KÜLÖNBÖZŐ KOLINTARTALMÚ KÉSZÍTMÉNYEK STABILITÁSA A BENDŐBEN, *IN SITU* MEGHATÁROZVA

A kísérlet célja különböző kolin források (Procol-25®, Norcol-25®, Sintocol-25®, Reashure®, mint bendőbeli lebontás ellen védett termékek, és kolin-klorid, mint nem védett termék) bendőbeli stabilitásának mérése volt, három bendőkanüllel ellátott anyajuhval. Termékenként három mintát inkubáltunk 0, 2, 4, 8, 16, 24 és 48 órán keresztül. A hagyományos *in situ* módszert úgy módosítottuk, hogy a nylon zacskókban elhelyezett mintákat a kolin kioldódás minimalizálása érdekében, a mosási fázis helyett, óvatos, hideg vízbe történő mártogatással tisztítottuk meg a bendőtartalomtól. A 12%/óra bendőbeni áthaladási sebességgel kalkulált kolin lebomlás, a lebontás ellen nem védett kolin esetében volt a leggyorsabb, 88,92%. A lebomlás ellen védett termékek közül a Procol-25®, a Norcol-25® és a Sintocol-25® lebomlása 69,42, 69,51 és 83,29% volt, ami a nem védett formához képest szignifikánsan ($P<0,05$) lassabb volt, de a védett termékek között is szignifikáns különbségek ($P<0,05$) voltak megfigyelhetők. A Reashure nevű termék bendőbeni lebomlása volt a leglassúbb, az eredeti kolintartalom 13,85%-a bomlott le. E termék bendőbeli stabilitása szignifikánsan ($P<0,5$) nagyobb volt, mint a vizsgált másik négyé. Az eredmények alapján, a módosított *in situ* eljárás, egy gyors és költséghatékony módszernek tűnik a különböző kolin források bendőbeni lebonthatóságának vizsgálatára.

INTRODUCTION

Fatty liver syndrome is one of the most common metabolic disorders in high-producing Hungarian dairy herds. The development of hepatic lipidosis is the result of a long and complex process. After calving, the quickly increasing energy and nutrient demand of the cow cannot be met by the energy and nutrient supply from feed (*Bell et al.,* 1995), and therefore body reserves are mobilized (*Van den Top et al.,* 1995). Non-esterified fatty acids (NEFA) originating from the adipose tissue enter the liver and are either oxidized in the tricarboxylic acid (TCA) cycle and serve as energy source or esterified into triacylglycerols. These can be stored in liver cells or exported from the liver as very low-density lipoproteins (VLDL). During excessive fat mobilization, the liver is not able to completely export triglycerides in VLDL, but stores them in the liver cells (*Reid et al.,* 1979; *Smith et al.,* 1997). There is increasing evidence that choline plays a central role in fat mobilization of dairy cattle (*Bell,* 1981; *Zeisel,* 1988). Recent studies (*Piepenbrink and Overton,* 2000; *Pinotti et al.,* 2002) suggest that high-producing dairy cows may be choline deficient around parturition, which leads to decreased liver functions, and especially to the impaired synthesis and secretion of VLDL. Choline supplementation given in unprotected form is degraded quickly by rumen microbes, and thus very limited amount of choline is available postruminally in the cow (*Neill,* 1979; *Sharma and Erdman,* 1988). Therefore, choline supplementation for ruminants should be given in rumen-protected form. There are several techniques to protect choline from ruminal degradation, which are the know-how of the producers. These methods provide different levels of protection, which determine the effectiveness of the product. Therefore, there is a need for a method to study the ruminal stability of different products. The aim of the experiment reported here was to develop and study a cost-effective method of measuring the ruminal stability of different choline sources.

MATERIALS AND METHODS

The rumen stability of the following five products was studied by the *in situ* method of *Ørskov and McDonald* (1979), originally developed for determining protein degradation in the rumen.

— Choline chloride: Not rumen-protected form, containing 50% choline chloride (Bóly Rt., Bóly, Hungary)

— Procol-25®: Rumen-protected product by coating a mixture of fatty acids, containing 25% choline chloride (Provimi Italia, Milan, Italy)

— Norcol-25®: Rumen-protected product by coating a mixture of fatty acids, containing 25% choline chloride (Throw Nutrition Italy, Bussolengo, Italy)

— Sintocol-25®: Rumen-protected product by coating a mixture of fatty acids, containing 25% choline chloride (Sintofarm SpA, Guastalla, Italy)

— Reashure®: Rumen-protected product by coating a mixture of fatty acids, containing 25% choline chloride (Balchem Co., New Hampton, NY, USA)

Five g of the five products were weighed into 10 x 12.5 cm size nylon bags of 40 µm mesh size. Triplicates of the samples were incubated in the rumen of

three rumen-canulated ewes (2 years old, Merino x Texel crossbred) for 0, 2, 4, 8, 16, 24 and 48 hours. The samples were placed into the rumen at 7.00 hours a.m. The ewes were housed in individual stalls during the experiment and were fed 0.5 kg ewe concentrate per day and *ad libitum* grass hay. The concentrate was offered in two equal doses at 6.00 and 18.00 o'clock; animals had continuous access to hay and drinking water. The ingredient and nutrient composition of the daily ration is summarized in *Table 1.*

Table 1.

Ingredient and nutrient composition of the daily ration

Daily intake, kg/day as fed(1)	
Grass hay(2)	1.5
Ewe concentrate*(3)	0.5
Nutrient composition, g/kg DM(4)	
DM(5)	893.0
CP(6)	127.5
NDF(7)	582.0
ADF(8)	353.0
Ether extract(9)	32.0
NEm, MJ/kg(10)	5.7
Ca	6.7
P	3.3
Mg	3.0
Daily nutrient intake, g/day(11)	
DM(5)	1786.0
CP(6)	227.7
NDF(7)	1039.5
ADF(8)	630.5
Ether extract(9)	57.2
NEm, MJ/kg(10)	10.2
Ca	12.0
P	5.9
Mg	5.4

* Ingredient composition: 56.0% gound corn, 10.0% wheat flour, 5.0% wheat bran, 15.0% alfalfa meal, 8.0% sunflower meal, 2.2% limestone, 1.0% salt, 0.6% monocalcium-phosphate, 1.2% urea, 1.0% vitamin and mineral premix; Nutrient composition: 88.0% DM, 15.8% CP, 16.4% NDF, 8.7% ADF, 9.7% ash, 1.12% Ca, 0.53% P, 0.11% Mg, 6500 IU Vitamin A/kg, 3100 IU Vitamin D/kg, 10.3 mg Vitamin E/kg, 220 mg/kg of Fe, 110 mg/kg of Zn, 45 mg/kg of Mn, 2.56 mg/kg of Co, 0.54 mg/kg of I, 0.28 mg/kg of Se(12)

1. táblázat: A napi takarmányadag összetétele és táplálóanyag tartalma
napi takarmányfelvétel, kg/nap eredeti nedvességgel(1), réti széna(2), anyajuh táp*(3), táplálóanyag összetétel, g/kg szárazanyag(4), szárazanyag(5), nyersfehérje(6), neutrális detergens rost(7), savdetergens rost(8), nyers zsír(9), létfenntartó nettóenergia, MJ/kg(10), napi táplálóanyag-felvétel, g/nap(11), *alapanyag-összetétel: 56,0% kukoricadara, 10,0% búzadara, 5,0% búzakorpa, 15,0% lucerna liszt, 8,0% extrahált napraforgó dara, 2,2% mész, 1,0% só, 0,6% monokalcium-foszfát, 1,2% karbamid, 1,0% vitamin és mikroelem premix; táplálóanyag-tartalom: 88,0% szárazanyag, 15,8% nyersfehérje, 16,4% neutrális detergens rost, 8,7% savdetergens rost, 9,7% hamu, 1,12% Ca, 0,53% P, 0,11% Mg, 6500 NE vitamin A/kg, 3100 NE vitamin D/kg, 10,3 mg vitamin E/kg, 220 mg/kg Fe, 110 mg/kg Zn, 45 mg/kg Mn, 2,56 mg/kg Co, 0,54 mg/kg I, 0,28 mg/kg Se(12)

A modification of the method of *Ørskov and McDonald* (1979) was used: instead of thorough washing after incubation the samples were cleaned from the rumen content by dipping the bags tenderly in a bucket of cold water several

times, to minimize the loss of choline during the cleaning process. The bags were dried at 40 °C for 48 h. The use of a relatively low temperature during drying is explained by the high fat content of the samples, which would melt at higher temperatures. Choline content of the samples was determined indirectly via nitrogen analysis by the Kjeldahl method (*Helrich,* 1990), because the samples did not contain any nitrogen source other than choline and probably some microbial protein from the ruminal fluid. The predicted degradability of choline was calculated from the equation $p=a+b(1-b^{-ct})$ (*Ørskov and McDonald,* 1979). Where *a* is the immediately soluble fraction at 0 hour incubation, *b* is the difference of total degradable fraction and fraction *a*, *c* is the rate of degradation of the fraction *b,* and *t* is time of incubation. *Nocek and Russell* (1988) reported, that the ruminal passage rate of small feed particles are 0.12–0.15 unit/hour in an intensively fed high producing cow, therefore predicted choline degradability were calculated for 12%/hour ruminal passage rate.

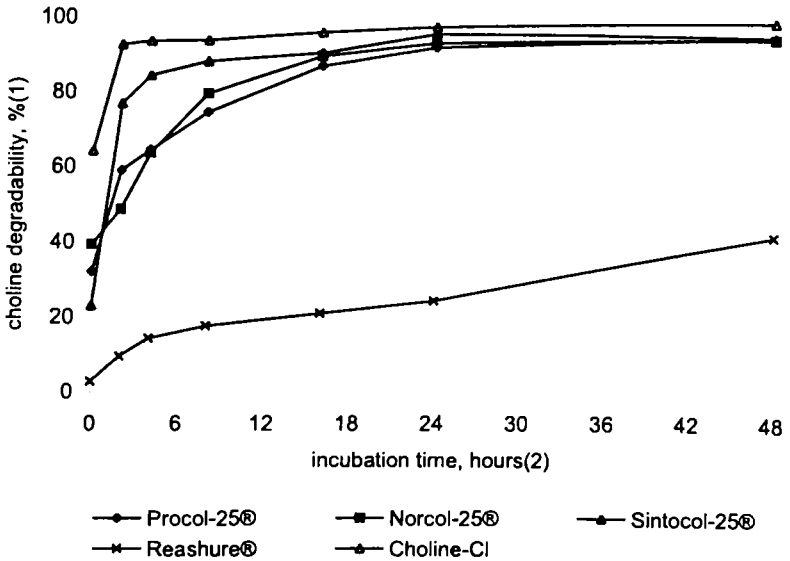
The experiment was approved by the Animal Use and Care Administrative Advisory Committee of Municipal Veterinary Service for Animal Protection and was in agreement with the Ethical Codex of the Hungarian Association for the Animal Care of Laboratory Animals (protocol number: 210/1/2003).

The data were analyzed by analysis of variance (ANOVA) using the SPSS 9.0 programme package. Means were compared post hoc by Tukey's multiple comparison test (*Shott,* 1990) at every incubation time and homogeneous subsets were formed. The level of significance was set at $P<0.05$.

RESULTS

Large differences could be detected among the products already at the beginning of the incubation period (*Fig. 1. and Table 2.*). The major proportion of the non-protected choline dissolved during the tender dips of the bags and 92.7±4.3% of it was degraded during the first 2 h of incubation. All the four rumen-protected choline preparations tested shoved significantly ($P<0.05$) higher ruminal stability than the non-protected form, but differences could be observed in degradation characteristics especially at the beginning of incubation. Sintocol-25® degraded significantly ($P<0.05$) faster then the other three rumen protected products. There were no significant differences between Procol-25® and Norcol-25® during the whole period of incubation. These two products had significantly ($P<0.05$) higher ruminal stability than Sintocol-25® after 2, 4, 8 and 16 hours of incubation. These differences disappeared between Procol-25®, Norcol-25® and Sintocol-25® after 24 hours of incubation, because 91.95±0.4, 93.18±0.3 and 95.54±0.9% of their choline content, respectively, went into the solution. Reashure® was found to show the highest stability in the rumen of sheep, because significantly ($P<0.05$) less choline was degraded from it than from any other products after each incubation.

Fig. 1.: **Degradation characteristics of the examined choline sources in the rumen**



1. ábra: A vizsgált kolinforrások bendőbeli lebomlási üteme
kolin lebonthatóság, %(1), inkubációs idő, óra(2)

Table 2.

Choline degradability (%) of choline products after different incubation times in the rumen of sheep

Incubation time, hours(1)	Choline-Cl	Procol-25®	Norcol-25®	Sintocol-25®	Reashure®	SE
0	64.32^{a}	32.26^{b}	39.45^{b}	23.14^{c}	2.87^{d}	4.28
2	92.71^{a}	59.15^{b}	48.81^{b}	76.98^{c}	9.69^{d}	4.79
4	93.67^{a}	64.53^{b}	63.64^{b}	84.56^{c}	14.43^{d}	2.98
8	93.81^{a}	74.60^{b}	79.66^{b}	88.25^{c}	17.71^{d}	2.82
16	95.98^{a}	86.99^{b}	89.69^{b}	90.52^{b}	21.02^{c}	1.88
24	97.49^{a}	91.95^{b}	93.18^{ab}	95.54^{ab}	24.39^{c}	1.71
48	99.37^{a}	95.10^{a}	94.85^{a}	95.46^{a}	41.99^{b}	2.25
Predicted degradability at 12%/h ruminal passage rate*(2)	88.92^{a}	69.42^{b}	69.51^{b}	83.29^{c}	13.85^{d}	2.78
C**	0.998^{a}	0.179^{b}	0.140^{b}	0.588^{c}	0.047^{d}	0.049

Means with different superscripts within a row differ (P<0.05)(3)
* Prediction is based on the *Ørskov and McDonald* (1979) equation $p=a+b(1-b^{-ct})$(4)
** Rate of degradation of fraction *b*(5)

2. táblázat: Kolin termékek bendőbeni lebonthatósága (%) juhban, különböző inkubációs idő esetén
inkubációs idő, óra(1), *várható lebomlás 12%/óra bendőpasszázs mellett(2), az eltérő betűjellel ellátott értékek a sorokon belül szignifikánsan különböznek (P<0,05)(3), *a becslés az *Ørskov és McDonald* (1979) egyenleten alapul $p=a+b(1-b^{-ct})$(4), **lebomlási sebesség(5)

DISCUSSION

In their experiment, *Nocek and Russell* (1988) proved that the ruminal passage rate of small feed particles was 0.12–0.15 unit/hour in an intensively fed high producing cow. Based on this finding, rumen-protected products are exposed to microbial fermentation for 7–9 h; therefore, the choline content of samples after 8 h of incubation reflects the actual ruminal stability of the products. Only the rumen-protected products contained considerable amounts of choline after 8 h of incubation, and therefore only these products can be used effectively for supplementing the diet of a high-producing dairy cow. About 20–25% of the original choline content of Procol-25® and Norcol-25® and 10–15% of Sintocol-25® may reach the duodenum, where it has a chance to be absorbed. The ruminal stability of Reashure® was the highest, because more than 80% of its original choline content remained available for duodenal absorption.

The *in situ* method — developed for studying the ruminal degradation characteristics of protein sources — seems to be suitable for routine comparison of the ruminal stability of rumen-protected choline products following the modification of the washing phase. The better ruminal stability unfortunately does not prove the better bioavailability of a product, because even if choline is successfully protected from ruminal degradation, it still must be released and subsequently absorbed in the intestine. Free choline can be fermented by the bacteria in the small and large intestine (*Deuchler et al.*, 1998), therefore the fecal choline content does not reflect necessarily the bioavailability of choline, which should be the subject of further investigation.

ACKNOWLEDGMENT

The technical support of the staff of the Department of Animal Science, Georgikon Faculty, at the University of Pannonia was much appreciated.

REFERENCES

Bell, A.W.(1981): Lipid Metabolism of Ruminant Animals. Pergamon Press, Oxford, England, 363–410.

Bell, A.W. – Sleptis, R. – Ehrhardt, R.A.(1995): Growth and accretion of energy and protein in the gravid uterus during late pregnancy in Holstein cows. J. Dairy Sci., 78. 1954–1961.

Deuchler, K.N. – Piperova, L.S. – Erdman, R.A.(1998): Milk choline secretion as an indirect indicator of postruminal choline supply. J. Dairy Sci., 81. 238–242.

Helrich, K.(1990): Association of Official Analytical Chemists: Official Methods of Analysis. *Padmore, J.M.*: 4. Animal feed. 15th ed. AOAC, Arlington, VA., 69–88.

Neill, A.R.(1979): The low availability of dietary choline for the nutrition of sheep. Biochem. J., 180. 559–565.

Nocek, J.E. – Russell, J.B.(1988): Protein and energy as an integrated system: relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci., 71. 2070–2107.

Ørskov, E.R. – McDonald, I.(1979): The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. J. Agric. Sci., Cambridge 92. 499–503.

Piepenbrink, M.S. – Overton, T.R.(2000): Liver metabolism and production of periparturient dairy cattle fed rumen-protected choline. J. Dairy Sci., 83. Suppl. 1., 257.

Pinotti, L. – Baldi, A. – Dell'Orto, V.(2002): Comparative mammalian choline metabolism with emphasis on the high-yielding dairy cow. Nutr. Res. Rev., 15. 315–331.

Reid, R.H. – Collins, R.A. – Baird, G.D. – Roberts, C.J. – Simmons, H.W.(1979): Lipid production rates and the pathogenesis of fatty liver in fasted cows. J. Agric. Sci., 93. 253–261.

Sharma, B.K. – Erdman, R.A.(1988): Effects of high amounts of dietary choline supplementation on duodenal choline flow and production responses in dairy cow. J. Dairy Sci., 71. 2670–2676.

Shott, S.(1990): Statistics for Health Professionals. W. B. Saunders Co., Philadelphia, PA.

Smith, T.R. – Hippen, A.R. – Beitz, D.C. – Young, J.W.(1997): Metabolic characteristics of induced ketosis in normal and obese dairy cows. J. Dairy Sci., 80. 1569–1581.

Van den Top, A.M. – Wensing, T. – Geelen, M.J.H. – Wenting, G.H. – Want'Kloster, A.T. – Beenen, A.C.(1995): Time trends of plasma lipids and hepatic triacylglycerol synthesizing enzymes during postpartum fatty liver development in dairy cows with unlimited access to feed during the dry period. J. Dairy Sci., 78. 2208–2220.

Zeisel, S.H.(1988): Modern Nutrition and Health and Disease. Lea and Febiger, Philadelphia. 440–452.

Érkezett: 2007. március

Szerzők címe: *Elek, P.:* AGROKOMPLEX C. S. ZRt.
AGROKOMPLEX C.S. Ltd.
H-8112 Zichyújfalu, Pf.: 1.
Husvéth, F.: Pannon Egyetem, Állattudományi és Állattenyésztéstani Tanszék
Department of Animal Science, Georgikon Faculty of Agriculture
H-8360 Keszthely, Deák F. u. 16.

Authors' address: