STSM – Short scientific report

Dr Mislav Didara
University of Josip Juraj Strossmayer
Faculty of Agriculture in Osijek
Kralja P. Svacica 1D,
31000 Osijek Osijek
Croatia

Subject: Short Term Scientific Mission – Short scientific report
Reference COST Action FA1308
Reference code: COST-STSM-ECOST-STSM-FA1308-150115-051396

Location: Nutrition Sciences N.V., Drongen, Belgium
Host:
Dr Geert Bruggeman
Nutrition Sciences N.V.
geert.bruggeman@nutrition-sciences.com
Period: 15/01/2015 to 28/02/2015

Project name:
Development of a rumen model to measure by-pass protein and improve dairy care

Purpose of the STSM:

Our goal was to build a model for *in vitro* assessment of rumen by-pass fat coated/encapsulated products digestibility. We adapted models of Calsamiglia/Cargallo (*in vitro* protein digestion) and the RUSITEC model of rumen simulation.

Description of the work carried out during the STSM:

Our goal was to build a model for *in vitro* assessment of rumen by-pass fat coated/encapsulated products digestibility. We adapted models of Calsamiglia/Cargallo (*in vitro* protein digestion) and the RUSITEC model of rumen simulation. Our model is based on 3 steps: 1. Rumen fluid simulation, 2. Gastric fluid simulation and 3. Intestinal fluid simulation. In vitro protein digestion model had to be modified because it lacked bile as a component of a model. Bile contains bile acids which are essential for normal digestion of

fat. Small amount (0.5 g) of bypass fat product or feed was placed inside nylon bags. Bags were sealed, weighted and incubated. After incubation bags were freezdried and weighted again, the difference in weight was used to calculate digestibility. Soxhlet analyses were done to confirm freezdry weight from pure fat products. Commercial substitute for bile, which contained some of the bile acids necessary for fat digestion, and dried complete bovine bile (Sigma-Aldrich, Germany) were used in the trial. Higher *in vitro* intestinal digestion was determined using bovine bile so model was build up using bovine bile. Optimal digestibility was determined when using bovine bile at 10 g/L concentration in comparison to 5 or 15 g/L of bovine bile. Optimal concentration of pancreatin was set to 3.0 g/L, concentrations of 1.5 g/L and 4.5 g/L showed no significant difference. Better digestibility was determined at pH 7.8 compared to the pH 5. During intestinal phase digestibility was determined at 8, 16, 24 and 32 hour intervals. Gastric fluid simulation was done using pepsin and 0.0125 M HCl, pH adjusted to 1.9 during 1 hour. Using the soybean meal as a protein source for digestion validity of method was tested. Digestion of protein source did not differ significantly from Calsamiglia/Cargallo paper. To test the method for fat digestion number of fat rich feed and commercial bypass fat products have been used: coconut oil, coconut powder, linseed, sunflower seed, brasilian nut, toasted soybean, Ca soap, bypass palm oil, fat coated bypass selenium, fat coated bypass choline chloride. For the rumen fluid simulation rumen fluid from the three canulated sheep was used. Rumen fluid was diluted with the sheep artificial saliva acting as buffer. Minifors benchtop bioreactor was used to simulate rumen digestion. Anaerobic conditions were achieved by flushing the mixture of rumen fluid and the buffer with the CO_2. Bioreactor was airtight sealed so no air could come in and the excess of gas was evacuated with the use of waterlog. Temperature was constantly kept at 39˚C. pH was monitored and adjusted using the NaOH and HCl. Second step lasted for 1 h, and included incubation in 1 g/L pepsin and 0.0125 M HCl at pH 1.9. Third step, the intestinal phase was done either for 8 or 24 h using the 3 g/L of pancreatin and 10 g/L of bovine bile. Starting pH was adjusted at 5 using the phosphate buffer. In both cases pH was gradually increased from 5.0 to 7.8 during the 2 or 6 h time period depending on the total intestinal phase time 8 or 24 h respectively. In this way we tried to simulate *in vivo* conditions as ruminant intestine pH is lower for a longer time in comparison to the monogastric animals. Three step in vitro fat digestion method was compared with the *in vivo* dairy cow trials with some rumen bypass products. Good correlation was find both after

rumen digestion step and intestine digestion step. In that matter we can conclude that three step *in vitro* model for digestion of fat works with rumen bypass fat products. Adding bovine bile improves model of three step protein digestion and makes it more appropriate for the in vitro assessment of digestibility of rumen by-pass fat products.

Conclusion:

Three step *in vitro* model for digestion of fat works with rumen bypass fat products. Adding bovine bile improves model of three step protein digestion and makes it more appropriate for the in vitro assessment of digestibility of rumen by-pass fat products.

Future collaboration with the host institution:

The collaboration with the host institution was very successful. Based on the successful collaboration during this short term scientific visit, the host and guest institutes are willing to continue the collaboration. Future exchange of experts working in the host institution Nutrition Sciences N.V., Belgium and the applicant's institution Faculty of Agriculture in Osijek, Croatia is arranged.

Foreseen publications/articles resulting from the STSM:

Publication of the results and a model for in vitro assessment of rumen by-pass fat coated/encapsulated products digestibility in one of the European peer-review journal will be considered. The results will also be presented at one of the upcoming DairyCare FA-1308 meetings.

Applicant

Dr.sc. Mislav Didara

February, 28th 2015.

Mislav Didara