Не виконання технологічних вимог утримання свинопоголів'я, невчасне виявлення свиноматок в охоті та осіменіння їх з порушенням зоотехнічних вимог, годівля свиней незбалансованими кормовими сумішами – призводить до зниження середньодобових приростів та кількості опоросів на 1 свиноматку в рік, підвищення технологічного відходу, а в цілому по селекційній піраміді до додаткового утримування 15923 основних свиноматок (+24,8 %), збільшення операторів на 504 особи (+10,6 %), щорічної перезатрати 96,3 тис тонн комбікормів (+18,9 %) та зниження рівня рентабельності галузі на 16,8 %.

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BULGARIAN RESEARCH PROGRAM FOR REDUCING METHANE PRODUCTION FROM RUMINANTS THROUGH INNOVATION IN THEIR NUTRITION

Mariana Petkova
Institute of Animal Science Kostinbrod 2232, Bulgaria

The paradox of modern living is that that the better the living conditions of people are becoming the more the risks to their health. The processed of urban development, economic expansion & consumption all pose threats to our environment. It is not accidental that "the Earth's vital signs are in a danger zone: human population and consumption stretch the capacity of the planet "Scientists' worldwide alarm about global warming and the necessity to prevent global climate catastrophe from the last century. It is now well recognized that the rising levels of greenhouse gases (GHG) in the atmosphere and the forecasts for the consequences that may result in this process are very alarming. It is clear that all countries in the world should unite their efforts in helping to save the planet. This has led to the signing of the Kyoto Protocol. Now GHG emissions are a global issue receiving a lot of attention. Animal scientists need to be aware of this issue.

Interdisciplinary and multidisciplinary aspects of the bulgarian research program

The problem of greenhouse gases is a well known issue now. The global release of methane from agricultural sources accounts for two-thirds of the anthropogenic CH_4 sources. Due to their population size, ruminants are the largest present-day contributor to GHG emissions (80 million tonnes of methane annually). Ruminal fermentation is not a very efficient process (at least from a biochemical point of view) and results in several by-products considered as waste, mainly carbon dioxide (CO_2) and methane (CH_4). Methane is a potent GHG and domestic ruminants have been blamed by many for a large

portion of the global GHG emissions, thus having a significant impact on climate change. Methane in the rumen is produced by microbes from the *Archaea* group, commonly referred to as methanogens. The other end by-products in the rumen, carbon dioxide and hydrogen (H_2), are combined to form CH_4 and water (H_2O) in the following equation: $2CO_2 + 4H_2 = CH_4 + 2 H_2O$. The animal cannot utilize CH_4 and it is eructated or exhaled with some of the CO_2 and other rumen gases that are produced in the reticulo-rumen. Emissions of CH_4 from feeds can account for 2–15 % of the animal's gross energy intake and it is a loss of net energy for the animal.

Thus, not only is methane a GHG, but it also represents an energy loss of feed gross energy from the animal. Methane producing bacteria in the rumen use metabolic hydrogen produced during microbial fermentation, which ensures that it does not accumulate in the rumen and stop the fermentative process. How much methane is released depends on the amount of H_2 produced and the supply of alternative acceptors that serve as traps. The rumen fermentation pattern has great influence on the balance between H_2 produced and the amount of available H_2 traps. The diet has a great potential to influence hexose partitioning. Acetic and butyrate production promotes H_2 formation and consequently CH_4 production, whereas propionate is a net trap of H_2. The molar proportion of the various volatile fatty acids produced in the rumen may provide some insight into the extent of ruminal CH_4 production, but direct measurement of methane emissions are far more precise at estimating emissions.

There is still a large potential for increased global warming from CH_4 due to its two main characteristics: 1. It has great potential to contribute to global warming because 1 ppm released into the atmosphere increases its temperature by 0.2°C. 2. Methane has high warming potential (25 times CO_2) and short atmospheric lifetime (12 years).

Previous studies in this field demonstrate that animals create 130 times more waste products than people and ruminant animals (particularly cattle, buffalo, sheep, goat and camels) produce CH_4 under the anaerobic conditions of the digestive processes. There have been attempts to quantify methane emissions from ruminants: mature dairy cow typically produces 80–110 kg/year or 185 g/head/day or 250–500 l/day or 21g/kg DMI or presented as MJ/kg ECM or as g CH_4/ ha. Wild ruminants (bison, elk, caribou, deer, sheep) produce about 0.19-0.37 Tg/yr (1 teragram = 10^{12} grams) of methane, which on average comes to about 49 g of methane (or about 1 kg of CO_2 Eq.; the global warming potential of GHG is expressed as CO_2 equivalents/animal/d). By way of comparison, an average car emitted between 15 and 22 kg of CO_2/d in 2010.

Significant progress has been made in studies on this problem in the past 20 years. These were focused primarily on: (1) Contribution of livestock to GHG emissions; (2) Measuring emissions; (3) Potential for mitigation; (4) Whole farm modelling; (5) Future perspectives. Many factors with a specific and principle effect were clarified. Today it can be concluded with certainty that there will be real results with respect to reducing the reduction of the greenhouse gases CH_4, CO_2, NO_2 from ruminants, if the diet allows for low degrad-

ability of nutrients in the rumen and subsequent higher digestibility in the intestines and digestive tract as a whole. Moreover, feed evaluation today has new content: environmental aspects. Hence, one of the main tasks of the proposed program in the time ahead will be to chart methane emissions based on different Bulgarian feed rations. The first step will be to measure cow's methane emissions. As today there is not yet a "best" method (accurate, fast, and cheap, with sustainable results) the final point of the research program will be to determine the most appropriate method to measure GHG emissions and to find a balance between feeds and emissions as well as to associate all parameters in one model that contribute to improving the results of feeding of ruminants, in other words to develop a whole-farm greenhouse gas emissions model. The production of methane and the secretion of nitrogen in ruminant manure depend on the feed the animals are given. Other aspects, such as how the manure is handled, will also influence the total quantity of methane released in the cowshed.

In our research program, through application of current and new methods for determining the production of methane from ruminants, in conjunction with establishing the actual effect of various factors on methanogenesis, we will make practical suggestions to reduce methane production and propose innovations that will bring useful economic efficiency on this problem. The results may have added value in improving the feeding strategy for ruminants to reduce methane emissions into the environment at global and regional level.

As it is demonstrated later, our research program represents an interdisciplinary approach involving studies and knowledge in several fields of agriculture and animal's biology and husbandry: plant production, environmental science, ruminant physiology, microbiology, chemistry, biochemistry, biotechnology, food science and technology, economical science.

The created research program is both analytically and experimentally based and as such, relies on analytical feasibility and sample availability. As detailed below, we have experience in all of the analytical methods proposed. We will focus on recently collected samples from the *in vivo* experiments on digestibility with different sources of protein and waste feeds from the processing of oil crops-ethanol processing, which were carried out in Bulgaria and additional samples from the main feedstuffs used and available in Bulgaria. Moreover, we have an extensive samples collection with undegradable protein (UDP) after rumen incubation ready for incubation in the following sectors of the digestive tract. These samples present an exceptional opportunity for new evaluation of feeds with environmental aspects. Methane production can be measured both *in vivo* and *in vitro.* The present program will employ four methods. The individual animal technique respiration chamber [6] is a research approach, which will be used as a gold standard. The computer system directly accounts O_2, CO_2, CH_4 and heat production. The most common tracer technique with sulphur hexafluoride (SF_6 tracer method) gas as a tracer for CH_4 will be applied. This method involves the use of permeation tube containing SF_6 placed into the rumen and the gas samples are collected from around the nose and mouth of the animal via a harness and evacuated PVC canister.

These samples are then analysed using gas-chromatograph with electron capture detector (ECD). This technique and method of estimation CH4 emissions have the advantage that both individual grazing animals groups of animals can be measured simultaneously. In addition it is a relatively cheap method for measurement of methane emissions. It is also a safe – method: SF6 is a colourless, odourless, non-toxic and non-flammable gas. Both *in vitro* gas production techniques will be applied in our study. The RUSITEC technique is a simple, but special technique that attempts to simulate fermentation in the rumen. The equipment contains several fermenters. The fermenters are filled with filtered rumen fluid collected from cows and artificial saliva is continuously infused. The feed samples under investigation feed for the microbes are contained – placed in nylon bags. The nylon bags usually remain in the fermenters for 48 h before they are replaced. Gas sample from the bag go on with analyzing for CH_4 by gas chromatograph. Several diets simultaneously in replicated fermenters could be measured for methane production. The principle of the *in vitro* batch culture method, known as *in vitro* gas production technique (IVGPT), is incubation of the substrate in bottles containing buffered rumen fluid (i.e. McDougall buffer) which is combined with the water displacement technique. The fermentation products methane, VFA, NH_3 etc. are measured over a relatively short period of time. Batch culture incubates make it possible to conduct many treatments and replicates in parallel.

A tool to estimate and reduce greenhouse gases from farms is the HOLOS model. The present program intends to master and employ this successful model, which includes: Canadian methodology for enteric CH_4 estimation and total CO_2 eq, as well as recent research findings (NRC nutrition requirements and Basic Structure) (IPCC Tier 2). Therefore, this program culminated in the development of “Holos”, a model that enables the GHG emissions from a single farm to be estimated from a systems perspective.

Our program also emphases on certain methane prediction models: static empirical; dynamic empirical; dynamic mechanistic etc. To this end the methodology of the updated EMEP/CORINAIR will be used, which in the aspects that concern the estimation of greenhouse gases emissions adopts the IPCC revised Guidelines. For the estimation of NH_3 emissions we will apply the methodology of the EMEP/EEA.These methodologies have been selected among others as they are internationally approved emission inventory systems and it has been shown – especially for estimating livestock emissions – that they score highly in transparency, consistency, completeness, comparability and accuracy, the general criteria an improved emission inventory system ought to have. Moreover, nowadays international and national scientific efforts tend to unify methodologies used.

Thus, having used of all the above techniques and methods, the main challenge will be to find a simple measurement technique of measuring the quantity of methane emission. Every research approach in this program is a team approach finally.

While it has been considered that feed manipulations are essential for the rumen fermentation pattern to and could decrease methane production by

10–15 % it is presently unclear which is the more balanced nutrition from the environmental point of view with emphasis on intensive production systems. Because of before the animals begin to produce; we must give feeds in certain combinations which will meet their requirements. Addressing this question for feeding strategy for reducing methane is critical since many of the world's feed resources are with specific composition and content.

Previous experience, sampling and analyses of feeds and rumen fermentation pattern have provided surprising results on the relationship between nutrition and environmental pollution. For instance, the separation of feed evaluation into systems for energy and systems for protein (and minerals) is not very suitable where the aim is to achieve reduction in methane production. More attention must be paid to the use of low rumen degradable protein concentrates. In this respect, feeds with relatively low rumen starch degradability have a contradictory effect on the nitrogen balance. More studies on N balance as a dietary efficiency factor need to be carried out because it is not clear what the N redirecting is between urine and faces when the N intake with diets is low. In this program, through the study of different diet conditions (more concentrates, improved forage quality, more by-pass starch, fats and oils, secondary plants metabolites, feed additives), we will provide new evidence to define the essential role of feed manipulations for methane production. Specifically, we will address the following original hypotheses:

Hypothesis 1: Dietary manipulations can provide unique potential to reduce methane emissions and to mitigate greenhouse gases emissions as a whole.

Rationale: It is considered that two nutritional strategies have the greatest impact on degradation in the rumen and digestibility in the intestines and thus on the production of methane: Strategy I. Increasing dry matter intake (DMI); and Strategy II: Increasing the proportion of concentrates in the diet. The first one is based on the principle that changing the DMI alters the rumen fermentation and digestion process. The proportion of methane (MJ/d) increases with increasing the feeding level, but when expressed in percentage of Gross Energy, it decreases. Nutritional options for changes in DMI not only affect the amount of substrate available for microbial degradation, but also changes fermentation conditions and the size of the microbial population. For example, the fate of ingested starch changes with changes in the amount of dry matter ingested, as increased intake levels will lead to a proportionally higher amount of starch digested in the small intestine rather than fermented in the rumen. According the second strategy methane production changes in a curve-linear way or is only slightly reduced. There is lack of quantitative knowledge on the degradation and passage behaviour of starch in the digestive tract of dairy cows, both in terms of site and place of digestion and in terms of VFA profile. This is considered as a research area that deserves high priority, particularly as regards starch in maize silage. Aspects, which need to be considered, are the storage of starch by micro-organisms with increasing concentrations in the rumen, or an altered passage rate which alters the time available for microbial degradation. The effects of the feed intake level, how-

ever, are strongly confounded with most of the factors. A strict interpretation of these relationships awaits measurement, which is a major goal and will be one of the major novelties of this program.

Once more, experiments are needed to conclusively decide on the best mitigation options for the farmer in different regions of the world. Exchange of information between countries, is essential for improving our understanding of climate change and emissions of greenhouse gases.

Approach: An integrated approach in which an efficient animal production is combined with limited nutrient losses to the environment is needed. This requires integrated systems, preferably based on nutrients rather than energy and protein. As a first approach the nutrients can be separated in groups such as ketogenic, aminogenic, glucogenic nutrients. The second approach is to deliver the nutrients in a required ratio to the animal. This ratio can be manipulated by shifting the digestion, notably that of protein and starch, from the forestomach to the small intestine. A further manipulation is considering the rumen. The present program emphasises on the next challenges: Roughage based milk and meat production will probably be even more important in the future. Consequently, research to minimize emission of CH_4 may focus on the factors related to roughage, among others: replacing grasses partly with legumes; improved pasture management; substituting a part of grass silage with maize- and cereal silages; impact of forage maturity; forage with tannins.

As shown before, the hexose partitioning in the rumen could be influenced by some external factors. The program addresses fundamental non-nutritional questions on this subject, which have not been solved so far by applying conventional approaches and combined with biotechnology and microbiology. They will be answered by studying various factors on methanogenesis that are at the cutting edge of modern ruminant nutrition.

Hypothesis 2: Redirection of rumen fermentation can provide unique chances to reduce methane loss in cattle.

Rationale: Research studies carried out in this field so far have convincingly demonstrated that significant results are achieved in the manipulation of methane production in the rumen by redirecting the fermentation process. Firstly, it is clear that the conditions in the rumen are very unstable. They are created for short periods and the ecology of the system is such that it frequently reverts back to the initial levels of fermentation though a variety of adaptive mechanisms. However, the task to create sustainability in the rumen is made particularly difficult since the rumen contains many different types of microbes and any inhibitor needs to be specific in its mode of action. If ruminal fermentation patterns are shifted from acetate to propionate, both hydrogen and methane will be reduced. But the conditions in the rumen strongly favour methanogenesis over acetogenesis. If acetogenesis could be promoted at the expense of methanogenesis this could result in a greater supply of acetic acid and an improved energy supply to the animal. More experiments could be carried out on this point of view. Secondly, hydrogen flow in the rumen can be modelled stoichiometrically, but accounting for H_2 by direct measurement of reduced substrates often does not concur with the predictions of stoichiometric

models. Clearly, substantial gaps remain in our knowledge of the intricacies of hydrogen flow within the ruminal ecosystem. Further characterisation of the fundamental microbial biochemistry of hydrogen generation and methane production in the rumen may provide insight for development of effective strategies for reducing methane emissions from ruminants. There has been n investigation yet, which not only covers energy efficiency, but also the extent of feed digestions.

Approach: The hypothesis will be tested using the following integrated approach: (1) establishment of biodegradable and digestible feeds and rations characteristics; (2) investigation of the rumen fermentative pattern under a wide range of conditions (feedstuffs, diets). Rumen degradability will be compared with intestinal (duodenal, ileal) digestibility. In addition, ruminal fermentative parameters NH3, VFA, pH will be assessed. Special attention will be paid to the shift of rumen fermentation from an acetate dominated profile of VFA to a propionate dominated one. Besides might shift the excretion of N from the faces to urine. Also, the fatty acids profile of milk will be assessed as an alternative tool to monitor rumen fermentation, including CH4 production; (3) examine *in vitro* CH4 and CO2 productions under wide range of conditions (feedstuffs, diets). The validity of the results will be assessed; (4) redirection of rumen fermentation to reduce methanogenesis will be validated. A new approach, based on using cell fractions as opposed to whole cells, will be used; (5) finally, the contribution of the all these parameters aiming to reduce the emission of methane and other GHG from dairy cows should be integrated and implemented at farm level rather than at animal level. The current HOLOS model offers sufficient scope to evaluate methane mitigation options.
М. ПЕТКОВА

Викладено основи забруднення навколишнього середовища жуйними та шляхи можливого скорочення метанових викидів.

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EFFECT OF FEEDING WHEAT DDGSS TO WEANED PIGS ON PERFORMANCE AND BLOOD SERUM CHOLESTEROL

Maia Ignatova, Maria Todorova
Institute of animal science – Kostinbrod, Bulgaria

The objective of this experiment was to study the effect of inclusion of 20 % wheat dried distillers grain with solubles in diet on weaned pig performance and blood cholesterol concentration.

Twenty six weaned pigs cross-breed Youna (initial body weight 13,8 ± 0,06 kg) with two replicates were randomly allocated to two experimental