Journal of Zhejiang University SCIENCE
ISSN 1009-3095
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

Review:

Acute phase reactants, challenge in the near future of animal production and veterinary medicine*

GRUYS E.[†1], TOUSSAINT M.J.M.[1], UPRAGARIN N.[1,2], VAN EDEREN A.M.[1], ADEWUYI A.A.[1], CANDIANI D.[1,3], NGUYEN T.K.A.[1,4], SABECKIENE (BALCIUTE) J.[1,5]

([1]*Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands*)

([2]*Department of Farm Resources and Production Medicine, Faculty of Veterinary Medicine, Kasetsart University, Nakhon Pathom, Thailand*)

([3]*Department of Veterinary Morphophysiology, University of Torino, Italy*)

([4]*Department of Embryo Transfer and Reproduction, the National Institute of Animal Husbandry, Hanoi, Vietnam*)

([5]*Department of Infectious Diseases, Lithuanian Veterinary Academy, Kaunas, Lithuania*)

[†]E-mail: e.gruys@vet.uu.nl

Received July 25, 2005; revision accepted Aug. 26, 2005

Abstract: The future of acute phase proteins (APPs) in science is discussed in this paper. Many functions and associated pathological processes of APPs are unknown. Extrahepatic formation in local tissues needs attention. Local serum amyloid A (SAA) formation may be involved in deposition of AA-amyloid induced by conformational change of SAA resulting in amyloid formation, having tremendous food safety implications. Amyloidogenesis is enhanced in mouse fed beta pleated sheet-rich proteins. The local amyloid in joints of chicken and mammary *corpora amylacea* is discussed. Differences in glycosylation of glycoproteins among the APPs, as has been shown for α1-acid glycoprotein, have to be considered. More knowledge on the reactivity patterns may lead to implication of APPs in the diagnostics and staging of a disease. Calculation of an index from values of several acute phase variables increases the power of APPs in monitoring unhealthy individuals in animal populations. Vaccinations, just as infections in eliciting acute phase response seem to limit the profitability of vaccines because acute phase reactions are contraproductive in view of muscle anabolism. Interest is focused on amino acid patterns and vitamins in view of dietary nutrition effect on sick and convalescing animals.

When inexpensive methodology such as liquid phase methods (nephelometry, turbidimetry) or protein array technology for rapid APP measurement is available, APPs have a future in routine diagnostics. Specific groups of patients may be screened or populations monitored by using APP.

Key words: Acute phase protein, Amino acid, Joint, Mammary gland, Mastitis, Serum amyloid A (SAA), Vitamin A
doi:10.1631/jzus.2005.B0941 **Document code:** A **CLC number:** Q95

INTRODUCTION

The systemic acute phase reaction known to occur on infection, inflammation, trauma, burns, malignancies and tissue damage in general, has been studied by scientists from various disciplines. In the last decade, emphasis has been laid on application of blood tests for acute phase reactants to monitor animal health in general, as well as for human patients suffering from specified classes of diseases (Gruys *et al*., 2005). However, basic mechanistic patterns associated with biological reaction mechanisms, such as local production of acute phase proteins by cells of organs involved in specific physiological mechanisms and disease processes which may be associated with defence functions and with development of, even worse, tissue alteration, still remain to be uncovered.

Precolostrum mammary tissue (McDonald *et al*., 2001) and mastitic mammary epithelium have been

* The paper presented at the 5th International Conference on Animal Acute Phase Proteins, Dublin, Ireland, March 14th~15th, 2005

shown to form mammary serum amyloid A (mSAA) (Vivanco *et al*., 2003; Nguyen *et al*., 2003). Moreover, haptoglobin (Hp) and other acute phase reactants are found in the milk. These factors are supposed to have functions in regulating the inflammatory process and to be beneficial for the enteric milieu of the young mammal including protection of the gut mucosa by mucus formation (Gruys *et al*., 2005).

In birds with chronic arthritis the synovial cells may reveal SAA upregulation, SAA protein formation (Ovelgönne *et al*., 2001; Upragarin *et al*., 2002; 2005a) and amyloid formation (Landman, 1998; Upragarin *et al*., 2005b).

This paper on the expected future developments of acute phase protein (APP) science and APP-related disorders is based on (1) present scientific activities concerning basic mechanistic patterns not discovered sofar, (2) development of technology for quantitative measurement of APPs or the cellular upregulation of their formation, and (3) assessment of disorders, health and welfare with APP values. Some topics in these fields will be mentioned.

BASIC MECHANISTIC PATTERNS

Specific organs

Differentiated reactivity patterns of parenchymal cells in the organ involved, such as mammary gland, depend on locally active cytokines and are unraveled in this paper. Moreover, cell specific factors may be revealed. Just like enzymes known from clinical enzymology, specific cell proteins such as the fatty acid-binding gut protein (Niewold *et al*., 2004), may be discovered and have applications in diagnostics and therapy.

Functional aspects of acute phase reactants in milk and blood need further attention. Knowledge of the activities which activate or just mitigate the inflammatory reaction can lead to new ways for therapy and prevention of inflammatory processes in organs involved, such as lung or mammary gland.

Extrahepatic, local SAA formation (Landman, 1998) and its possible precipitation as amyloid (Upragarin, 2005) as has been found in avian amyloid arthropathy, needs further investigations. From the pathogenetic mechanisms preventive measures may be developed. Once the beta-pleated sheets of amyloid have been formed, the substance has tremendous food safety implications. In murine studies orally administered AA-amyloid appeared to enhance inflammation-/acute phase reaction-induced amyloidosis, where the administered material served as nidus for amyloidogenesis. This indicates that like prions, this pathological material should be banned for risk groups of consumers (Lundmark *et al*., 2002 & 2003). In mammary tissue, colostrum and fresh milk *corpora amylacea* may occur which contain amyloid (described to be derived from casein (Niewold *et al*., 1999)). Recently this amyloid was found to be positive for SAA (Toussaint *et al*., 2005) and this possibly originates from mammary epithelium after in situ hybridization results (Toussaint *et al*., 2005). By others the local formation of mSAA has been mentioned (Larson *et al*., 2005). The amyloidotic corpora should be regarded as unwanted beta pleated factors in colostrum and milk and the relationship to this mSAA is recommended for futher investigation.

Various proteins

In birds acute phase proteins such as (ovo)transferrin appear to have special characteristics differing from those of mammals. In mammals and possibly in avian species as well, some acute phase proteins may reveal differences in glycosylation patterns associated with different diseases and stages of those diseases, as had been shown for feline α1-acid glycoprotein (AGP) (Ceciliani *et al*., 2004; Pocacqua *et al*., 2005). Further analysis may unravel basic biological mechanisms and indicate specificity of the glycosylation patterns for disease and reveal new concepts for therapy.

Reaction of different analytes in various situations, separate and combined

Viral, bacterial, and protozoal infections may be associated with different patterns in cytokine release and acute phase reactivity. Inflammatory processes in internal organs appeared to result in more severe reactivity patterns than diseases of the skin and the enteric system (Alsemgeest, 1994). Specific knowledge of pattern details may lead to implication of the parameters in diagnostics and staging of the disease.

Calculation of an index from values of rapid- and slow-reacting positive and negative APPs had been reported (Gruys and Toussaint, 2001; Gruys, 2002;

Toussaint *et al*., 1995; 2002; 2004; Niewold *et al*., 2003), because it appeared to increase statistical sensitivity and specificity for detecting unhealthy subjects. It covers a broad time span and includes changes in blood values resulting from the body's reactivity as well as starvation. In layer chickens, on contact with *Staphylococcus aureus* or turpentine, an acute phase protein reaction was induced. Measurement of values for SAA, transferrin, serum albumin and apolipoprotein A-1 in blood samples of these birds (Upragarin, 2005; Upragarin *et al*., 2005b) showed that calculation of an acute phase index for this species offers promising results in this species. Outcome was as has been calculated for cows with various diseases (Toussaint *et al*., 1995) and for pigs with a *Streptococcus suis* infection (Toussaint *et al*., 2002).

Infections and vaccination

To prevent spontaneous disease frequent vaccination is recommended. It has been shown, however, that upon vaccination an acute phase protein reaction may develop. This appears to limit the advantages of vaccines, because acute phase reactions are known to be contraproductive in view of muscle anabolism. Future interest will be more on amino acid pattern differences in muscle and APPs (Table 1) and on the negative acute phase reactants.

Table 1 Major amino acid composition differences between muscle protein and two major acute phase proteins after a gross amino acid composition list (in arbitrary units) (Reeds *et al*., 1994)

Amino acid	Muscle protein	*CRP*	*SAA*
Phe	40	105	103
Tyr	36	50	67
Trp	13	42	45
Arg	69	36	116
Ala	59	31	106

CRP: C-reactive protein; SAA: serum amyloid A

On starvation and negative energy balance associated with most diseases, muscle proteins are catabolised for amino acid supply of the hepatic APP formation and as source of energy. Especially for those APPs which rapidly and quantitatively increase in blood, their formation may have amino acid impact. An increased hindquarter protein catabolism exceeding the hepatic protein synthesis, and efflux of glutamine and alanine from the hindquarter was measured during a porcine induced endotoxemia study (Bruins *et al*., 2003). For growth during and after recovery from a disease, food requirements for amino acids thus may differ from the formula in ordinary food. Some pig studies indicate positive influences of additional dietary tryptophan (Le Floc'h *et al*., 2004) or L-arginine (Bruins *et al*., 2002).

Negative APPs may be associated with a change in concentration of bound compounds. A decrease of retinol-binding protein and of vitamin A values may be vice versa interrelated, vitamin A-deficiency being well known to decrease immune reactivity of children in developing countries. It is astounding to encounter a huge negative variation from normal blood vitamin A values of around 1~0.75 μmol/L to around <0.1 μmol/L in fattening pigs, as was revealed in a local investigation by a Dutch veterinarian (Hogendoorn, 2004).

Association of APPs with parturition, starvation and ketosis in cattle has been described. Rise in non-esterified fatty acids (NEFAs) occurs; their level increase might parallel those of some APPs. It is to be expected that due to negative APPs, blood vitamin A levels decrease as suggested for the pig. The NEFAs are toxic and have negative influence on metabolism. It is hypothesized that the NEFAs may decrease with increased muscular activity (walking). An inverse association with walking activity had been shown (Adewuyi, 2004).

DEVELOPMENT OF TECHNOLOGY TO QUANTATIVELY MEASURE PROTEINS OR CELLULAR UPREGULATION OF THEIR FORMATION

Several groups, after many laboratories used radio-immunoassays (RIA) and enzyme-linked methodology (ELISA) for APP measurement in particular of C-reactive protein (CRP) in human hospitals, are developing methods for rapid measurements of APP values. Multiple immunoassays with simultaneous analysis of various different serum analytes have been recommended (Lukacs *et al*., 2005; Ray *et al*., 2005; Petrou *et al*., 2002). Different nephelometry systems are compared during application for human CRP (Maggiore *et al*., 2005). Turbidimetry is developed for APP in the dog (Kjelgaard-Hansen *et al*., 2003), horse (Jacobsen *et al*., 2005a; 2005c) and for the cat (Kjelgaard-Hansen *et al*., 2005). Other methods of liquid phase analysis with the option of recycling of the sample (Philips, 2001), such as those possible with Surface Plasmon Resonance (Biacore® system), have been tested for APP in bovine milk (Åkerstedt *et al*., 2005). Two-dimensional electrophoresis with mass spectrometry has been shown to be applicable to animal samples with the aim to measure acute phase reactants (Miller *et al*., 2004; 2005a) as has been done by others for analytes of human cancer growth and activity (Mian *et al*., 2005; Wang *et al*., 2005). A protein chip has been developed for measurement of haptoglobin and SAA in human patients (Tolson *et al*., 2004). Protein microarray methodology on slides has been proposed for APP in pigs (Toussaint *et al*., 2004). Preliminary experiments with a monoclonal anti porcine CRP and pig acute phase sera using methodology as described (Timmerman *et al*., 2004), offered the possibility to measure more than 1000 pig blood sample spots on a single slide.

Indirectly, acute phase protein formation may be measured in biopsies by methods to assess upregulation of protein synthesis (quantitative PCR). Especially the technique may be applied on samples after slaughter, or in histopathology and together with assessment of cytokines.

These technological developments may have crucial importance in the future if done rapidly, and at low costs, many samples can be handled, the APPs have a good future in diagnostics. This technique is for general assessment just as the erythrocyte sedimentation rate is used in internal medicine, but more sensitive, and for special groups of patients such as horses after castration (Jacobsen *et al*., 2005c) or laparotomy (Miller *et al*., 2005b).

ASSESSMENT OF DISORDERS, HEALTH AND WELFARE

Specific groups (Petersen *et al*., 2004; Murata *et al*., 2004), such as castrated horses (Alsemgeest, 1994; Jacobsen *et al*., 2005b), cows with inflammatory processes including mastitis (Alsemgeest, 1994; Eckersall *et al*., 2001), peri-parturient cattle (Alsemgeest *et al*., 1993; Alsemgeest, 1994; Koets *et al*., 1998), periparturient sows (Zhu *et al*., 2005), or dogs and cats with infectious disorders (Ceron *et al*., 2005) benefited from acute phase reactant measurement. In milk samples of cows (McDonald *et al*., 2001; Eckersall *et al*., 2001; Jacobsen *et al*., 2005d) and sheep and goats (Winter *et al*., 2005) mastitis may be monitored.

Combination of various APP values in an index increases the diagnostic power of the APPs to discern between normal animals in a population and non-healthy ones. After it was shown that an index combining values of fast and slow, positive and negative APPs can be used to assess human cancer patients (Ingenbleek and Young, 1994), such an index has been mentioned to assess health in populations of cattle, pig, dog and chicken (Toussaint *et al*., 1995; 2000; Martinez-Subiella and Ceron, 2005; Upragarin *et al*., 2005a; 2005b; Upragarin, 2005). Not only blood or milk samples can be used, meat juice (Petersen *et al*., 2005) and other body fluids such as saliva (Parra *et al*., 2005), are tested and found useful.

Involvement of multi-analysis technology in measuring the APPs and when coupled with pattern recognition software, the power of selective diagnostics of the APPs can increase.

Finally, because with respect to reaction of acute phase analytes in slaughter pigs (Heegaard *et al*., 2005) and in cows with experimental induction (Jacobsen *et al*., 2005d) individual variability is striking, in the future the APP reactivity pattern of an individual breeding animal may be used for selection on robustness.

CONCLUSION

Future possibilities for acute phase reactants depend on basic new mechanistic findings of known proteins, new discoveries such as organ specific components, and on technological possibilities for rapid immunological or chemical multi-analyses coupled with computer analysis of the patterns found. A shared cost European Union (EU) project (No. QLK5-2001-02219) on porcine acute phase proteins has helped to spread the knowledge to scientists of member states involved, and to develop a base for practical applications. For other species such as cattle, horse, dog and cat, but also chicken and even human, a similar field is open for development of clinical and health management applications. Finally, new fields of research and application are in the negative metabolic influences of acute phase processes and their relationship with growth and nutrition.

References

Adewuyi, A.A., 2004. Relationship between Plasma NEFAs Concentration and Physical Activity in Postpartum Ruminants. Thesis at Van Hall Institute, Leeuwarden, the Netherlands, Project No. 33410.

Åkerstedt, M., Björk, L., Waller, K.P., Sternesjö, A., 2005. Development of a Rapid Biosensor Method for Determination of Haptoglobin in Bovine Milk. The 5th International Colloquium on Animal Acute Phase Proteins, Dublin, Ireland, p.60.

Alsemgeest, S.P.M., 1994. Blood Concentrations of Acute-Phase Proteins in Cattle as Markers for Disease. PhD Thesis Utrecht, the Netherlands, ISBN: 90-393-0573-0.

Alsemgeest, S.P.M., Taverne, M.A., Boosman, R., van der Weyden, B.C., Gruys, E., 1993. Peripartum acute-phase protein serum amyloid-A concentration in plasma of cows and fetuses. *Am. J. Vet. Res.*, **54**:164-167.

Bruins, M.J., Soeters, P.B., Lamers, W.H., Deutz, N.E.P., 2002. L-Arginine supplementation in pigs decreases liver protein turnover and increases hindquarter protein turnover both during and after endotoxemia. *Am. J. Clin. Nutr.*, **75**:1031-1044.

Bruins, M.J., Deutz, N.E.P., Soeters, P.B., 2003. Aspects of organ protein, amino acid and glucose metabolism in a porcine model of hypermetabolic sepsis. *Clin. Sci.*, **104**:127-141.

Ceciliani, F., Grossi, C., Giordano, A., Pocacqua, V., Paltrinieri, S., 2004. Decreased sialylation of the acute phase protein alpha1-acid glycoprotein in feline infectious peritonitis (FIP). *Vet. Immunol. Immunopathol.*, **99**:229-236.

Ceron, J.J., Eckersall, P.D., Martinez-Subiela, S., 2005. Acute phase proteins in dogs and cats: current knowledge and future perspectives. *Vet. Clin. Patho.*, **34**:85-99.

Eckersall, P.D., Young, F.J., McComb, C., Hogarth, C.J., Safi, S., Weber, A., McDonald, T., Nolan, A.M., Fitzpatrick, J.L., 2001. Acute phase proteins in serum and milk from dairy cows with clinical mastitis. *Vet. Rec.*, **148**:35-41.

Gruys, E., 2002. Acute Phase Proteins in Bovine Medicine. Proceedings American Veterinary Medical Association Congress 2002. Nashville, USA, p.317-321.

Gruys, E., Toussaint, M.J.M., 2001. Monitoring Animal Hygiene, Welfare and Health by Analytes of the Acute Phase Reaction. *In*: Non-specific Assessment of Infection, Inflammation, Bruising, Stress and Starvation. Proceedings 19th ESVP Meeting. Thessaloniki, Greece, p.113-131.

Gruys, E., Toussaint, M.J.M., Niewold, T.A., Koopmans, S.J., 2005. Acute phase reaction and acute phase proteins. *J. Zhejiang Univ. SCI.*, in Press.

Heegaard, P.M.H., Stockmarr, A., Sorensen, N.S., 2005. Porcine Acute Phase Proteins in Experimental Models of Infectious Diseases. The 5th International Colloquium on Animal Acute Phase Proteins. Dublin, Ireland, p.5.

Hogendoorn, M.P., 2004. Vitamin A in Fattening Pigs. Internal Report, Veterinary Practice Hogendoorn, Pijnacker, the Netherlands (in Dutch Language).

Ingenbleek, Y., Young, V.R., 1994. Transthyretin (prealbumin) in health and disease: nutritional implications. *Annu. Rev. Nutr.*, **14**:495-533.

Jacobsen, S., Kjelgaard-Hansen, M., Petersen, H.H., Jensen, A.L., 2005a. Evaluation of a commercially available human serum amyloid A (SAA) turbidometric immunoassay for determination of equine SAA concentrations. *Vet. J.*, in Press.

Jacobsen, S., Frei, S., Jensen, J.C., Thoefner, M.B., 2005b. Serum Amyloid A and Other Acute Phase Reactants in Horses with Normal or Protracted Recovery after Castration. The 5th International Colloquium on Animal Acute Phase Proteins. Dublin, Ireland, p.41.

Jacobsen, S., Kjelgaard-Hansen, M., Petersen, H.H., Jensen A.L., 2005c. Equine Serum Amyloid A Measurements Using a Commercially Available Turbidometric Immunoassay. The 5th International Colloquium on Animal Acute Phase Proteins. Dublin, Ireland, p.45.

Jacobsen, S., Niewold, T.A., Kornalijnslijper, E., Toussaint, M.J.M., Gruys, E., 2005d. Kinetics of local and systemic isoforms of serum amyloid A in bovine mastitic milk. *Vet. Immunol. Immunopathol.*, **104**:21-31.

Kjelgaard-Hansen, M., Jensen, A.L., Kristensen, A.T., 2003. Evaluation of a commercially available human C-reactive protein (CRP) turbidimetric immunoassay for determination of canine serum CRP concentration. *Vet. Clin. Pathol.*, **32**:81-87.

Kjelgaard-Hansen, M., Hansen, A.E., Schaap, M.K., 2005. Determination of Feline Serum Amyloid A (SAA) by Means of a Commercially Available Human SAA Turbidimetric Immunoassay. The 5th International Colloquium on Animal Acute Phase Proteins. Dublin, Ireland, p.51.

Koets, A.P., de Schwartz, N., Tooten, P., Kankofer, M., Broekhuijsen-Davies, J.M., Rutten, V.P., van Leengoed, L.A., Taverne, M.A., Gruys, E., 1998. Release of proinflammatory cytokines related to luteolysis and the periparturient acute phase response in prostaglandin-induced parturition in cows. *Theriogenology*, **49**:797-812.

Landman, W.J.M., 1998. Amyloid Arthropathy in Chickens. PhD Thesis, Utrecht, the Netherlands, ISBN: 90-393-1667-8.

Larson, M.A., Weber, A., Weber, A.T., McDonald, T.L., 2005. Differential expression and secretion of bovine serum amyloid A3 (SAA3) by mammary epithelial cells stimulated with prolactin or lipopolysaccharide. *Vet. Immunol. Immunopathol.*, **107**:255-264.

Le Floc'h, N., Melchior, D., Sève, B., 2004. The Importance of

Dietary Tryptophan for Preserving Growth and Controlling Inflammatory Response of Weaned Pigs Submitted to Immune Stress. *In*: Madec, F., Clement, G. (Eds.), Animal Production in Europe: the Way Forward in a Changing World. Proceedings of the in-between Congress of the ISAH. Vol I, St Malo, France, p.239-240.

Lukacs, Z., Dietrich, A., Ganshow, R., Kohlschutter, A., Kruithof, R., 2005. Determination of HIV antiobodies, hepatitis C antibodies, and hepatitis B antigens in dried blood spots–feasibility study using a multi-analyte immunoassay. *Clin. Chem. Lab. Med.*, **43**:141-145.

Lundmark, K., Westermark, G.T., Nystrom, S., Murphy, C.L., Solomon, A., Westermark, P., 2002. Transmissibility of systemic amyloidosis by a prion-like mechanism. *Proc. Natl. Acad. Sci. USA*, **99**:6979-6984. With erratum in 2003. *Proc. Natl. Acad. Sci. USA*, **100**:3543.

Maggiore, U., Cristol, J.P., Canaud, B., Dupuy, A.M., Formica, M., Pozzato, M., Panichii, V., Consani, C., Metelli, M.R., Sereni, L., *et al*., 2005. Comparison of three automated assays for C-reactive protein in end-stage renal disease: clinical and epidemiological implications. *J. Lab. Clin. Med.*, **145**:305-308.

Martinez-Subiella, S., Ceron, J.J., 2005. Evaluation of acute phase protein indexes in dogs with leishmaniasis at diagnosis, during and after short-term treatment. *Vet. Med. Chech.*, **50**:39-46.

McDonald, T.L., Larson, M.A., Mack, D.R., Weber, A., 2001. Elevated extrahepatic expression and secretion of mammary-associated serum amyloid A3 (M-SAA3) into colostrum. *Vet. Immunol. Immunopathol.*, **83**:203-211.

Mian, S., Ugurel, S., Parkinson, E., Schlenzka, I., Dryden, I., Lancashire, L., Ball, G., Creaser, C., Rees, R., Schadendorf, D., 2005. Serum proteomic fingerprinting discriminates between clinical stages and predicts disease progression in melanoma patients. *J. Clin. Oncol.*, **23**:5088-5093.

Miller, I., Friedlein, A., Tsangaris, G., Maris, A., Fountoulakis, M., Gemeiner, M., 2004. The serum proteome of Equus caballus. *Proteomics*, **4**:3227-3234.

Miller, I., Fountoulakis, M., Duvigneau, C., Hartl, R., Dobretsberger, M., Sieber, K., Prosl, H., Gemeiner, M., 2005a. The Plasma Proteome of Equus Caballus: Patterns under Baseline Conditions and during Anthelmintic Treatment. The 5th International Colloquium on Animal Acute Phase Proteins. Dublin, Ireland, p.48.

Miller, M.S., Moritz, A., Röcken, M., Litzke, L.F., 2005b. The Acute Phase Response after Different Laparoscopic Procedures in the Horse. Proceedings of Eurpean Congress on Comparative and Veterinary Clinical Pathology. Utrecht, the Netherlands, p.116.

Murata, H., Shimada, N., Yoshioka, M., 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. *Vet. J.*, **168**:28-40.

Nguyen, T.K.A., Stockhofe, N., Toussaint, M.J.M., Vivanco, V., Gruys, E., Niewold, T.A., 2003. Serum Amyloid A in Acute Phase Response of Cows with Mastitis Experimentally Induced by *Streptococcus Uberis*. *In*: Animal Welfare and Acute Phase Proteins. Proceedings Book of Fourth European Colloquium on Acute Phase Proteins. Segovia, Spain, p.104-105.

Niewold, T.A., Murphy, C.L., Hulskamp-Koch, C.A., Tooten, P.C., Gruys, E., 1999. Casein related amyloid, characterization of a new and unique amyloid protein isolated from bovine corpora amylacea. *Amyloid.*, **6**:244-249.

Niewold, T.A., Toussaint, M.J.M., Gruys, E., 2003. Monitoring Health by Acute Phase Proteins. *In*: Animal Welfare and Acute Phase Proteins. Proceedings of Fourth European Colloquium on Acute Phase Proteins. Segovia, Spain, p.57-67.

Niewold, T.A., Meinen, M., van der Meulen, J., 2004. Plasma intestinal fatty acid binding protein (I-FABP) concentrations increase following intestinal ischemia in pigs. *Res. Vet. Sci.*, **77**:89-91.

Ovelgönne, J.H., Landman, W.J.M., Gruys, E., Gielkens, A.L.J., Peeters, B.P., 2001. Identical amyloid precursor proteins in two breeds of chickens which differ in susceptibility to develop amyloid arthropathy. *Amyloid: J. Prot. Fold. Disord.*, **8**:41-51.

Parra, M.D., Tecles, F., Martinez-Subiela, S., Ceron, J.J., 2005. C-reactive protein measurement in canine saliva. *J. Vet. Diagn. Invest.*, **17**:139-144.

Petersen, H.H., Nielsen, J.P., Heegaard, P.M.H., 2004. Application of acute phase protein measurements in veterinary clinical chemistry. *Vet. Res.*, **35**:163-187.

Petersen, B., Gymnich, S., Nielsen, J.P., Pineiro, C., 2005. The Use of Acute Phase Proteins in Pig Production. The 5th International Colloquium on Animal Acute Phase Proteins. Dublin, Ireland, p.1-4.

Petrou, P.S., Kakababos, S.E., Christofidis, I., Argitis, P., Misiakos, K., 2002. Multi-analyte capillary immunosensor for the determination of hormones in human serum samples. *Biosens. Bioelectron.*, **17**:261-268.

Philips, T.M., 2001. Multi-analyte analysis of biological fluids with a recycling immunoafinity column array. *J. Biochem. Biophys. Methods*, **49**:253-262.

Pocacqua, V., Provasi, E., Paltrinieri, S., Gelain, E., Comunian, C., Ceciliani, F., 2005. Glycan moiety modifications of feline alpha1-acid glycoprotein in retrovirus (FIV, FeLV) affected cats. *Vet. Immunol. Immunopathol.*, **107**:17-26.

Ray, C.A., Bowsher, R.R., Smith, W.C., Devanarayan, V., Willey, M.B., Brandt, J.T., Dean, R.A., 2005. Development, validation, and implementation of a multiplex immunoassay for the simultaneous determination of five cytokines in human serum. *J. Pharm. Biomed. Anal.*, **36**:1037-1044.

Reeds, P.J., Fjeld, C.R.F., Jahoor, F., 1994. Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? *J. Nutr.*, **124**:906-910.

Timmerman, P., van Dijk, E., Puijk, W., Schaaper, W., Slootstra, J., Carlisle, S.J., Coley, J., Eida, S., Gani, M., Hunt, T., 2004. Mapping of a discontinuous and highly

conformational binding site on follicle stimulating hormone subunit-β (FSH-β) using domain Scan^{TM} and matrix Scan^{TM} technology. *Mol. Diversity.*, **8**:61-77.
Tolson, J., Bogumi, R., Brunst, E., Beck, H., Elsner, R., Humeny, A., Kratzin, H., Deeg, M., Kuczyk, M., Mueller, G.A., *et al*., 2004. Serum protein profiling by SELDI mass spectrometry: detection of multiple variants of serum amyloid alpha in renal cancer patients. *Lab. Invest.*, **84**:845-856.
Toussaint, M.J.M., van Ederen, A.M., Gruys, E., 1995. Implication of clinical pathology in assessment of animal health and in animal production and meat inspection. *Comp. Haematol. Int.*, **5**:149-157.
Toussaint, M.J.M., Lipperheide, C., Eckersall, P.D., Alava, M., Jobert, J.L., Heegaard, P.M.H., Meloen, R.H., Madec, F., 2000. Assessment of Health in Pigs by Acute Phase Protein Assays. *In*: Tielen, M.J.M., Voets, M.T. (Eds.), Proceedings of the *X*th International Congress on Animal Hygiene. Vol. 1, Animal Health Service, Boxtel, the Netherlands, ISBN: 90-71649-04-0.
Toussaint, M.J.M., Jobert, J.L., Gruys, E., 2002. Combination of Values for Acute Phase Proteins (APP) in an Index in a Pig Model with Induced *Streptococcus Infection*. *In*: Abstract Book of First International Congress on Transthyretin in Health and Disease. Strasbourg, p.100.
Toussaint, M.J.M., Hogarth, C.J., Nguyen, T.K.A., Gruys, E., 2004. Acute Phase Variables to Assess Health in Various Species. *In*: Madec, F., Clement, G. (Eds.), Animal Production in Europe: the Way Forward in a Changing World. Proceedings of the in-between Congress of the ISAH, Vol. 1, St Malo, France, p.119-120.
Toussaint, M.J.M., Hogarth, C.J., Nguyen, T.K.A., Loeb, E., Balciute, J., Vivanco, V., van Ederen, A.M., Jacobsen, S., Niewold, T.A., Gruys, E., 2005. Serum Amyloid A (SAA) in Mammary Tissues with Inflammatory Processes and in Mammary Corpora Amylacea. *In*: Grateau, G., Kyle, R.A., Skinner, M. (Eds.), Amyloid and Amyloidosis. CRC Press, Boca Raton, p.203-205.
Upragarin, N., 2005. In vitro Studies on the Pathogenesis of AA Amyloid Arthropathy in Chicken. PhD Thesis, Utrecht, the Netherlands, ISBN: 90-393-4038-2.
Upragarin, N., Landman, W.J.M., van Asten, A.J.A.M., Toussaint, M.J.M., van Ederen, A.M., Gruys, E., 2002. Serum Amyloid A (SAA) mRNA and Protein Expression in Primary Culture Chicken Synoviocytes. Proceedings Third Colloquium on Acute Phase Proteins. Kaap Doorn, Doorn, the Netherlands, p.72.
Upragarin, N., van Asten, A.J.A.M., Tooten, P.C.J., Landman, W.J.M., Gruys, E., 2005a. AA-amyloid formation by primary chicken fibroblast-like synoviocytes. *Vet. Immunol. Immunopathol.*, **106**:39-51.
Upragarin, N., Toussaint, M.J.M., Tooten, P.C.J., van Asten, A.J.A.M., Wajjwalku, W., Gruys, E., 2005b. Acute Phase Protein Reaction in Layer Chickens. A Calculated Acute Phase Protein Index as Measure to Assess Health during the Rearing Period. Proceedings 14th World Veterinary Poultry Congress. Istabul, Turkey, p.344.
Vivanco, V., Balciute, J., van Ederen, A.M., Toussaint, M.J.M., Nguyen, T.K.A., Loeb, E., Gruys, E., 2003. Immunohistochemical Investigation on Serum Amyloid A (SAA) in Bovine Tissues with Inflammatory Processes. *In*: Animal Welfare and Acute Phase Proteins. Proceedings Book of Fourth European Colloquium on Acute Phase Proteins. Segovia, Spain, p.106-107.
Wang, J.X., Zhang, B., Yu, J.K., Liu, J., Yang, M.Q., Zheng, S., 2005. Application of serum protein fingerprinting coupled with artificial neural network model in diagnosis of hepatocellular carcinoma. *Chin. Med. J.*, **118**:1278-1284.
Winter, P., Miny, M., Baumgartner, W., 2005. Interpretation of SAA Concentrations in Milk from Dairy Ewes and Goats. The 5th International Colloquium on Animal Acute Phase Proteins. Dublin, Ireland, p.31.
Zhu, Y., Österlundh, I., Hulten, F., Magnusson, U., 2005. Can Acute Phase Reactants be Used at Parturition to Identify Sows with Subclinical Mastitis? The 5th International Colloquium on Animal Acute Phase Proteins. Dublin, Ireland, p.59.