# Acute Phase Proteins In Veterinary Medicine

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## Introduction

Acute phase proteins (APPs) are non-specific innate immune components involved in the restoration of homeostasis and the restraint of microbial growth before the acquired immunity takes place. They are synthesized mainly by hepatocytes upon stimulation by, mainly, the cytokines IL-1-beta, IL6 and TNF-α. (Murata *et al*., 2004)

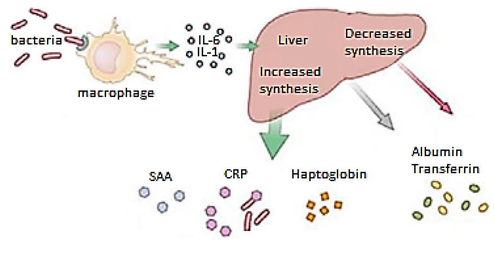


*Fig. 1. The main components of the acute phase response (www.medical-art.eu)*

Some of the APPs increase during the acute phase response (positive APPs) and some of them decrease (negative APPs).

| Positive APPs | Negative APPs |
|---|---|
| Haptoglobin (Hp), C-Reactive Protein (CRP) , Serum Amyloid A (SAA), Caeruloplasmin (Cp), Fibrinogen (Fb), α1-Acid Glycoprotein (AGP). | Albumin, Transferrin, Apolipoprotein A1, Retinol Binding Protein. |

*Fig. 2. Classification of APPs* (Murata *et al*., 2004, Paltrinieri, 2008)

APP genes can be classified depending on their response to cytokines:

| | Type 1 APPs | Type 2 APPs |
|---|---|---|
| **Synthesis incremented by** | IL-1 (IL-1a, **IL-1b**, **TNF-α**), synergy with **IL-6**, glucocorticoids | **IL-6**, glucocorticoids, inhibited by IL-1 |
| **They are positive APPs** | Major | Moderate |
| **Examples (varies between species)** | SAA, CRP | Fb, Hp, α1 Proteinase Inhibitor (α1-PI), Cp, AGP |

*Fig. 3. Type 1 and Type 2 APPs* (Paltrinieri, 2008; Petersen *et al*., 2004; Safi, 2012)

In general, APPs inhibit the inflammatory reaction, promote the healing of wounds and re-establish homeostasis (Saco Rodriguez, 2013).

## APPs in various animal species

Positive APPs are classified as major, moderate or minor depending on the degree of increase during the acute phase response. This classification, however, may vary depending on the species. (Murata et al., 2004)

| Species | Major APPs | Moderate APPs |
|---|---|---|
| **Dog** | CRP, SAA | Hp, AGP, Cp |
| **Cat** | SAA, AGP*, Hp* | Fb |
| **Pig** | CRP, Pig-MAP, SAA | Hp, Cp |
| **Cow** | Hp, SAA | AGP, CRP, Fb |
| **Horse** | SAA | Hp, Fb |
| **Mouse** | SAA | Hp, AGP |
| **Rat** | α2-macroglobulin | Hp, AGP |
| *AGP considered as moderate by some authors | | |

*Fig. 4. Positive APPs* (Murata *et al*., 2004; Paltrinieri, 2008; Cray *et al*., 2009; Eckersall & Bell, 2010; Tóthová *et al*., 2011; Saco Rodriguez, 2013)

Furthermore, the APP response depends on the animal species, individuals (depending on age, sex, pregnancy and polymorphisms) and the agent that triggered the response (Saco Rodriguez, 2013).

## APPs as biomarkers

APP analysis presents some advantages compared to WBC (white blood cells) analysis, as they are more rapid and sensitive markers of acute inflammation. They are useful for monitoring, diagnosing and stablishing a prognostic for inflammatory processes, stress, subclinical infections, pregnancy… They can even facilitate the differential diagnosis, although most of the times they only indicate the presence of an infectious-inflammatory condition.

The diseases that alter APP levels have been described in dogs better than cats, but it is known that the major APPs in dogs are CRP and SAA, and in cats SAA and AGP. In cats, AGP is a reliable diagnostic biomarker for FIP (feline infectious peritonitis).

As the experimental work of this thesis is based on the study of Hp in pigs, here are shown some facts about APPs utility as biomarkers in this species.

These are some diseases that have been proven to alter APPs levels in pigs:

| | Hp | Pig-MAP | CRP | SAA | References |
|---|---|---|---|---|---|
| *Streptococcus suis* | ■ | ■ | ■ | ■ | Saco Rodriguez, 2013 |
| *Actinobacillus pleuropneumoniae* | ■ | ■ | ■ | ■ | Cray et al., 2009; Saco Rodriguez, 2013 |
| *Haemophilus parasuis* | ■ | ■ | ■ | | Saco Rodriguez, 2013 |
| *Yersinia enterocolitica* | ■ | | ■ | | Saco Rodriguez, 2013 |
| *Pasteurella multocida* | ■ | ■ | ■ | ■ | Saco Rodriguez, 2013 |
| *Mycoplasma hyopneumoniae* | ■ | | ■ | ■ | Cray et al., 2009; Saco Rodriguez, 2013 |
| African Swine Fever | ■ | ■ | ■ | ■ | Saco Rodriguez, 2013 |
| Classical Swine Fever | ■ | | ■ | ■ | Saco Rodriguez, 2013 |
| Aujeszky | ■ | ■ | ■ | | Saco Rodriguez, 2013 |
| PRRS | ■ | | ■ | | Saco Rodriguez, 2013 |
| Type 2 swine circovirus | ■ | ■ | ■ | | Saco Rodriguez, 2013 |
| Swine flu | ■ | | ■ | ■ | Saco Rodriguez, 2013 |
| Tail and ear biting | ■ | ■ | ■ | ■ | Cray et al., 2009 |
| Arthritis | ■ | | ■ | ■ | Cray et al., 2009 |
| 48h transport | ■ | ■ | ■ | | Cray et al., 2009 |

■ Only in clinically affected animals
■ Only in experimental infecions

*Fig. 5. Some diseases that alter APP levels in pigs*.

Some studies explain variations in APP levels in different conditions in pigs (Saco Rodriguez, 2013):

- Experimentally induced inflammation. Hp, CRP and Pig-MAP are the APPs which serum levels increase the most.
- APP in natural infections. Generally, they increase more in animals that show symptoms or in those infected with bacteria.
- APP in subclinical conditions. Subclinical illnesses are common in the porcine industry and they affect productivity and decrease animal welfare. APPs will help us detect these conditions before the animals show any symptoms.

Furthermore, APPs can be used as a marker of production efficiency. Animals that have low APP levels will gain more weight. And Hp determination at the slaughterhouse can be an indicator of sanitary problems at the farm where the animals come from. (Saco Rodriguez, 2013)

## Experimental work

The experimental work of this thesis consists of the analysis of serum samples from 44 pigs to determine their Haptoglobin levels.

The pigs were distributed in 4 different corrals): animals in corrals A and B had 1,2 $m^2$ per animal and some straw available, while animals in corrals C and D only had 0,7 $m^2$ per animal and no straw.
The objective of this analysis is to prove the effect of welfare and stress on Hp levels, which has been used as a marker of these conditions in pigs.

Materials and methods: the serum samples were analysed using an automatic analyser (Olympus AU400) and the Tridelta Development Ltd. diagnostic kit.

Results and discussion: The obtained absorbances were used to determine the Hp concentration. The results are represented in Fig. 6:

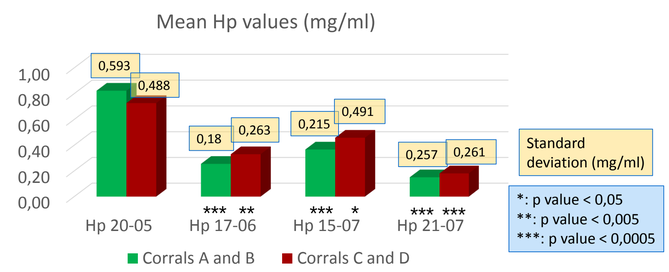


*Fig. 6. Mean Hp values, standard deviation and p-values.*

Note: all the Hp levels were within the normal range (0,00-2,20 mg/ml). Therefore, we can say that all the animals were in a good sanitary and welfare situation.

The higher levels of Hp were found on the first day, as a result of the stress caused by the mixing of the animals into the four corrals. After that, Hp levels stabilise and we can appreciate how levels of the animals in corrals C and D are slightly higher, as expected.

Standard Deviation is higher on first day as well, and after that the variability of the Hp values is lower, as a result of the stability of the living conditions.

The last samples were obtained at the moment the animals were slaughtered. We can't observe an increase in Hp levels because the changes in Hp synthesis (which involve transcriptional changes) require at least 6 hours to be carried out, and all the animals were transported and slaughtered within one morning.

P-values were calculated, for both conditions (better / worse living conditions), and they all were lower than 0,05, proving that the changes of Hp levels within both groups of corrals were statistically significant.

However, p-values between both conditions (calculated those 4 days separately) were all greater than 0,05.
Thus, we can conclude that, in this particular farm, better living conditions don't cause a statistically significant reduction of Hp levels. This is probably due to the fact that the living conditions in this farm are better than in most farms. So we would probably see more significant results if we did an evaluation of Hp levels in a farm with worse living conditions.

## Conclusions

APP analysis is widely used in veterinary medicine and in recent years there has been an exponential increase in APP research in all animal species. They have been included in routine biochemical profiles at a number of laboratories all over the world.
They are biomarkers for a great number of infectious/inflammatory conditions and stressful situations, and give us early (although unspecific) information of their presence. They are useful for diagnostic and prognostic purposes, and for monitoring such processes as well.
It seems logical, then, that their use in laboratories all over the world will continue to increase as cheaper and easier techniques are developed.

**Bibliography**:
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