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Review

ACUTE PHASE PROTEINS IN SHEEP AND GOATS – FUNCTION, REFERENCE RANGES AND ASSESSMENT METHODS: AN OVERVIEW

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Summary

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Acute phase response (APR) is activated by disorders in systemic homeostasis. The main purpose of APR is to prevent further tissue damage by isolation and destruction of causative agent, removing the toxic products and providing conditions for tissue repair. Probably the most significant change during APR is a transformation in the liver protein spectrum expressed by increased rate of synthesis of acute phase proteins (APPs) or acute phase reactants. Numerous APPs that perform specific functions are established whose quantitative variations vary considerably among different animals. Some of them are mediators (C-reactive protein, fibrinogen), others act as inhibitors (protease inhibitors – α1-antitrypsin) and especially in small ruminants, the most specific APPs are scavengers and transporters (haptoglobin, serum amyloid A). Despite considerable progress, many of the characteristics of APPs in domestic animals are still poorly understood especially in sheep and goats. Little is known about some other proteins such as lipopolysaccharide binding protein, hemopexin, alpha-1 antitrypsin, lactoferrin, transferrin, C-reactive protein, ceruloplasmin, fibrinogen and alpha-1 acid glycoprotein. The aim of this study is to present information concerning the most important functions of APPs, as well as their ranges in healthy sheep and goats and laboratory assay methods.

Key words: acute phase proteins, sheep, goats

INTRODUCTION

The acute phase response (APR) or acute phase reaction is a complex early, antigen non-specific, defense systemic reaction appearing before a specific immune response is raised (Petersen *et al.,* 2004). It is activated by trauma, neoplastic growth, bacterial, parasitic and viral infection, burns, surgery, immunological disorders etc. (Gruys *et al.,* 2005; Cray *et al.,* 2009; Eckersall & Bell, 2010; Tothova *et al.,* 2014). The main purpose of APR is to restore the homeostasis by isolating and destroying the harmful agent and to activate the repair process (Janeway *et al.,* 2001; Ceciliani *et al.,* 2002). The damaged tissue is the primary source that initi-

ates APR. The early changes involve activation of cells belonging to the innate immunity – macrophages and monocytes which synthesise pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α) and some others (Murata *et al.,* 2004). These mediators are involved in the activation of fibroblasts, leukocytes and endothelial cells. The latter, by producing more cytokines (IL-6, IL-8, IL-11, PGE2, LIF, nitric oxide), increase the permeability of blood vessels, enhance leukocyte migration and induce local changes – swelling, pain, redness and warmth which accompany the inflammatory process (Gruys *et al.,* 2005). The increased concentration of cytokines may induce systemic APR and resultant activation of the hypothalamic-pituitary-adrenal system (increased adrenocorticotropic hormone and glucocorticoids production), reduction of growth hormone secretion and a considerable number of metabolic and biochemical changes (complement cascade and blood coagulation system activation; decreased serum concentration of iron, zinc, calcium, vitamin A, α-tocopherol; negative nitrogen balance; changes in the concentration of some plasma proteins) clinically manifested by pyrexia, anorexia, weight loss etc. (Moshage, 1997; Gabay & Kushner, 1999; Gruys *et al.,* 1999; Pyorala, 2000; Gruys *et al.,* 2005; Jain *et al.,* 2011; Ceciliani *et al.,* 2012; O'Reilly & Eckersall, 2014; Tothova *et al.,* 2014). Down–regulation of APR is controlled by many inflammatory mediators (IL-4, IL-10) and hormones (glucocorticoids) as well as by the production of antagonists to pro-inflammatory cytokines (Gruys *et al.,* 2005). Usually APR continues for 1–2 days and after this period the affected organism should return to normal function (Ceciliani *et al.,* 2002). If the acute inflammation becomes chronic, APR is still induced (Baumann & Gauldie, 1994; Jain *et al.,* 2011).

One of the most important metabolic alterations accompanying APR occurs mainly in the liver but also in other organs. It involves the synthesis of a large group proteins, collectively known as acute phase proteins (Ceciliani *et al.,* 2012). Their expression depends on cytokines' levels, whereas the cytokine activity modulation is regulated by glucocorticoids and growth factors (van Miert, 1995; Ceciliani *et al.,* 2002; Gruys *et al.,* 2005).

ACUTE PHASE PROTEINS

The acute phase proteins are a large group of plasma proteins which originate mainly from the liver in response to pro-inflammatory cytokines (IL-1, IL-6, TNF-α). They are released very quickly into the circulation, where each performs a specific activity. During APR the serum of APPs concentrations increase substantially while in healthy animals and humans, APPs are undetectable or in negligible quantities. However, some APPs collectively called "permanent" are continuously secreted and released into the bloodstream while the "induced" ones are present in plasma only during APR. In general, the main function of APPs is to defend the host against pathological damages, assist in the restoration of homeostasis and in the regulation of different stages of inflammation (Petersen *et al.,* 2004; Tothova *et al.,* 2014).

APPs can also be synthesised extrahepatically in some tissues such as testicular tissue, adipose tissue, lung, ovary, uterus, mammary glands, digestive tract (Ceciliani *et al.,* 2012).

The type and magnitude of APP expression varies between the species. Plasma concentrations of major APPs in healthy animals are very low. During the acute phase of inflammation their levels are greatly enhanced in the first few hours after exposure to the pathogenic factors (Gruys *et al.,* 1999; Ceciliani *et al.,* 2002; Petersen *et al.,* 2004). APPs kinetics depends on the animal species and extent of tissue damage. Serum APPs concentrations generally reach a peak within 24–48 hours. If a new stimulus does not appear and inflammation ceases, the feedback APPs regulation limits the response within 4 to 7 days after challenge. Chronic inflammation is considered as a series of individual inflammatory stimuli and is characterised by longer and slight increase in the serum concentration of APPs as compared to acute inflammation (Sipe, 1985; Murtaugh, 1994; Heegaard *et al.,* 2000; Jain *et al.,* 2011).

CLASSIFICATION OF ACUTE PHASE PROTEINS

Despite the uniformity in the development of APR between species, there is a lot of evidence showing that each animal has its own specific APPs set (Pyorala, 2000). The biological functions of some APPs studied in ruminants are summarised in Table 1.

In general terms, these proteins can be classified according to their quantitative variation and function.

A) Depending on the magnitude of the increase or decrease by at least 25% during APR (Murata *et al.,* 2004; Ceron *et al.,* 2005; Eckersall & Bell, 2010).

- Positive APPs – haptoglobin, serum amyloid A, C-reactive protein.
- Negative APPs – albumin, transferrin, transthyretin (prealbumin, thyroxine–binding protein).

B) Depending on the magnitude of increase in the concentration of positive

Table 1. Biological function of acute phase proteins (according to Ceciliani *et al.*, 2012; O'Reilly & Eckersall, 2014; Tothova *et al.*, 2014)

Proteins	Abbreviation	Biological function	Classification
Haptoglobin	Hp	Binds free haemoglobin	Positive
Serum amyloid A	SAA	Binds cholesterol, opsonin	Positive
Ceruloplasmin	Cp	Copper transport, iIron metabolism	Positive
Albumin	Alb	Amino acids source, transporter, osmotic pressure	Negative
C-reactive protein	CPR	Complement activation, binding to membrane phosphorylcholine, opsonization	Positive
Fibrinogen	Fb	Blood clotting	Positive
Lipopolysaccharide binding protein	LPB	Binds to bacterial LPS, macrophage cell activation	Positive
α_1-acid glucoprotein	AGP	Bind drugs, inflammatory mediators and bacterial derived molecules	Positive
α_1-antitrypsin	AAT	Serine protease inhibitor	Positive
Transferrin	TF	Binds iron	Negative
Hemopexin	Hpx	Heme binding	Positive
Lactoferrin	LF	Binding and transferring Fe^{3+} ions	Positive

APPs (Eckersall & Bell, 2010; Gomez-Laguna *et al.*, 2011; Tothova *et al.*, 2014) (Table 2).

- Major APPs – their concentrations increase 10 to 100-fold, reaching a peak 24–48 hours after pathological stimulus and decline quickly due to short half – life (Niewold *et al.,* 2003).
- Moderate APPs – their concentrations increase 5 to 10-fold, reaching a peak 2–3 days after pathological stimulus and decline more slowly than major APPs (Eckersall, 2006a).
- Minor APPs – gradually increase by between 50% and 100% of their normal level (Eckersall & Bell, 2010).

C) Depending on their role during inflammation (Tirziu, 2009; Khan & Khan, 2010).

- Mediators (C-reactive protein, fibrinogen)
- Modulators (complement proteins, inhibitors to coagulation cascade)
- Inhibitors (protease inhibitors – α1-antitrypsin, α2-macroglobulin)
- Scavengers and transporters (haptoglobin, serum amyloid A, ceruloplasmin)
- Immunomodulators (α1-acid glycoprotein)

D) Depending on their cytokine induction (Murata *et al.*, 2004; Gruys *et al.*, 2005)

- Type 1 dependent (induced by IL-1 and TNF-α)
- Type 2 dependent (induced by IL-6)

The synthesis of type-1 dependent APPs is synergistically affected also by IL-6, in contrast, the secretion of type-2 APPs is neither induced nor synergistically affected by IL-1, moreover it has a inhibitory effect on their synthesis. The reduction of negative APPs in plasma during inflammation is due to the priority of positive APPs synthesis. Furthermore, some of negative APPs are hormone-binding proteins and their reduction provides more available, biologically active forms of hormones, some of which (glucocorticoids) are very important for induction of APR. Other APPs as transthyretin have also an inhibitory effect on IL-1 production by monocytes and endothelial cells, thus its declining concentration may be regarded as a part of the adaptive mechanisms of APR induction (Ceciliani *et al.,* 2002). Some APPs inhibit protease enzymes synthesised by phagocytes and pathogenic microorganisms and therefore protect tissues from damage e.g. α1-antitrypsin, α2-macroglobulin (Gruys *et al.,* 1999). Other major APPs as haptoglobin, serum amyloid A and C-reactive protein act as scavengers by binding metabolites formed during inflammation (Wagener *et al.,* 2001). Some APPs (α1-acid glycoprotein) have an antibacterial activity and thus positively influence the immune response (Eckersall, 2006b).

Table 2. APPs in sheep and goats

	Sheep	Goats
Major APPs	Haptoglobin, SAA	Haptoglobin, SAA
Moderate APPs	AGP	Fibrinogen, AGP
Minor APPs	Fibrinogen, Ceruloplasmin	Ceruloplasmin
Negative APPs	Albumin	Albumin

SAA= serum amyloid A; AGP=α_1-acid glucoprotein

ACUTE PHASE PROTEINS IN SMALL RUMINANTS

Haptoglobin

Haptoglobin (Hp) is considered to be a major APP in sheep and goats (Table 2).

It refers to a group of transporter (metal-binding) plasma proteins that increase during APR (Pannen & Robotham, 1995). Hp is synthesised mainly in the liver, although its coding gene is expressed in other tissues such as lung, skin, spleen, kidney and adipose tissue (D'Armiento *et al.,* 1997; Yang *et al.,* 2003). The synthesis of Hp is mediated and regulated by the growth hormone, insulin, bacterial endotoxin, prostaglandins, pro-inflammatory cytokines – IL-1, IL-6, tumour necrosis factor (Raynes, 1994).

Hp has several distinctive features. According to Yang *et al.* (2003) and Kato (2009) the most important one is to bind the free haemoglobin (Hb) in equimolar ratio with very high affinity (Table 1). The complex Hp-Hb is too large and cannot pass through the glomerulus of the kidney and may only be removed by reticuloendothelial system. It is accomplished by binding to CD163 receptors presented on macrophages and monocytes surface (Schaer *et al.,* 2002). Hp–Hb half-life is up to 50 min (Ceciliani *et al.,* 2012). The quantity of Hp does not increase in cases in which red blood cells are destroyed in the spleen or liver but not in the vessels (Jain *et al.,* 2011) and it may decrease during massive erythrolysis (Smith & Roberts, 1994).

The free Hb in the bloodstream is toxic and has oxidative activity (Wagner *et al.,* 2001). Hp binds it and thus prevents oxygen radicals formation (stimulated by iron), reduces the oxidative damages associated with haemolysis which defines its role as an antioxidant (Smith & Roberts, 1994; Murata *et al.,* 2004).

Hb contains iron which is one of essential elements required for bacterial growth. After the Hp-Hb complex is formed, the iron becomes unavailable for bacteria which explains the bacteriostatic effect of Hp (Ceciliani *et al.,* 2012).

It is important to note that Hp may inhibit mast cell proliferation and directly interact the effector cells by binding to CD11/CD18 receptor and also to suppress T-cell proliferation (by inhibition of Th2 response) which expresses its immunomodulatory and anti-inflammatory role (El Ghmati *et al.,* 1996; Murata *et al.,* 2004).

Serum amyloid A

Serum amyloid A (SAA) is also a major APP in small ruminants (Table 2). Its function is not fully understood but some activities have been well described and documented. Several effects of SAA are well explored, e.g. binding, transporting and scavenging of cholesterol from destroyed cells and lipid debris from bacteria to the liver during inflammation (Ceciliani *et al.,* 2012); inhibition of phagocyte oxidative burst (Linke *et al.,* 1991; Gruys *et al.,* 2005), inhibition of platelet aggregation (Petersen *et al.,* 2004), detoxification of endotoxin (binding to lipopolysaccharide) (Schroedl *et al.,* 2001; Murata *et al.,* 2004), binding to Gram negative bacteria and opsonisation (Hari-Dass *et al.,* 2005; Tothova *et al.,* 2011), inhibition of lymphocytes and endothelial cells proliferation (Murata *et al.,* 2004). It exhibits chemotactic recruitment of inflammatory cells to localised areas of inflammation (Xu *et al.,* 1995; Uhlar & Whitehead, 1999) and down regulates some systemic (pyrexia) events during AP (Shainkin-Kestenbaum *et al.,* 1991; Uhlar & Whitehead, 1999) (Table 1). SAA is synthesised by IL-1, IL-6 and TNFα mainly from hepatocytes but intestinal epithelial cells, macrophages and smooth-muscle cells can also release it by the same pro-inflammatory cytokines (Vreugdenhil *et al.,* 1999; McDonald *et al.,*

2001; Murata *et al.,* 2004). Based on the abovementioned information, it can be concluded that SAA plays a role as an opsonin, prevents accumulation of cholesterol on the site of inflammation and modulates innate immune reactions.

It is established that the normal ovine colostrum contains an isoform of SAA (mammary-associated SAA) which stimulates mucin secretion from the intestine and prevents bacteria development and adapts postparturient period of the newborn animals (McDonald *et al.,* 2001; Mack *et al.*, 2003).

Ceruloplasmin

Ceruloplasmin (Cp) is a metal-binding APP that stores and transports copper in the body (O'Reilly & Eckersall, 2014) and also plays a role in iron metabolism (Lovstad, 2006) (Table 1). It is classified as a minor APP in sheep and goats (Table 2). Cp is a ferroxidase and acts as an antioxidant that converts the toxic ferrous ion (Fe^{2+}) into the non-toxic ferric ion (Fe^{3+}) and thus protects the tissues from damaging effects of free radicals (Patel *et al.*, 2002; Murata *et al.,* 2004). Summarised data (Murata *et al.,* 2004) show that Cp is synthesised primarily in the liver but other tissues can be involved including respiratory epithelium of the lung.

Fibrinogen

Fibrinogen (Fb) is a soluble glycoprotein belonging to β-globulin fraction of the blood plasma. It is classified as a minor APP in sheep but moderate in goats (Table 2). The tissue injury is one of the primary stimulus responsible for activation of the coagulation cascade involving many and different types substances. Fb is a coagulation protein and its synthesis increased during the APR (Davalos & Akassoglou, 2012). It is important to note that if intravascular coagulation occurs, Fb plasma concentration decreases. Fb is a substrate for fibrin formation and also serves as a matrix for migration of inflammation-related cells and healing of wounds (Raynes, 1994; Thomas, 2000; Murata *et al.,* 2004). It binds specifically to the CD11/CD18 receptors on the migrated phagocytes surface and releases a cascade of intracellular signals that lead to increased degranulation, phagocytosis, antibody-dependent cellular cytotoxicity and delayed apoptosis (Rubel *et al.,* 2001; Murata *et al.,* 2004).

Alfa-1 acid glycoprotein

Alpha-1 acid glycoprotein (AGP) or orosomucoid is highly glycosylated protein synthesised mainly by liver but extrahepatic production (notably epithelial and endothelial cells) have also been confirmed (Fournier *et al.,* 2000; Murata *et al.,* 2004). AGP is considered as a moderate APP in small ruminants (Table 2). It has at least three important biological functions (Table 1). Like serum albumin, AGP is a binding protein in plasma (O'Reilly & Eckersall, 2014). In physiological conditions, AGP is able to bind more than 300 different biologically active endogenous and exogenous substances such as heparin, histamine, serotonin, steroids, catecholamines and drugs (Fournier *et al.,* 2000; Israili & Dayton, 2001; Eckersall, 2008; Ceciliani *et al.,* 2012). Other suggested AGP functions are the inhibition of neutrophil activation, phagocytosis, platelet activating factor, natural killer cell activity and also a role in T- and B-cell maturation which determines its anti-inflammatory and immunomodulatory activities (Okumura *et al.,* 1985; Fournier *et al.,* 2000; Israili & Dayton, 2001; Murata *et al.,* 2004; Eckersall, 2008). It exhibits a moderate response in

most animals including small ruminants and is likely to be associated with chronic conditions (Tothova *et al.,* 2014). AGP has a role in the innate defense and may act as a non-specific antimicrobial agent by binding directly with lipopolysaccharide and neutralising its toxicity (Moore *et al.,* 1997; Murata *et al.,* 2004; Raich, 2012).

Hemopexin

Hemopexin (Hpx) is a heme-binding plasma glycoprotein consisting of a single polypeptide chain which is synthesised by hepatocytes in response to IL-6 stimulation (Immenschuh *et al.,* 1995). Heme (iron-protoporphyrin IX) is a component of haemoglobin, myoglobin and some enzymes such as cytochrome, heme peroxidase and others (Smith, 1999). It is released mainly after the destruction of red blood cells. The main function of Hpx is to bind free heme and to protect from oxidative stress, therefore, it acts as an antioxidant (Gutteridge, 1995). Together with Hp and transferrin, Hpx plays a role in the iron homeostasis (Delanghe & Langlois, 2001). Other important role of Hpx is to bind nitric oxide (Shipulina *et al.,* 1998) and carbon monoxide (Shaklai *et al.,* 1981) and thus to neutralise their toxicity. Information concerning Hpx in small ruminants is limited but there are some data about its behaviour during protozoan diseases (Sousa Almeida *et al.,* 2012).

Alfa-1 antitrypsin

Alpha-1 antitrypsin (AAT) is an APP belonging to serpins with a broad spectrum of antiprotease activity (Table 1). AAT is a serine protease inhibitor which protects tissues from neutrophil proteolytic enzymes (mainly elastase and proteinase) at the site of imflammation (Murata *et al.,* 2004; Tothova *et al.,* 2014).

Lactoferrin

Lactoferrin (LF) is a part of the innate immunity system and represents one of the first defense reactions against pathogens invading via mucosal tissues (Legnard *et al.,* 2005). LF activities affect the growth and proliferation of infectious agents such as bacteria, protozoan, viruses and fungi (Ward *et al.,* 2002). LF is presented in most mucosal secretions such as colostrum, milk, saliva, small intestine secretion, nasal secretion (Tothova *et al.,* 2014). It is known that neutrophils are the main source of plasma LF (Iyer & Lonnerdal, 1993). In healthy organisms the plasma LF concentration is low but during infection or other tissue injuries it rapidly increases due to inflammatory cells activation (Adlerova *et al.,* 2008). For this reason several authors classify LF as an APP (Kanyshkova *et al.,* 2001). LF plays an important role during infection by binding the iron in stable compound (Adlerova *et al.,* 2008). The complex LF-Fe does not disintegrate even at low pH, notably at the site of inflammation due to metabolic changes occurred by pathogen activities (Valenti & Antonini, 2005). It expresses a bacteriostatic effect of LF that makes the iron unavailable from bacterial growth and proliferation (Tothova *et al.,* 2014).

Albumin

Albumin (Alb) is the main member of negative APP in ruminants (Table 2). It is the most abundant protein in the blood of animals and humans, representing 35–50% of total protein. Alb is responsible for about 75% of the osmotic pressure of plasma and is the main source of amino acids that may be utilised for synthesis of positive APPs during APR (Ceron *et al.,* 2005; Tothova *et al.,* 2014). Furthermore, the initiation of APR triggers down-regulation of Alb production (Gabay &

Kushner, 1999). Beyond its role as a reservoir for amino acids, Alb also serves as a carrier protein for many organic and inorganic biologically active substances such as thyroxin, estrogen, bilirubin, penicillin, cortisol, free fatty acids, calcium, magnesium, drugs and other which is possibly due to the variety of binding sites on the albumin molecule (Nicholson *et al.,* 2000). The values of plasma Alb may be used as an indicator of nutritional status due to its relatively long half-life (Tothova *et al.,* 2014).

Transferrin

Transferrin (TF) is a glycoprotein belonging to the family of metal binding transport proteins, synthesised in the liver and together with Alb described as a negative APP (Table 1). TF has a high capability to bind ferric ions in pH of 7.4 but reversibly when the acidity increase (Gomme & McCann, 2005). According to Broch *et al.* (1987), Kaplan *et al.* (1991) and Gkouvatsos *et al.* (2012), iron chelation by transferrin has some important functions such as maintaining Fe^{3+} in soluble form under physiological conditions; supporting iron transport and cellular uptake; maintaining Fe^{3+} in a redox-inert state, preventing the generation of toxic free radicals and inhibition of the proliferation and growth of bacteria by limiting the access to iron.

C-reactive protein

C-reactive protein (CPR) is an APP belonging to the pentameric protein family (pentraxins) (Bottazzi *et al.,* 1997). It performs several important functions such as the ability to activate the complement cascade by the classical pathway, modulation of activity of blood platelets, erythrocyte aggregation and opsonisation of necrotic tissues and cellular debris (Vojtic & Krajnc, 2000). Khan & Khan (2010) also reported that CRP binds with highest affinity to phosphocholine residues in a calcium dependent manner, apoptotic cells, glycans, somatic components of bacteria, fungi and parasites. A number of physiological functions of CPR and its behaviour during various infectious and non-infectious diseases in sheep and goats are not fully elucidated. However, there are data showing that the CRP plasma levels are increased in the case of various types of pneumonia in sheep and goats (Haligur & Ozmen, 2011).

Lipopolysaccharide binding protein

Lipopolysaccharide binding protein (LBP) is a serum glycoprotein belonging to a small family of lipid-binding proteins together with some other proteins (bactericidal permeability-increasing protein, phospholipid transfer protein, cholesteryl ester transfer protein) (Gustsmann *et al.,* 2001). LBP is synthesised in the liver and intestinal epithelial cells (Ramadori *et al.,* 1990; Vreugdenhil *et al.,* 1999). It plays a key role of innate immune response against bacteria (Ceciliani *et al.,* 2012). Lipopolysaccharide (LPS) molecules are components of external membrane of Gram negative bacteria (Gustsmann *et al.,* 2001) and responsible for the biological toxicity (Raetz, 1990; Rietschel & Brade, 1992). The biological actions of LPS are mediated by both LPS-binding proteins and LPS receptors (Fenton & Golenbock, 1998). LBP binds and transfers a bacterial LPS to the CD14, CD11/18 receptors of antigen-presented cells (Fenton & Golenbock, 1998) and granulocytes (Lamping *et al.,* 1996). This leads to cellular activation resulting in the release of systemically active pro-inflammatory molecules, which in turn mediate systemic toxicity (Fenton & Golenbock, 1998). Enhancement or

inhibition of LPS-induced cellular activation depends on LBP concentration. Low LBP concentration performs a pro-inflammatory role (activation of mononuclear cells), whereas high concentration has an anti-inflammatory role by inhibition LPS-induced cellular stimulation (Lamping *et al.,* 1996; Gustsmann *et al.,* 2001; Ceciliani *et al.,* 2012). LPB is able to bind not only with LPS but also with lipoteicholic acid, a major constituent of the cell wall of Gram-positive bacteria (Ceciliani *et al.,* 2012).

ASSESSMENT METHODS AND REFERENCE RANGES

It is known that the plasma levels of APPs are low in the absence of the pathological process. However, there are number of sensitive methods developed for establishing their values both in patients and in healthy subjects which are presented in Table 3.

Several studies have been conducted in goats and sheep which are designed to determine the reference ranges of APPs in healthy animals (Table 4). In this table are also mentioned some studies that have been conducted in sheep and goats in experimental settings but the data concerning the ranges of APPs values are obtained only from the healthy animals included in the control groups.

CONCLUSION

A wide range of investigations have been conducted to determine the usefulness of APPs in various diseases especially in cattle, but data concerning these reactants are still relatively under-utilised in sheep and goats. Overall, they are a useful indi-

Table 3. Assays for measuring APPs in small ruminants

APPs	Measurement method	Reference
Hp	Colorimetric method based on the haemoglobin-binding capacity and preservation of the peroxidase activity of the bound haemoglobin at low pH (Phase Haptoglobin Assay kit, Tridelta, Ireland).	Eckersall (2006b)
SAA	Solid phase sandwich ELISA (Tridelta, Ireland)	Colom-Cadena *et al.* (2014)
Cp	Colorimetric enzyme assay by p-phenylenediamine dihydrochloride oxidation measurement	Meling *et al.* (2012)
Fb	Heat precipitation method. Nephelometric method.	Ulutas & Ozpinar (2006) Fasulkov *et al.* (2014)
CPR	Turbidimetric immunoassay by liquid phase immunoprecipitation reaction with rabbit polyclonal antibodies against human CRP (Protiline, BioMérieux)	Vojtic & Krajnc (2000)
ASG	Precipitation method by perchloric acid and determination using bicinchoninic acid protein assay reagent	Eckersall *et al*. (1996) Gonzalez *et al.* (2008)
AGP	Radial immunodiffusion by kit (J-Path Inc. Tokyo, Japan).	Eckersall *et al.* (2007)
LBP	Commercial ELISA kits (Jiancheng Biology Co., Nanjing, China)	Zhong *et al.* (2014)

Table 4. APPs ranges in healthy small ruminants

APPs, species	Normal range (examined)	Reference
Hp, Sheep	0.16±0.13 –0.19±0.10 g/L	Ulutas & Ozpinar (2006)
	0.197±0.015 g/L	Aziz & Taha (1997)
	0.99±0.12 g/L	Eckersall *et al.* (2007)
	0.30±0.06 mg/mL	Wells *et al.* (2013)
	0.038–0.112 g/L	Zhong *et al.* (2014)
	0–1.0 mg/dL	Jain *et al.* (2011)
	0.06-0.12 g/L	Lepherd *et al.* (2009)
	0.048±0.008 mg/mL	Gurdogan *et al.* (2014)
	0.99 (±0.062) – 0.122 (±0.101) g/L	Nowroozi-Asl *et al.* (2008)
	0.215 (±0.068) – 0.260 (±0.081) mg/mL	Pfeffer & Rogers (1989)
Hp, Goats	0.12–0.14 mg/mL	Ulutas *et al.* (2008)
	0.1 g/L	Hashemnia *et al.* (2011)
	3.05±1.08 g/L	Fasulkov *et al.* (2014)
	0–0.05 g/L	Gonzalez *et al.* (2008)
	0.39–1.26 (0.784) mg/dL	Heller & Johns (2015)
	41.6 mg/L	Gonzalez *et al.* (2011)
	0.58 mg/L	Rahman *et al.* (2010)
	0.056±0.009 mg/L	Balikci *et al.* (2013)
SAA, sheep	2.67±0.23 mg/L	Eckersall *et al.* (2007)
	0.82±0.53 µg/mL	Wells *et al.* (2013)
	0.963–8.54 mg/L	Zhong *et al.* (2014)
	0–2.0 µg/mL	Lepherd *et al.* (2009)
	5.62±0.84 µg/mL	Gurdogan *et al.* (2014)
SAA, goats	4.20–12.65 µg/mL	Ulutas *et al.* (2008)
	6.1 µg/mL	Hashemnia *et al.* (2011)
	1.69–11.94 (4.88) mg/L	Gonzalez *et al.* (2008)
	0.42–2.2 (0.92) µg/ML	Heller & Johns (2015)
	8.7 µg/mL	Rahman *et al.* (2010)
	6.06±0.89 µg/mL	Balikci *et al.* (2013)
AGP, sheep	2.14±0.24 g/L	Eckersall *et al.* (2007)
	0.246–0.470 g/L	Zhong *et al.* (2014)
AGP, goats	4.6–40.5 (18.43) mg/dL	Heller & Johns (2015)
LBP, sheep	1.20–2.79 g/L	Zhong *et al.* (2014)
LBP, goats	0–0.68 (0.125) ng/mL	Heller Johns (2015)
Cp, sheep	11.8±2.3 –13.5±1.8 mg/dL	Ulutas & Ozpinar (2006)
	0.030–0.039 g/dL	Sousa Almeida *et al.* (2012)
Cp, goats	4.97–11.85 mg/dL	Ulutas *et al.* (2008)
	1.1 mg/L	Rahman *et al.* (2010)
CRP, sheep	0.51±0.19 – 0.55±0.10 mg/dL	Ulutas & Ozpinar (2006)
	5.235±0.429 – 9.115±0.647 mg/L	Vojtic & Krajnc (2000)
Fb, sheep	436.2±39.5 – 437.5±47.8 mg/dL	Ulutas & Ozpinar (2006)
	1.6–4.1 g/L	Lepherd *et al.* (2009)
Fb, goats	3.2±0.69 g/L	Fasulkov *et al.* (2014)
	2–4 (2) g/L	Gonzalez *et al.* (2008)
	2.67 g/L	Gonzalez *et al.* (2011)
Hpx, sheep	0.013–0.018 g/dl	Sousa Almeida *et al.* (2012)
ASG, goats	0.86–1.78 (1.25) g/L	Gonzalez *et al.* (2008)
	1.38 g/L	Gonzalez *et al.* (2011)
TF, sheep	0.352–0.448 g/dL	Sousa Almeida *et al.* (2012)
AAT, sheep	0.155–0.193 g/dL	Sousa Almeida *et al.* (2012)

cator for detecting animals with subclinical infections, determining the prognosis of clinical infection, differentiation between viral and bacterial disease, treatment monitoring, vaccine effectiveness and stress conditions. It is known that the increased APPs concentration is not specifical for a particular disease but reflects the overall health of the animals. Based on these data it can be concluded that the knowledge of APPs could be useful for establishment of herd health in small ruminants.

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