The Effect of Nutrition on Serum Acute Phase Reactants, Inflammatory Mediators and Gangliosides in Healthy Ostriches

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Abstract: In the present study the effects of nutrition on serum acute phase reactants Haptoglobin (Hp), Serum Amyloid A (SAA) and α1-Acid Glycoprotein (AGP), inflammatory mediators (Tumor Necrosis Factor-α (TNF-α)), Interferon-γ (IFN-γ) and gangliosides (Total Sialic Acid (TSA)), Lipid Bound Sialic Acid (LBSA) and Protein Bound Sialic Acid (PBSA) were investigated. Blood samples were collected from the jugular vein of ninety apparently healthy ostriches and the concentrations of serum acute phase reactants, inflammatory mediators and gangliosides were measured.

Key words: Nutrition, acute phase proteins, inflammatory mediators, gangliosides, ostriches, Iran

INTRODUCTION

The Acute-Phase Proteins (APPs) are a group of blood proteins that change in concentration in animals subjected to external or internal challenges such as infection, inflammation, surgical trauma or stress (Eckersall, 2004; Murata et al., 2004; Gruys et al., 2005). APPs and their changes due to various inflammatory and non-inflammatory conditions have been studied intensively in many animal species (Kansko, 1997; Murata et al., 2004; Murata, 2007). Glycoproteins are defined as proteins which contain glycans chains linked glycosidically to selected amino acid residues. Sialic acids as monosaccharides are linked to the terminal galactose, N-acetylgalactosamine or to other sialic acids in carbohydrate chains attached to glycoproteins and glycolipids. Sialic acids are often involved in important cell surface communications and infection processes and are present in normal serum in humans and animals; their content in serum changes in various diseases (King and Cavanagh, 1991; Ekin et al., 2003; Citil et al., 2004). Sialic acid concentration increases rapidly following the inflammatory and injury process (Ekin et al., 2003). The mechanism underlying the induction of sialic acid increase is not clearly understood. However, investigators have reported that sialic acid localized at the end chain of many acute-phase proteins can be used as marker for the determination of acute-phase protein concentrations (Thougaard et al., 1998; Enjuanes et al., 2000; Ekin et al., 2003) because serum acute-phase proteins, especially α1-acid glycoprotein are sialyted glycoproteins.

Tumor necrosis factor is a cytokine involved in systemic inflammation and is a member of the group of cytokines that stimulate the acute phase reaction. The primary role of TNF-α is in the regulation of immune cells. TNF-α is able to induce apoptotic cell death, induce inflammation and to inhibit tumorogenesis and viral replication (Walsh et al., 1991).

There is some evidence that shows immune response can be influenced by nutrition for example, a previous study indicates Conjugated Linoleic Acids (CLA) have a favorable influence on immune competence in nursery pigs (Bassaganya-Riera et al., 2001). Other favorable effects include improved feed efficiency, change in body composition and decreased incidence of conditions associated with adverse effects of the immune response, e.g., allergic reactions and autoimmune disorders (Crock, 1993). The change in diet and environment post-weaning can be an important stressor for piglets which can result in increased disease susceptibility (Fraser et al., 1994). Any condition which impairs Acute Phase Protein Response (APPR) and hence the ability to restore homeostasis may limit survival from injury. Malnutrition prior to surgery attenuates aspects of the APPR (Jennings et al., 1992) and this may in part, explain the associated increase in post-operative morbidity in malnourished patients (Allison, 1992). The cause of the attenuated APPR in malnutrition is unknown but may be cytokine mediated.

There are no studies on the effects of nutrition on serum acute phase reactants, inflammatory mediators and gangliosides in healthy ostriches. The aim of the present...
study was to find and compare the concentrations of serum acute phase proteins, inflammatory mediators and gangliosides in healthy ostriches that are fed with different diets.

**MATERIALS AND METHODS**

**Animals:** After clinical examinations and laboratory tests, 90 clinically healthy ostriches were selected from three ostrich farms located in the provinces around Shiraz.

**Ostrich dietary**

**Farm 1 (Group 1):** Corn 21%, alfalfa 36%, soybean 20.5%, barley 15%, CaCO3 4%, calcium phosphate 2.5%, mineral complex 0.25%, vitamin complex 0.45%, lysine 0.1%, methionin 0.1% and NaCl 0.3%.

**Farm 2 (Group 2):** Corn 22%, alfalfa 38.2%, soybean 18.95%, wheat 11%, CaCO3 4.7%, phosphate 2.7%, mineral complex 0.65%, vitamin complex 0.65%, Vitamin A 0.2%, Vitamin B 0.1%, soyan oil 1.3%, lysine 0.15%, B complex 0.2% and NaCl 0.3%.

**Farm 3 (Group 3):** Corn 14.1%, alfalfa 41.5%, soybean 10.36%, barley 10%, bran 15%, CaCO3 4.56%, phosphate 2-2.5%, mineral complex 0.5%, vitamin complex 0.5%, lysine 0.3%, methionin 0.12%, alaktin 0.52% and NaCl 0.54%.

**Blood sampling and processing:** Blood samples were collected from the jugular vein of healthy ostriches into tubes without anticoagulant. The sera were separated by centrifugation at 750 g for 15 min and stored at -20°C until used. In the serum of different groups of birds, acute phase proteins and inflammatory mediators were measured using validated standard procedures.

**Acute phase reactants determination**

**Haptoglobin determination:** Haptoglobin (Hp) was measured according to prevention of the peroxidase activity of hemoglobin which is directly proportional to the amount of Hp. The analytical sensitivity of this test in serum has been determined as 0.0156 mg mL⁻¹ for Hp by the manufacturer (Tridelta Development Plc, Wicklow, Ireland).

**Serum amyloid A determination:** Serum Amyloid A (SAA) was measured by a solid phase sandwich ELISA. The analytical sensitivity of this test in serum has been determined as 0.3 μg mL⁻¹ for SAA by the manufacturer (Tridelta Development Plc, Wicklow, Ireland).

**Serum α1-acid glycoprotein determination:** Serum α1-acid glycoprotein was measured by Radial-Immunodiffusion Method (Tridelta Development Plc, Wicklow, Ireland).

**Gangliosides determination**

**Total sialic acid determination:** Serum total sialic acid concentration was determined by the thiobarbituric acid method previously described by Warren (1959). The amount of total sialic acid was determined by use of a standard curve developed from a standard sample of N-acetyl neuraminic acid.

**Lipid bound sialic acid determination:** Lipid bound sialic acid concentration was determined by the method described by Katopodis et al. (1982). The amount of lipid bound sialic acid was determined by use of a standard curve developed from a standard sample of N-acetyl neuraminic acid.

**Protein bound sialic acid determination:** Protein bound sialic acid concentration was measured by subtracting serum total sialic acid from lipid bound sialic acid.

**Inflammatory mediators determination**

**IFN-γ and TNF-α determination:** IFN-γ and TNF-α were measured by a solid phase sandwich ELISA (AbC 606 and AbC 607, respectively, Your fournisseur AbCys S.A. Paris, France).

**Statistical analysis:** Data were analyzed by SPSS Software, Version 11.5. Descriptive statistics including mean and standard error were calculated for all variables. In the present study ANOVA, Duncan and t-test were done. A p<0.05 was considered as statistically significant.

**RESULTS AND DISCUSSION**

The mean±SE of acute phase proteins, inflammatory mediators and gangliosides in 90 clinically healthy ostriches belonging to 3 groups was shown in Table 1. The significant statistical differences between different parameters in the 3 groups was shown in Table 2. The statistical evaluations showed that there were no significant differences between acute phase proteins, LBSA, TNF-α and IFN-γ in the different groups (p>0.05). There was a statistical significant difference between TSA in Group 2 and 3 (p = 0.007) and there were statistical significant differences between PBSA and in Group 3 with Group 1 and 2 (p = 0.024 and p = 0.013, respectively).

The concentrations of serum Hp in clinically healthy ostriches in Farms 1-3 were 0.078±0.0027, 0.073±0.0017,
Table 1: Mean±SE of serum concentrations of acute phase proteins, inflammatory mediators and gangliosides in 30 clinically healthy ostriches in 3 groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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<tr>
<td></td>
<td>Mean</td>
<td>Standard error</td>
<td>Mean</td>
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<tr>
<td>Hp (g L⁻¹)</td>
<td>0.078</td>
<td>0.0027</td>
<td>0.073</td>
</tr>
<tr>
<td>SAA (µg mL⁻¹)</td>
<td>1.7200</td>
<td>0.0090</td>
<td>1.73</td>
</tr>
<tr>
<td>TSA (nmol L⁻¹)</td>
<td>0.00044</td>
<td>0.0</td>
<td>0.00039</td>
</tr>
<tr>
<td>LBSA (nmol L⁻¹)</td>
<td>0.000178</td>
<td>0.0</td>
<td>0.000175</td>
</tr>
<tr>
<td>PBSA (nmol L⁻¹)</td>
<td>0.00026</td>
<td>0.00001</td>
<td>0.00028</td>
</tr>
<tr>
<td>TNF-α (pg dL⁻¹)</td>
<td>16.61</td>
<td>0.57</td>
<td>16.48</td>
</tr>
<tr>
<td>IFN-γ (pg dL⁻¹)</td>
<td>9.37</td>
<td>0.2415</td>
<td>9.5500</td>
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The concentrations of serum amyloid A in Farms 1-3 were 1.72±0.039 1.72±0.026 and 1.77±0.044 mg mL⁻¹ and are in agreement with the other research on ostriches and also the normal value of avian that was observed by Nazifi et al. (2011). Statistical evaluation showed that there were no significant differences between Hp and SAA so, the nutrition does not have any effect on acute phase proteins in ostriches.

Addition of cholesterol to a high-fat diet increases plasma SAA levels and atherosclerosis independent of an adverse effect on plasma lipoproteins, consistent with the hypothesis that SAA may promote atherosclerosis directly by mediating retention of SAA-enriched HDL to vascular proteoglycans (Lewis et al., 2004).

Diet high in saturated fat and cholesterol which are associated with increased risk of cardiovascular disease in humans increase SAA levels in mice (Grundy and Denke, 1990; Liao et al., 1994). An atherogenic diet has previously been shown to increase SAA levels in C57BL/6 mice. The diet used in that study contained very high levels of cholesterol and cholic acid (Liao et al., 1994).

In this study, it was observed that α1-acid glycoprotein was not detectable in serum of any of the farm’s ostriches which is in agreement with the previous study. This is in agreement to Curtis et al. (1995) finding about malnutrition effect on α1-acid glycoprotein. These researchers did a study on the effect of nutritional status on the magnitude of the acute phase protein response and determined whether this is associated with changes in the magnitude of the related cytokine responses. They reported a significant reduction in the plasma C-reactive protein response in the malnourished group but no difference between the groups in the responses of α1-antitrypsin, α1-acid glycoprotein or in the trace elements iron or zinc which reflect induction of ferritin and metallothionein. Dietary CLA had no statistical effects on serum levels of α1-acid glycoprotein. α1-acid glycoprotein is an acute inflammatory phase reactant mediated by interleukin-1, interleukin-6 and tumor necrosis factor α (Eckersall et al., 1996).

The concentration of acute phase proteins is generally low to non detectable in healthy animals and

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<th>Parameters</th>
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<tr>
<td>Hp (g L⁻¹)</td>
<td>1</td>
<td>2</td>
<td>0.400</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.983</td>
<td></td>
</tr>
<tr>
<td>SAA (µg mL⁻¹)</td>
<td>1</td>
<td>2</td>
<td>0.984</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.571</td>
<td></td>
</tr>
<tr>
<td>TSA (nmol L⁻¹)</td>
<td>1</td>
<td>2</td>
<td>0.376</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.367</td>
<td></td>
</tr>
<tr>
<td>PBSA (nmol L⁻¹)</td>
<td>1</td>
<td>2</td>
<td>0.676</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.210</td>
<td></td>
</tr>
<tr>
<td>LBSA (nmol L⁻¹)</td>
<td>1</td>
<td>2</td>
<td>0.967</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.024*</td>
<td></td>
</tr>
<tr>
<td>TNF-α (pg dL⁻¹)</td>
<td>1</td>
<td>2</td>
<td>0.967</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.043*</td>
<td></td>
</tr>
<tr>
<td>IFN-γ (pg dL⁻¹)</td>
<td>1</td>
<td>2</td>
<td>0.024*</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.013</td>
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*Significant in p<0.05; **Significant in p<0.01

0.07±0.0036 µg mL⁻¹, respectively which are in agreement with the concentrations of serum Hp in clinically healthy ostriches found in another study on healthy ostriches and in clinically healthy avian reported by Nazifi et al. (2010a, b).
elevations are used to diagnose and monitor inflammatory diseases (Feldman et al., 2000). Albumin concentration is associated with levels of several acute phase proteins, C-reactive protein, α1-acid glycoprotein or ceruloplasmin and with nutritional markers such as normalized protein catabolic rate. Albumin concentration in dialysis patients changes with inflammation and nutritional status through their effects on albumin catabolism and synthesis, respectively. Within the range of albumin levels in these patients, nutritional variables primarily affected albumin synthesis while inflammation caused hypoalbuminemia by increasing the albumin Fractional Catabolic Rate (FCR). Albumin synthesis also increased in proportion to Plasma Volume (PV) as % body weight. The PV expansion does not contribute to hypoalbuminemia (Kaysen et al., 2002).

Dietary CLA supplementation increases IgG levels compared to controls (p<0.05) and a significant linear effect of the dietary treatment is also found (p = 0.03) (Corino et al., 2002). Sugano et al. (1997) observed increased serum IgG in rats fed diets supplemented with 0.5 and 1% CLA. According to these researchers, CLA regulate the production of immunoglobulins, increasing the concentrations of IgA, IgG and IgM and decreasing IgE concentration (Pescevitz, 1998).

The serum APPs have been used as nonspecific clinical markers of health problems in humans and other mammalian species (Gabay and Kushner, 1999). APPs can be used for prognosis and diagnosis of disease and may provide a similar use in identifying poultry health problems.

The acute phase response is mediated by the pro-inflammatory cytokines which induce the synthesis of acute phase proteins by the liver, particularly Interleukin-1 (IL-1), Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6) (Zetterstrom et al., 1998). The pro-inflammatory cytokines induce the synthesis of acute phase proteins by the liver. There are major differences between various animal species in the APP response in disease (Eckersall, 2006). Several acute phase proteins have been analyzed in chicken in association with common poultry diseases. Most of these APPs do not change to the same level as mammalian APPs and could not be analyzed in natural infections. In mammals, the increased synthesis of acute phase proteins in response to an immune challenge varies in magnitude from a 50% increase in ceruloplasmin to a several hundred-fold increase in C-reactive protein (Barnes et al., 2001). In chickens, only a few APPs have been described so far. Of these, the plasma α1-acid glycoprotein, SAA, transferrin, TSA, LBSA, PBBSA and ovotransferrin (Inoue et al., 1997; Chamanza et al., 1999; Holt and Gast, 2002; Xie et al., 2002, Nazifi et al., 2010a-c, 2011) can be mentioned.

Inflammation or tissue injury causes the release of pro-inflammatory cytokines such as IL-1, IL-6 and tumour necrosis factor which alter the blood concentration of a variety of proteins that are produced primarily in liver. Tracheitis, bronchitis, tracheal edema, bronchial caseous plugs and air sacculitis indicate widespread inflammatory reaction in IBV and this causes release and elevation of SAA and Hp concentrations. Kovacs et al. (2007) showed a mild increase in SAA in the goose by administration of a fowl cholera vaccine containing inactivated Pasteurella multocida. Vaccination was an inflammatory factor and produced increased SAA levels.

Nazifi et al. (2010a-c) showed a significant increase in SAA levels of infectious bursal disease virus-infected chicks. Chamanza et al. (1999) reported that administration of terpentint to pullet and Staphylococcus aureus infection in chicks cause an elevation in SAA and transferrin concentration. The concentrations of serum LBSA in ostriches of Farms 1-3 were 0.00026±0.0001, 0.00028±0.0001 and 0.00026±0.0001 mmol L⁻¹, respectively. The concentrations of serum PBBSA in ostriches of Farms 1-3 were 0.000178±0.0000, 0.0000175±0.0000 and 0.00021±0.00001 mmol L⁻¹, respectively and the concentrations of serum TSA in ostriches of Farms 1-3 were 0.00034±0.0000, 0.00039±0.0000 and 0.00047±0.0000 mmol L⁻¹, respectively.

The concentrations of serum TNF-α in ostriches of Farms 1-3 were 16.61±0.57, 16.48±0.434 and 15.996±0.870 pg DL⁻¹, respectively and the concentrations of serum INF-γ in ostriches of Farms 1-3 were 9.37±0.2415, 9.55±0.2617 and 9.79±0.436 pg DL⁻¹, respectively. The comparison of concentrations of serum LBSA, PBBSA, TSA, TNF-α and INF-γ in this study are in agreement with the other research also, these parameter levels in ostriches are in agreement with the normal value of avian and are lower than the normal value of cattle serum LBSA, PBBSA, TSA, TNF-α and INF-γ that have been reported before (Nazifi et al., 2011).

The cytokine responses in the three groups were similar and hence, the different nutrition in different groups was not sufficient to influence the cytokine response. There is an early increase in IL-6, soluble receptors of TNF and IL-1 receptor antagonist in the malnourished and well nourished groups but no detectable increase in plasma IL-1 or TNF. There is no difference between either group for any of these markers of activation of the cytokine network. Weight loss is therefore associated with a reduction in aspects of the acute phase response but this is due to impaired effectiveness rather than reduced magnitude of the cytokine response (Curtis et al., 1995).
They are capable of stimulating mononuclear phagocytes and endothelial cells to release immune modulators such as TNF-α, members of the interleukin family (IL-1, IL-6, IL-8 and IL-12) and interferon-α (Hamaun et al., 1998). There were statistical significant differences between TSA in Groups 2 and 3 (p = 0.007) also, between PBSA in Group 3 with Groups 1 and 2 (p = 0.024 and 0.013, respectively), on the other hand, TSA and PBSA have been affected by nutrition.

The concentrations of TSA, LBSA and PBSA are significantly higher in diseased birds than healthy avian (Farsang et al., 2002; Nazifi et al., 2011). Malnutrition is well-known to cause a reduction in the plasma concentration of many proteins, especially those with a short half life such as prealbumin and transferring (Fleck et al., 1985). This reflects the diversion of amino acids from the synthesis of these proteins into other proteins which are more essential for immediate survival. It may also partially explain the fall in the concentrations of these proteins when an acute phase response is induced and when there is synthesis of a large number of proteins necessary for containment of inflammation and repair of diseased tissue (Whicher and Westcott, 1992).

At the same time, however, increased transcapillary escape of proteins occurs as a result of local inflammatory mediators acting on the capillary wall. It seems that in the study, all of the groups received enough of the necessary materials and the different diets had no effect on Hp, SAA, α1-acid glycoprotein, TNF-α, IFN-γ and LBSA.

To summarize, these findings suggest that TSA and PBSA are affected by nutrition but Hp, SAA, α1-acid glycoprotein, TNF-α and IFN-γ are not affected by nutrition.

CONCLUSION

The results of the study showed that nutrition has no effect on the serum concentration of Hp, SAA, LBSA, TNF-α and IFN-γ but diet does have an effect on TSA and PBSA.

REFERENCES


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