

Effect of dietary protein concentration on responses to *Escherichia coli* endotoxin in broiler chickens

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(Received 8 September 1994 – Revised 6 December 1994 – Accepted 6 January 1995)

The effect of dietary protein concentration on stress responses against injection of *Escherichia coli* lipopolysaccharide (LPS) was studied in male broiler chickens. Chickens (7 d of age) were fed on a 100 (low-protein; LP) or 300 g protein/kg (high-protein; HP) diet for 2 weeks. LPS was injected intraperitoneally every 2 d during the final 6 d, or once 16 h before the end of the experiment, at a concentration of 900 µg/chick. The LPS injection did not affect body-weight gain, feed intake, gain:intake ratio, or plasma Fe concentration. The single injection of LPS reduced plasma Zn concentration, but the repeated injections did not. Feeding the HP diet increased the response of plasma Zn concentration to the single injection of LPS. Plasma albumin concentration was reduced by LPS injection. Feeding the HP diet resulted in a higher plasma α 1-acid glycoprotein (AGP) concentration than feeding the LP diet, in chicks untreated with LPS. An increase in plasma AGP concentration observed after LPS injection in chicks fed on the LP diet was greater than that seen in chicks fed on the HP diet. No significant changes in plasma AGP concentration in response to repeated injections of LPS were observed in chicks fed on the HP diet. Plasma interleukin-1 (IL-1)-like activity was greater in chicks fed on the LP diet than in those fed on the HP diet, when LPS was injected. The response of plasma IL-1-like activity to the single injection of LPS in chicks fed on the LP diet was the greatest among the treatment groups. These results suggest that acute-phase responses to LPS injection are much greater in chicks fed on a LP diet than in those fed on a HP diet, and multiple injection of LPS weakens the responses.

Acute-phase response: Dietary protein: Lipopolysaccharide

Exposure of animals to infectious or inflammatory agents not only induces immune responses in the host, but also causes metabolic changes that lead to decreased rates of gain and feed consumption. Cytokines are involved in communication between immune cells, and between immune cells and other cells of the animal body. Cytokines also act indirectly on the liver by creating a hormonal milieu for enhancing production of acute-phase proteins. Thus, leucocyte products, cytokines, play significant roles in immunity, nutrient metabolism and the endocrine system. It is well known that of all cytokines, interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF) are important regulators of metabolic responses during the early stages of inflammation.

Nutrition can influence the release of IL-1, IL-6 and TNF following an immune response and consequently the outcome of infectious challenges (Klasing, 1988; Klasing & Barnes, 1988; Grimble, 1990, 1992; Klasing & Johnstone, 1991). Clinical and experimental animal studies suggest that protein deficiency influences the metabolic responses to endotoxin and modifies the ability of monocytes to produce cytokines (Hoffman-Goetz & Kluger, 1979;

Hoffman-Goetz *et al.* 1981, 1985; Kauffman *et al.* 1982; Keenan *et al.* 1982; Hoffman-Goetz & Marcon, 1983; Drabik *et al.*, 1987). There is evidence that both the release rate of IL-1 in stimulated monocytes and the hepatic and hypothalamic responses to the released IL-1 are lowered by protein deficiency, as a decrease in febrile response to endotoxin has been shown in protein-deficient rats (Hoffman-Goetz *et al.* 1985; Bradley *et al.* 1987; Bradley & Kauffman, 1988). However, it is not known how feeding a low-protein (but not protein-free) diet affects IL-1 production or activity and how dietary protein concentration affects acute-phase responses following the repeated stimulation of an immunogen.

Acute-phase proteins are concerned with defence mechanisms against tissue damage and infections (Fleck, 1989; Wan *et al.* 1989), and may possibly be an indicator of activity or production of IL-6 in chickens (Klasing & Johnstone, 1991). α 1-Acid glycoprotein (AGP) is an acute-phase protein also present in chicks (Takahashi *et al.* 1994). Acute-phase-protein synthesis is probably also influenced by nutritional status, but nutritional modification of the synthesis is not clear as yet even in mammals.

The present study was undertaken to determine the effects of dietary protein concentration, and single or repeated administration of LPS on the acute phase of stress responses in broiler chickens.

MATERIALS AND METHODS

Animals and diets

Non-vaccinated male broiler chicks (1 d old) were housed in a battery brooder with electric heating for 1 week. Chicks were selected from a twofold larger population to obtain uniform body weight, and randomly assigned to eight groups of eight chicks, with four replicates of two chicks per cage. The birds were fed *ad lib.* on a low-protein (LP, 100 g/kg) or a high-protein (HP, 300 g/kg) diet (Table 1) without any antibiotics or anticoccidial drugs for 2 weeks. The protein concentration in the diets was calculated from the nutrient composition of feedstuffs used in poultry diets (National Research Council, 1984). Body weights were determined individually before injection of lipopolysaccharide (LPS) and at the end of the experiment. Feed intake for 14 d was determined per cage of two chicks and body-weight gain/100 g feed intake was calculated using the data from two chicks per cage for 14 d.

Preparation and injection of lipopolysaccharide

Escherichia coli LPS was obtained from Difco Laboratories (Detroit, MI, USA) and dissolved, in sterilized saline (9 g NaCl/l) at a concentration of 900 μ g/ml. Half of the chicks in each dietary group were injected intraperitoneally once with either 900 μ g LPS or sterilized saline 16 h before the end of the experiment. The other half were injected with either the same amount of LPS or the saline every 2 d during the final 6 d of the experiment, and the final injection was made at the same time as the groups receiving the single injection.

Preparation of the interleukin-1 fraction from blood

Blood was taken via a wing vein and plasma was separated by centrifugation (500 g, 15 min). To prepare the IL-1 fraction, 3 ml plasma, a mixture of equal volumes of plasma obtained from two chicks kept in the same cage, was concentrated by using ultrafiltration (Amicon PM 100; WR Grace & Co. Conn., Beverly, MA, USA). The resulting solution was passed through membrane filters (Amicon PM 10 and PM 30), yielding filtrates with proteins in the molecular ranges 10000 to 30000. The filtrates were sterilized by membrane filtration, lyophilized, and stored at -80° until analysis.

Table 1. *Compositions (g/kg) of the low-protein and high-protein chick diets**

| Ingredient | Low protein | High protein |
|----------------------------------|-------------|--------------|
| Maize | 890.0 | 369.3 |
| Soyabean meal | 43.4 | 445.4 |
| Soyabean protein | — | 70.0 |
| DL-Methionine | 0.6 | 3.9 |
| L-Lysine | 1.8 | — |
| L-Arginine | 2.0 | — |
| Soyabean oil | 18.7 | 72.6 |
| Calcium carbonate | 13.2 | 11.9 |
| Calcium phosphate | 19.4 | 16.1 |
| Sodium chloride | 2.9 | 2.9 |
| Vitamin mixture† | 4.0 | 4.0 |
| Mineral mixture† | 4.0 | 4.0 |
| Calculated crude protein (g/kg)‡ | 100.0 | 300.0 |

* Calculated metabolizable energy was 13.4 MJ/kg diet for each diet.

† See Akiba & Matsumoto (1978).

‡ Crude protein content was calculated from feedstuff composition tables (National Research Council, 1984).

Determination of interleukin-1-like activity

IL-1-like activity was determined by its capacity to augment mitogen-stimulated thymocyte proliferations. Thymuses were obtained from 6-week-old chicks and thymocytes were teased from the thymus through a stainless steel mesh ($< 100 \mu\text{m}$). Mononuclear cells of the thymus were isolated by layering the cell sample over 1.077 specific gravity Ficoll-Isopaque and centrifuging (600 g at 15° for 50 min). The mononuclear layer was collected and washed three times by centrifuge (650 g at 15° for 10 min) in a RPMI-1640 medium. The filtrates containing the IL-1 fraction were dissolved in 300 μl RPMI-1640 medium. A 50 μl sample of the filtrate was incubated with 2×10^6 thymocytes in ninety-six-well plates and 1 μg phytohemagglutinin-P (PHA-P; Difco BRL, New York, USA) per well in the RPMI-1640 medium supplemented with 100 units/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin (Difco) and 50 ml/l autologous serum to give a final volume of 200 μl . Serum was inactivated at 56° for 30 min and sterilized. After incubation in a 50 ml/l CO_2 , humidified atmosphere at 42° for 54 h, each well was pulsed with 37 Bq [^3H]thymidine and incubated for an additional 16 h. Cells were harvested onto glass-fibre filters with a cell harvester and isotope incorporation into DNA was determined by scintillation counting. The results were expressed as disintegrations/min (dpm).

Determination of α 1-acid glycoprotein, albumin, iron and zinc concentrations

Plasma AGP concentration was determined using chicken AGP plate (single immunodiffusion; Saikin Kagaku Institute, Sendai, Japan), incorporating specific anti-chicken AGP serum of rabbit origin in agarose gel as described previously (Takahashi *et al.* 1994). Plasma albumin concentration was determined by an assay kit obtained from Wako Pure Chemicals Ltd (Osaka, Japan). Plasma Zn and Fe concentrations were determined by assay kits (Wako Pure Chemicals) based on colourimetric determinations using 2-(5-bromo-2-pyridylazo)-5-(N-n-propyl-N-3-sulfopropylamino)-phenol (Makino *et al.* 1982) and 2-nitroso-5-(N-propyl-N-sulfopropylamino)-phenol (Saito *et al.* 1981) respectively. Measurements of these variables were carried out in the plasma of individual chicks.

Table 2. *Effects of a single injection of Escherichia coli lipopolysaccharide (LPS; 900 µg/chick) and dietary protein concentration on body-weight gain, feed intake and gain:intake ratio in broiler chickens†*

(Mean values with their standard errors)

| Protein concentration (g/kg) | Body-wt gain‡ | | | | | | Feed intake§ | | Weight gain:feed intake for 14 d§ | |
|---------------------------------|--------------------------|----|-------------------------|----|------------------|----|--------------|----|-----------------------------------|-----|
| | Before LPS injection (g) | | After LPS injection (g) | | Overall (g/14 d) | | (g/14 d) | | (g/100 g) | |
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| 100 Saline | 208 | 10 | 15 | 6 | 225 | 12 | 687 | 11 | 33.1 | 1.6 |
| 100 LPS | 190 | 11 | 5 | 3 | 194 | 11 | 630 | 21 | 30.8 | 1.3 |
| 300 Saline | 495 | 17 | 35 | 7 | 530 | 19 | 801 | 11 | 66.1 | 2.0 |
| 300 LPS | 490 | 15 | 25 | 5 | 515 | 16 | 830 | 15 | 62.2 | 2.2 |
| Analysis of variance, <i>P</i> | | | | | | | | | | |
| Protein concentration | ** | | ** | | ** | | ** | | ** | |
| LPS injection | — | | NS | | NS | | NS | | NS | |
| Interactions | — | | NS | | NS | | NS | | NS | |

NS, not significant.

** *P* < 0.01.

† For details of diets and procedures, see Table 1 and p. 174.

‡ *n* 8.

§ Calculated on the basis of two chicks/cage (*n* 4).

Statistical analysis

A 2 (dietary protein concentration) × 2 (the injection procedure, the LPS and saline injections) factorial experimental test was applied to analyse the data in each of the experiments (single and repeated injections) and the calculation was carried out using the General Linear Model of SAS (SAS Institute, Cary NC, USA).

RESULTS

Table 2 shows the effect of a single injection of LPS on body-weight gain, feed intake and gain:intake ratio in chicks fed on the LP or HP diets. The LPS injection did not affect body-weight gain, feed intake or body-weight gain:feed intake ratio over 14 d.

Table 3 shows the effect of a single injection of LPS on plasma concentrations of Fe, Zn, albumin and AGP, and plasma IL-1-like activity in chicks fed on the LP or HP diets. Plasma Zn concentration was decreased by LPS injection and the decrease in chicks fed on the HP diet was substantial compared with that of chicks fed on the LP diet. Neither LPS injection nor dietary protein concentration affected plasma Fe concentration. In chicks fed on the HP diet plasma albumin concentration was higher than that of chicks fed on the LP diet, and was reduced by LPS injection. Plasma AGP concentration was higher in chicks fed on the HP diet than those fed on the LP diet. The single injection of LPS resulted in a fivefold increase in plasma AGP concentration in chicks fed on the LP diet compared with their saline-control counterparts, whereas those given the HP diet showed about a threefold increase. Plasma IL-1-like activity after LPS injection in chicks fed on the LP diet was five times greater than that in the saline-injected control, whereas LPS injection in chicks fed on the HP diet resulted in approximately the same activity as in the control birds.

Table 4 shows the effect of repeated injections of LPS on body-weight gain, feed intake

Table 3. *Effects of a single injection of Escherichia coli lipopolysaccharide (LPS; 900 µg/chick) and dietary protein concentration on plasma concentrations of iron, zinc, albumin and α 1-acid glycoprotein (AGP), and plasma interleukin-1 (IL-1) activity in broiler chickens†*

(Mean values with their standard errors)

| Protein concentration (g/kg) | Iron† (µg/l) | | Zinc† (µg/l) | | Albumin† (mg/ml) | | AGP† (µg/ml) | | IL-1-like activity§ (dpm × 10 ³) | |
|--------------------------------|--------------|----|--------------|-----|------------------|-----|--------------|----|--|------|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| 100 Saline | 910 | 70 | 1730 | 80 | 8.8 | 0.5 | 179 | 23 | 1.09 | 0.27 |
| 100 LPS | 650 | 80 | 1520 | 60 | 7.5 | 0.3 | 832 | 65 | 5.61 | 0.59 |
| 300 Saline | 690 | 80 | 1620 | 120 | 11.5 | 0.4 | 323 | 30 | 1.10 | 0.18 |
| 300 LPS | 640 | 80 | 980 | 80 | 10.2 | 0.5 | 983 | 73 | 1.14 | 0.34 |
| Analysis of variance, <i>P</i> | | | | | | | | | | |
| Protein concentration | NS | | * | | ** | | * | | ** | |
| LPS injection | NS | | ** | | ** | | ** | | ** | |
| Interactions | NS | | * | | NS | | NS | | ** | |

NS, not significant.

* *P* < 0.05, ** *P* < 0.01.

† For details of diets and procedures, see Table 1 and pp. 174–176.

‡ *n* 8.

§ *n* 4.

|| The value for [³H]thymidine in thymocytes incubated with the medium plus phytohaemagglutinin-P was 960 (SE 54) dpm (*n* 4).

Table 4. *Effects of repeated (three times) injections of Escherichia coli lipopolysaccharide (LPS; 900 µg/chick) and dietary protein concentration on body-weight gain, feed intake and gain: intake ratio in broiler chickens†*

(Mean values with their standard errors)

| Protein concentration (g/kg) | Body-wt gain‡ | | | | | | Weight gain: feed intake for 14 d§ (g/100 g) | | | |
|--------------------------------|------------------------------|----|-----------------------------|----|------------------|----|--|----|--|-----|
| | Before LPS injection (g/8 d) | | After LPS injection (g/6 d) | | Overall (g/14 d) | | Feed intake§ (g/14 d) | | Weight gain: feed intake for 14 d§ (g/100 g) | |
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| 100 Saline | 104 | 8 | 90 | 5 | 193 | 11 | 656 | 8 | 29.5 | 1.4 |
| 100 LPS | 110 | 12 | 78 | 10 | 188 | 18 | 632 | 12 | 29.7 | 2.8 |
| 300 Saline | 238 | 16 | 264 | 14 | 500 | 25 | 822 | 15 | 61.3 | 3.6 |
| 300 LPS | 258 | 14 | 265 | 9 | 523 | 19 | 828 | 16 | 63.3 | 2.4 |
| Analysis of variance, <i>P</i> | | | | | | | | | | |
| Protein concentration | ** | | ** | | ** | | ** | | ** | |
| LPS injection | — | | NS | | NS | | NS | | NS | |
| Interactions | — | | NS | | NS | | NS | | NS | |

NS, not significant.

** *P* < 0.01.

† For details of diets and procedures, see Table 1 and p. 174.

‡ *n* 8.

§ Calculated on the basis of two chicks/cage (*n* 4).

Table 5. *Effects of repeated injections (three times) of Escherichia coli lipopolysaccharide (LPS; 900 µg/chick) and dietary protein concentration on plasma concentrations of iron, zinc, albumin and α 1-acid glycoprotein (AGP), and plasma interleukin-1 (IL-1) activity in broiler chickens†*

(Mean values with their standard errors)

| Protein concentration (g/kg) | | Iron† (µg/l) | | Zinc† (µg/l) | | Albumin‡ (mg/ml) | | AGP‡ (µg/ml) | | IL-1-like activity§ (dpm × 10 ³) | |
|--------------------------------|--------|--------------|----|--------------|-----|------------------|-----|--------------|----|--|------|
| | | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| 100 | Saline | 1020 | 80 | 1790 | 60 | 8.8 | 0.6 | 179 | 6 | 1.21 | 0.24 |
| | LPS | 990 | 70 | 1620 | 80 | 7.5 | 0.3 | 522 | 64 | 2.01 | 0.65 |
| 300 | Saline | 920 | 40 | 1750 | 140 | 12.4 | 0.5 | 338 | 14 | 1.10 | 0.13 |
| | LPS | 800 | 60 | 1840 | 100 | 11.2 | 0.3 | 364 | 32 | 1.71 | 0.68 |
| Analysis of variance, <i>P</i> | | | | | | | | | | | |
| Protein concentration | | NS | | NS | | ** | | * | | NS | |
| LPS injection | | NS | | NS | | ** | | ** | | * | |
| Interactions | | NS | | NS | | NS | | * | | NS | |

NS, not significant.

* $P < 0.05$, ** $P < 0.01$.

† For details of diets and procedures, see Table 1 and pp. 174–176.

‡ n 8.

§ n 4.

|| The value for [³H]thymidine in thymocytes incubated with the medium plus phytohaemagglutinin-P was 960 (SE 54) dpm (n 4).

and gain: intake ratio in chicks fed on the LP or HP diets. LPS injection did not affect body-weight gain for 6 d after starting the injections, and did not affect feed intake, or body-weight gain: feed intake ratio for 14 d.

Table 5 shows the effect of repeated injections of LPS on plasma concentrations of Fe, Zn, albumin and AGP, and plasma IL-1-like activity in chicks fed on the LP or HP diets. Neither plasma Fe nor Zn concentration was influenced by LPS injection or dietary protein concentration. Plasma albumin concentration was higher in chicks fed on the HP diet than in chicks fed on the LP diet, and was reduced by LPS injection. Plasma AGP concentration was increased by repeated LPS injections in chicks fed on the LP diet, but not in chicks fed on the HP diet. Plasma IL-1-like activity was increased by 1.5 to 2 times the control after repeated LPS injections, whereas no significant effect of dietary protein level on the IL-1-like activity was observed.

DISCUSSION

Klasing *et al.* (1987) reported reduction of growth and feed utilization in a model system for studying immunologic stress by frequent injections of immunogens such as LPS, sheep erythrocytes and Sephadex in chicks under controlled laboratory conditions. In the present experiment we were not able to induce any changes in growth and feed utilization by repeated injections of LPS even using a similar model to that of Klasing *et al.* (1987). Changes in growth following immunogen injections appeared to be modified by the diet composition: feeding a soyabean–maize-based diet caused a greater response to immunogen than when maize–maize–gluten-based and carbohydrate-rich diets were fed (Klasing *et al.* 1987; Benson *et al.* 1993). The diets used in the present experiment were similar in composition and ingredients to those of Klasing *et al.* (1987), in which significant

immunogen-related reductions in body weight and feed intake were found after repeated injections of immunogen. A possible explanation of the conflict in results may be the differences in serotype of LPS used (Curtis & Flack, 1980) and/or dosage of LPS (Johnson *et al.* 1993).

Repeated injections of LPS reduced the response of plasma IL-1-like activity compared with the single LPS injection in the present experiment. Friedman *et al.* (1992) showed that continuous endotoxin infusion suppressed rat spleen cell production of cytokines including IL-1. It is, therefore, concluded that repeated or continuous LPS administration weakens the response of IL-1 production in chicks as well. It is known that immunogen increases the production or release of IL-1 from monocytes and/or macrophages and glucocorticoid hormones through stimulation of the pituitary-adrenal axis (Klasing, 1988; Grimble, 1990, 1992; Klasing & Johnstone, 1991). The enhanced IL-1 activity also stimulates hepatocytes to elaborate acute-phase protein (Grimble, 1990, 1992). Glucocorticoids (Besedovsky *et al.* 1986; Guyre *et al.* 1988) and some of the acute-phase proteins, including AGP (Bories *et al.* 1990), inhibit IL-1 production. Thus, immunoregulatory feedback systems exist between IL-1 and glucocorticoids and/or some acute-phase proteins. Therefore, the reduced responsiveness to multiple injections of LPS in the present study with chickens may have been elucidated by the mechanisms considered in mammals.

A protein-deficient diet is known to reduce the metabolic responses to endotoxin and the ability of monocytes to produce cytokines (Hoffman-Goetz & Kluger, 1979; Hoffman-Goetz *et al.* 1981, 1985; Kauffman *et al.* 1982; Keenan *et al.* 1982; Hoffman-Goetz & Marcon, 1983; Drabik *et al.* 1987). In most of the studies with mammals the effects of IL-1 on animals fed on a low-protein or protein-free diet were estimated by thermoeffector responses to IL-1 from macrophage culture or endotoxin. The present study showed that the production of IL-1-like substances following the single injection of LPS was enhanced to a greater extent in chicks fed on the LP diet than in chicks fed on the HP diet, regardless of the LPS injection programme. These results indicate that a moderately low protein concentration in the diet increases the response of IL-1 production compared with a high dietary protein concentration. However, Hannum *et al.* (1990) and Eisenberg *et al.* (1990) showed that human monocytes induce the IL-1-receptor antagonist (18–22 kDa) which could be important for *in vivo* regulation of IL-1 activity, and that expression of the cDNA in *E. coli* yields IL-1-receptor-antagonist activity. The findings suggest that the enhanced IL-1 activity in chicks fed on the LP diet may be due to the lower production of the IL-1 antagonist rather than higher synthesis of IL-1, or that feeding the HP diet may induce the antagonist to a greater extent than the LP diet in the procedure for determination of the IL-1 activity. However, Bradley & Kauffman (1988) observed that, in rats fed on a normal-protein diet, there was a reduction in febrile response to IL-1-containing supernatant fractions from macrophage cultures prepared from rats fed on a low-protein diet, and suggested that IL-1 production estimated by macrophage yield in rats fed on a low-protein diet was lower than that in rats fed on the control diet. The observations of Bradley & Kauffman (1988) and those from the present study suggest that changes in the IL-1 activity in animals fed on a low-protein diet cannot be explained by changes in the production of IL-1 receptor antagonist alone. Moreover, there is no direct evidence that dietary protein concentration affects the production of the antagonist. Further studies are needed to account for the different responses of IL-1 to dietary protein concentration between the present experiment and the previous studies with mammals.

Although dietary protein deprivation has a greater effect on IL-1 production than energy restriction (Hoffman-Goetz *et al.* 1985), energy restriction also affects production of IL-1 in monocytes (Hoffman-Goetz & Marcon, 1983). Moreover, a low-energy diet and/or fasting enhance various immune effector mechanisms, possibly as a consequence of

increasing macrophage activity (Wing *et al.* 1983). It is likely that the enhanced response of IL-1 activity to the LP diet might be caused not only by dietary protein concentration, but also by energy intake, since feed intake was lower in chicks fed on the LP diet than the HP diet. Generally, nutrient deficiencies reduce IL-1 production or activity; e.g. deficiencies of dietary sulphur amino acids (Klasing & Barnes, 1988) in chicks, Zn (Flynn *et al.* 1984; Winchurch, 1988), Fe (Helyar & Sherman, 1987) in rats, or certain vitamins (Trechsel *et al.* 1985). However, the present study showed that chicks fed on the LP diet responded well to the LPS injection in terms of plasma IL-1 activity, although the LP diet did not contain enough protein to achieve optimal growth and feed utilization. In addition, combined with the responses of the other indicators of the acute-phase responses in this experiment, feeding a low protein (but not protein-free) diet might be enough to maintain or develop the ability for stress responses to LPS in chicks.

Amrani *et al.* (1986) showed that an IL-6-like factor appears to be an active monokine which augments the synthesis of acute-phase proteins. Thus, IL-6 activity can possibly be estimated from acute-phase-protein production in chicks (Klasing & Johnstone, 1991). AGP is an acute-phase protein produced following inflammation in chicks (Takahashi *et al.* 1994). The changes in plasma AGP concentration in the present study suggested that IL-6 production was not regulated completely in the same manner as IL-1 production.

We have already shown that multiple injection of LPS reduces the response of plasma AGP concentration (Takahashi *et al.* 1994). This is in good agreement with the present result. Acute-phase proteins are involved in defence mechanisms against tissue damage and infections (Fleck, 1989; Wan *et al.* 1989). Plasma AGP has been reported to inhibit blastogenesis in man (Bennett & Schmid, 1980) and secretion of antibodies (Chiu *et al.* 1977). Suppression of lymphocyte blastogenesis in cattle with experimentally induced hepatic abscesses was highly correlated to serum AGP concentration (Motoi *et al.* 1992). AGP appears to be an important binding protein for catecholamines (Sager *et al.* 1987) which are secreted during the acute-phase response to stress. Protein-binding has been shown to diminish the biological effects of catecholamines although the physiological significance of catecholamine-binding in serum is obscure (Powis, 1975). In addition, murine peritoneal macrophages released a factor with inhibitory activity towards the proliferative effect of IL-1 on thymocytes when thymocytes were exposed to AGP (Bories *et al.* 1990). Thus, AGP has a suppressive effect on IL-1 production in mammals. In the present study, plasma AGP concentration in chicks fed on the HP diet was higher than that of chicks fed on the LP diet, indicating that a HP diet has the potential to suppress the response to LPS compared with a LP diet. This would possibly be a reason for the relatively low acute-phase responses following LPS injections in chicks fed on the HP diet in this study, although the role of AGP in stress and immune response in chicks has not been well elucidated.

A recent study in cultured rat hepatocytes showed that production of acute-phase protein was directly associated with IL-6, corticosterone and Zn status, but not with IL-1 alone (Coyle *et al.* 1993). It also showed that incubation of IL-1 with corticosterone and Zn in rat hepatocytes enhanced production of acute-phase protein. Thus Zn status may be important in producing acute-phase protein in the liver. Generally, an immunogen injection reduces plasma Zn and Fe concentrations and then increases the mineral concentrations in the liver (Beisel, 1977). When animals cope with the stress, the concentrations recover before the injection (Beisel, 1977). Furthermore, Laurin & Klasing (1987) indicated with chicks that the changes in the status of trace minerals such as Fe, Zn and Cu, to immunogenic challenges were dependent on the number of immunogen injections and the nutritional state of the animals; the changes in the mineral concentration in the liver following repeated injection were greater than those due to the single injection. The present

study showed that multiple injections of LPS reduced or diminished the responses of Zn and IL-1. These observations suggest that lack of AGP response after multiple injections of LPS may be related, in part, to a reduced response of IL-1 and Zn.

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