

Effects of moderate feed restriction and dietary L-arginine supplementation on cellular and humoral parameters of an acute lipopolysaccharide induced innate immune response in cockerels of a dual-purpose breed

Einflüsse moderater Futterrestriktion und diätetischer L-Arginin-Supplementation auf zelluläre und humorale Parameter einer akuten Lipopolysaccharid-induzierten, angeborenen Immunantwort bei Junghähnen einer Zweinutzungsrasse

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L-arginine (Arg) is dietary indispensable for birds and possesses immunomodulatory properties due to its function as precursor of cytotoxic nitric oxides (NO) and cell proliferating polyamines. To maintain its basic functions and generate a protective innate immune response (IIR), the avian immune system requires a sufficient supply of energy and nutrients. However, as a result of its rapidly increasing metabolic activity and cytokine-mediated anorexia, the acute systemic IIR is accompanied by a severe dysregulation of energy and nutrient homeostasis as well as a subsequent mobilisation of endogenous resources. Considering this tense metabolic state as highly susceptible to nutritional stress, the present study examined effects of surplus dietary Arg on cellular and humoral parameters of an acute lipopolysaccharide (LPS)-induced IIR in moderate restrictively fed chickens.

Methods: A total of 32 one-day-old Lohmann Dual cockerels were commercially reared in a floor-range system for three weeks and in single metabolic cages from day 22 onwards. At day 28 birds were randomly assigned to two diets differing in their Arg concentration only (23.2 % CP; control-diet: 1.37 % Arg; Arg supplemented diet (Arg-diet): 2.04 % Arg; n=16 birds/diet). In addition, from day 28 to 50 both groups were subject to an *ad libitum* and restrictive regime of feeding, respectively (n = 8 birds/diet*regime). During the entire study the restrictive regime limited birds' feed intake to 75 % of *ad libitum* consumption. On day 50 four cockerels of each group were intramuscularly injected with 2 mg *E.coli* LPS/kg BW (Sigma-Aldrich) as IIR inducer and 1 ml of 0.9 % saline solution (NaCl; B. Braun) as negative control, respectively. At 0, 1, 3, 5, 7 and 24 hours *post injectionem* (h *p. inj.*) rectal body temperature was recorded and heparinized blood samples were collected from cockerels' wing vein. Blood samples were examined for parameters of the cellular IIR (e.g. absolute and relative leukocyte counts, heterophil/lymphocyte ratio; HLR) via Wright-Giemsa stained blood smears and those of the humoral IIR (e.g. plasma NO, measured as its metabolite nitrite, via Griess assay kit; Cayman Chemical). Statistical analysis was performed as 2 x 2 x 2 x 6 four-factorial ANOVA (diet, regime, injection and sampling) using SAS procedure MIXED (p<0.05).

Results: The regime of feeding and dietary Arg concentration did not affect body temperature (41.1±0.1°C), plasma nitrite level (3.0±0.7 µM), differential blood count and HLR (0.5±0.1) in NaCl treated chickens. Therefore, these birds were just graphically pooled in order to ease readability of Fig. 1. In contrast to NaCl, LPS induced an acute systemic inflammation characterised by an initial hypothermia (40.6±0.1°C) with subsequent moderate hyperthermia (41.5±0.1°C), a severe increase in plasma nitrite levels (Fig. 1b) and a marked leukopenia (Fig. 1a) with strong lymphopenia and heterophilia peaking in a HLR of 3.4±0.3 at 7 h *p. inj.* (p<0.001). Whereas surplus dietary Arg did not modify the examined parameters in LPS treated birds, the moderate feed restriction caused lower total leukocyte counts (Fig. 1a) and absolute heterophils counts at 5 to 7 h *p. inj.* as well as considerably higher plasma nitrite levels at 3 to 7 h *p. inj.* (Fig. 1b) compared to *ad libitum* feeding (p<0.01).

Conclusions: The present study did not reveal any modulatory effects of surplus dietary Arg on cellular and humoral markers of avian IIR. On the contrary, it indicated that cellular IIR components seem to be more susceptible to metabolic stress caused by moderate long-term feed restriction than humoral components. However, based on the high cytotoxicity of NO against pathogens as well as host tissues, a metabolically intensified NO synthesis has to be considered as two-edged sword for the outcome of an acute IIR further. In conclusion, the present study emphasised the strong dependence of functionality and controllability of avian IIR on immune system's supply of energy and nutrients.

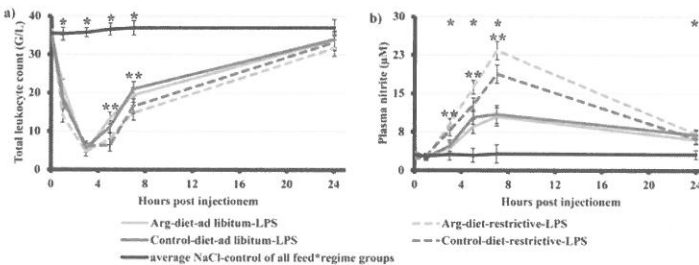


Figure 1. Changes in a) total leukocyte count and b) plasma nitrite concentration in moderate restrictively fed cockerels supplied with additional dietary Arg during 24 hours post LPS or NaCl injection (LSMeans ± SE; n=4). Values with * differ significantly between the NaCl and LPS treated groups (p < 0.001). Values with ** differ significantly between restrictively and *ad libitum* fed LPS-groups (p < 0.01).