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Effects of arginine and ornithine supplementation to a high-protein diet on selected cellular immune variables in adult cats

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Abstract

Background: Dietary protein and amino acid intake and composition can modulate immune function.

Objectives: To evaluate the effects of high-protein intake and arginine and ornithine supplementation on feline immune cells.

Animals: Ten healthy cats.

Methods: Experimental study. Cats received a high-protein basal diet as a single daily meal. A crossover design was applied with treatments being basal diet (w/o); basal diet with arginine supplementation (+50, 75, 100% compared to the arginine provision by the basal diet; Arg 1-3); and basal diet with ornithine supplementation (+100, 150, 200% compared to the arginine provision by the basal diet; Orn 1-3). Blood samples were collected at the end of each 11-day treatment period.

Results: Mitogen-stimulated proliferative activity of blood leukocytes revealed a quadratic effect for the dietary supplementation of arginine (P = .02) and ornithine (P = .03) (means for ConA-stimulation: w/o = 6.96; Arg 1 = 9.31; Arg 2 = 11.4; Arg 3 = 8.04; Orn 1 = 15.4; Orn 2 = 9.43; Orn 3 = 9.28; pooled SEM: 0.96). The number (% gated) of phagocytic granulocytes linearly decreased with increasing dietary concentrations of arginine (P = .05) and ornithine (P = .03) (means: w/o = 95.5; Arg 1 = 93.0; Arg 2 = 92.5; Arg 3 = 92.6; Orn 1 = 92.6; Orn 2 = 92.6; Orn 3 = 91.5; pooled SEM = 0.44).

Conclusions and Clinical Importance: This study could demonstrate immunomodulating properties of dietary arginine and ornithine in cats.

KEYWORDS

feline, immune system, phagocytic granulocytes, proliferation

1 | INTRODUCTION

Specific amino acids can modulate the immune function,[1] but there is little data for cats. Supplementation of arginine to a low-protein diet increases the proliferative and phagocytic activity of feline leukocytes.[2] Cytokine secretion and proliferative activity of the feline T-cell line MYA-1 are enhanced by high doses of arginine in the cell media.[3]

Abbreviations: ConA, concanavalin A; PHA-M, phytohemagglutinin; PWM, pokeweed mitogen.

Since there is little information available on the effects of arginine and ornithine in cats, the present study aimed at evaluating the dose-dependent immunomodulating properties of these amino acids in domestic cats.

2 | METHODS

2.1 | Study design

Ten healthy adult cats (4 female, intact cats, 6 male, neutered cats; 7.37 ± 2.00 years old) were included in this study. The study obtained approval by the Animal Welfare Committee (Landesamt für Gesundheit und Soziales) in Berlin, Germany (G 0120/15).

The cats received a high-protein diet without (w/o) or with the supplementation of arginine (+50%, +75% and +100% compared to the arginine provision by the basal diet; Arg 1-3) and ornithine (+100%, +150% and +200% compared to the arginine provision by the basal diet; Orn 1-3), using a randomized crossover design. The arginine concentration in the basal diet was 30.2 g/kg dry matter and the ornithine concentration 1.81 g/kg dry matter. The analyzed crude nutrient concentrations in the diets were (on a dry matter basis): 60.3% crude protein, 25.1% crude fat, 0.84% crude fiber, and 8.40% crude ash. More details about the diets can be found elsewhere.[4] The supplements (arginine, ornithine) were mixed with the daily amount of food. The cats were fed individually throughout the study in order to assess their daily feed intake.

Diets were fed for 11 days each, with a 7-day wash-out period in between, where only the basal diet was fed. The cats were housed in groups on days 1-7 of each treatment period, as well as during the wash-out periods. For the last 4 days of each treatment period, the cats were housed individually in metabolic cages to collect urine and feces (data not part of this study[4]). Blood was collected by routine cephalic venipuncture on the last day of each treatment period at 6.00 hours in the morning. For the present analyses, only fasting blood was used. Blood was collected using potassium EDTA tubes (Micro tube K3E, Sarstedt, Nümbrecht, Germany) for the differential blood count, and lithium heparin tubes (S-Monovette, Sarstedt, Nümbrecht, Germany) for the isolation of blood leukocytes (phenotyping, measurement of the proliferative activity, and number of phagocytic blood cells).

2.2 | Blood analyses

For the analysis of the white blood cell count and differential blood cell count, the Sysmex XT2000i (Sysmex Deutschland GmbH, Norderstedt, Germany) was used.

Details on the methods for phenotyping and the measurement of the proliferative activity and the number of phagocytic blood leukocytes are described by Paßlack et al.[5] In short, feline leukocytes were labeled with primary antibodies (mouse anti-cat CD4:FITC [Serotech; MCA1346F; 1:20 dilution], mouse anti-cat CD8ALPHA/BETA [Serotech; MCA1347PE; 1:20 dilution]), mouse anti-canine CD21 [Serotech; MCA1781S; 1:100 dilution], mouse anti-human CD14: FITC [Serotech; MCA1568F; 1:20 dilution], mouse anti-cat MHC Class II [Serotech; MCA2723; 1:20 dilution], and P-DH59B, Specificity CD172a [Monoclonal Antibody Center; P-BOV2049; 1:100 dilution]). Except for the samples with mouse anti-cat CD4:FITC and mouse anti-cat CD8ALPHA/BETA, the samples were additionally labeled with the following secondary antibodies: Goat F(ab')2 anti-mouse IgG1-RPE, Human adsorbed, Southern-Biotech, 1072-09 and goat anti-mouse IgG2b-RPE, Human adsorbed, Southern-Biotech, 1090-09.

For the measurement of the proliferative activity, peripheral blood mononuclear cells were stimulated with the mitogens pokeweed mitogen (PWM; concentration in the microplate well: 2.5 μg/mL), Concanavalin A (Con A; 5 μg/mL), and Phytohemagglutinin, M form (PHA-M; 10 μg/mL). Different mitogens were used, as not only different lymphocyte subpopulations are stimulated by these substances (T cells by PHA and ConA, T and B cells by PWM[6]), but also to a varying extent. For instance, a markedly higher stimulation of feline leukocytes has been observed for ConA than for PHA-M.[5]

For the measurement of the number of phagocytic granulocytes and monocytes in the blood of the cats, a commercial test kit (PHAGOTEST, Glycotope Biotechnology GmbH, Heidelberg, Germany) was used.

All measurements were performed using the flow cytometer MACSQuant (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany).

2.3 | Statistical data analysis

The data were analyzed using IBM SPSS Statistics 22 (SPSS Inc, Chicago, Illinois, 2013). A general linear model for repeated measures was applied to calculate linear and quadratic polynomial contrasts. Polynomial contrasts were calculated separately for the arginine and ornithine treatment, with the within-subject factor arginine resp. ornithine, and a number of levels = 4 (basal diet plus three [increasing] doses of arginine resp. ornithine). The data are presented in tables as means and pooled standard error of means (SEM). The level of significance was $P \leq .05$.

3 | RESULTS

3.1 | Health status and food intake

All cats were clinically healthy throughout the study. The diets did not affect the feed intake or body weight of the animals (data not shown, but published elsewhere[4]). The daily feed intake (% of the amount offered) was: 93.4% (w/o), 88.9% (Arg 1), 89.6% (Arg 2), 88.3% (Arg 3), 90.7% (Orn 1), 90.9% (Orn 2), and 92.1% (Orn 3) (SEM: 1.15%) (linear contrast for arginine: $P = .15$, quadratic contrast for arginine: $P = .39$, linear contrast for ornithine: $P = .53$, quadratic contrast for ornithine: $P = .32$).

As the supplements were mixed with the daily amount of food, but the remainder was not analyzed for the nitrogen concentration, the daily crude protein intake of the cats can only be calculated based on the intake of the basal diet and without regard to the additional

nitrogen intake by the supplements. The daily crude protein intake (per kilogram body weight and day) of the cats was 6975 mg (w/o), 6719 mg (Arg 1), 6697 mg (Arg 2), 6717 mg (Arg 3), 6830 mg (Orn 1), 6787 mg (Orn 2), and 6910 mg (Orn 3) (SEM: 118 mg) (linear contrast for arginine: $P = .23$, quadratic contrast for arginine: $P = .22$, linear contrast for ornithine: $P = .62$, quadratic contrast for ornithine: $P = .36$).

As published previously, the postprandial, but not the fasting blood urea concentrations' are above the reference range.[4]

3.2 | White blood cells and differential blood count

The dietary supplementation of arginine and ornithine had no effect on the white blood cell count (%) of the cats (Table 1). In some treatment groups, the absolute cell number of blood lymphocytes and monocytes was above the reference value. However, when the relative cell number (% of white blood cells) was considered, no increase of lymphocytes or monocytes could be detected.

All groups had eosinophilic granulocytes in the blood, both for the absolute and relative cell numbers above the reference range. An exception being the group with the highest arginine supplementation, where the percentage of blood eosinophils was within the reference range.

3.3 | Leukocyte subpopulations

The dietary supplementation of arginine and ornithine did not affect the numbers (% gated) of T cells, B cells, myeloid cells, or antigen-presenting cells (Table 2).

3.4 | Mitogen-stimulated proliferative activity of blood leukocytes

The stimulation with the mitogen PHA-M had no effect on the proliferative activity of blood leukocytes (Table 3). The dietary supplements first increased the proliferative activity of the leukocytes after stimulation with ConA (arginine, ornithine) and PWM (ornithine), but subsequently decreased their activity at higher supplementation doses (quadratic effects; $P < .05$).

3.5 | Number of phagocytic blood monocytes and granulocytes

Increasing doses of arginine and ornithine decreased the number of phagocytic blood granulocytes (linear contrasts; $P < .05$), but did not affect the number of phagocytic blood monocytes (Table 4).

TABLE 1 White blood cells and differential blood count of cats fed a high-protein diet without (w/o) or with arginine (Arg) or ornithine (Orn) supplementation[a]

									P values (polynomial contrasts)			
		Arg			Orn				Arg		Orn	
	w/o	Arg 1	Arg 2	Arg 3	Orn 1	Orn 2	Orn 3	SEM	Linear	Quadratic	Linear	Quadratic
White blood cells (G/l)	14.3	15.8	15.2	14.8	15.3	14.7	13.6	0.42	.82	.23	.38	.07
G/l												
Neutrophilic granulocytes	8.88	9.07	9.45	9.63	9.76	8.79	8.51	0.30	.61	.44	.45	.25
Lymphocytes	3.87	4.90	3.76	3.78	4.07	4.48	3.81	0.15	.02	.10	.75	.29
Monocytes	0.46	0.61	0.58	0.61	0.51	0.51	0.40	0.03	.41	.36	.24	.05
Eosinophilic granulocytes	1.06	1.11	1.06	0.77	0.97	0.95	0.87	0.07	.04	.36	.12	.96
Basophilic granulocytes	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.00	.68	.05	.82	.11
Percentage of white blood cells												
Neutrophilic granulocytes	62.2	57.8	63.3	64.5	62.7	60.1	62.1	0.73	.13	.08	.71	.74
Lymphocytes	27.3	31.0	25.9	25.9	27.5	30.1	28.4	0.73	.29	.30	.34	.61
Monocytes	3.24	3.76	3.89	4.17	3.30	3.49	3.06	0.15	.05	.91	.74	.38
Eosinophilic granulocytes	7.25	7.03	6.83	5.30	6.31	6.18	6.23	0.42	.07	.65	.25	.28
Basophilic granulocytes	0.09	0.13	0.11	0.11	0.15	0.11	0.12	0.01	.48	.17	.52	.24

Notes: Mean and pooled SEM. Reference values (G/L)[7]: white blood cells: 6-11 (18; when excited); neutrophilic granulocytes: 3-11; lymphocytes: 1–4; monocytes: 0.04-0.5; eosinophilic granulocytes: 0.04-0.6; basophilic granulocytes: 0-0.1.
Reference values (% of white blood cells)[7]: neutrophilic granulocytes: 60-78%; lymphocytes: 15-38%; monocytes: 0-4%; eosinophilic granulocytes: 0-6%; basophilic granulocytes: 0-1%.
[a]Arginine supplementation (Arg 1-3): +50%, +75%, and +100% compared to the arginine provision by the basal diet; ornithine supplementation (Orn 1-3): +100%, +150%, and +200% compared to the arginine provision by the basal diet.

TABLE 2 Phenotyping of blood leukocytes of cats fed a high-protein diet without (w/o) or with arginine (Arg) or ornithine (Orn) supplementation[a]

									P values (polynomial contrasts)			
		Arg			Orn				Arg		Orn	
	w/o	Arg 1	Arg 2	Arg 3	Orn 1	Orn 2	Orn 3	SEM	Linear	Quadratic	Linear	Quadratic
In % gated												
$CD4^+CD8^-$	31.8	33.5	32.5	31.5	30.9	35.8	32.6	0.77	.95	.08	.16	.33
$CD4^+CD8^+$	1.18	1.58	0.61	2.10	2.43	1.92	0.93	0.38	.55	.83	.58	.41
$CD4^-CD8^+$	18.1	18.9	16.2	16.1	16.3	17.7	17.5	0.69	.93	.37	.79	.26
$MHCII^+$	87.4	88.0	86.8	85.8	85.5	86.6	86.0	0.72	.87	.52	.87	.40
$SWC3^+$	98.0	97.3	97.3	98.0	97.1	98.1	96.4	0.31	.93	.94	.47	.64
$CD14^+$	13.9	12.5	15.0	14.9	13.5	12.7	12.0	0.65	.38	.96	.11	.82
$CD21^+$	23.6	22.1	22.4	21.4	23.1	20.3	20.9	0.90	.32	.33	.08	.50

Notes: Mean and pooled SEM. $CD4^+CD8^-$ and $CD4^+CD8^+$: T-helper cells; $CD4^-CD8^+$: cytolytic T cells; $MHCII^+$: antigen-presenting cells; $SWC3^+$ and $CD14^+$: myeloid cells; $CD21^+$: B cells.
[a]Arginine supplementation (Arg 1–3): +50%, +75%, and +100% compared to the arginine provision by the basal diet; ornithine supplementation (Orn 1-3): +100%, +150%, and +200% compared to the arginine provision by the basal diet.

TABLE 3 Proliferative activity[a] of lymphocytes of cats fed a high-protein diet without (w/o) or with arginine (Arg) or ornithine (Orn) supplementation[b]

									P values (polynomial contrasts)			
		Arg			Orn				Arg		Orn	
Mitogen stimulation	w/o	Arg 1	Arg 2	Arg 3	Orn 1	Orn 2	Orn 3	SEM	Linear	Quadratic	Linear	Quadratic
PWM	4.75	6.03	6.19	5.15	7.08	6.53	5.16	0.44	.77	.27	.90	.05
ConA	6.96	9.31	11.4	8.04	15.4	9.43	9.28	0.96	.50	.02	.92	.03
PHA-M	2.58	2.58	2.89	2.22	3.56	2.90	3.58	0.26	.81	.49	.46	.80

Notes: Mean and pooled SEM.
Abbreviations: ConA, Concanavalin A; PHA-M: Phytohemagglutinin, M form; PWM, pokeweed mitogen.
[a]Calculation of the proliferation index: proliferative activity of stimulated lymphocytes (% gated)/proliferative activity of unstimulated lymphocytes (% gated).
[b]Arginine supplementation (Arg 1-3): +50%, +75%, and +100% compared to the arginine provision by the basal diet; ornithine supplementation (Orn 1-3): +100%, +150%, and +200% compared to the arginine provision by the basal diet.

TABLE 4 Number of phagocytic blood monocytes and granulocytes of cats fed a high-protein diet without (w/o) or with arginine (Arg) or ornithine (Orn) supplementation[a]

									P values (polynomial contrasts)			
		Arg			Orn				Arg		Orn	
	w/o	Arg 1	Arg 2	Arg 3	Orn 1	Orn 2	Orn 3	SEM	Linear	Quadratic	Linear	Quadratic
Percentage gated												
Phagocytic monocytes	76.8	71.7	74.2	73.5	72.1	71.4	71.5	0.89	0.54	.40	.07	.28
Phagocytic granulocytes	95.5	93.0	92.5	92.6	92.6	92.6	91.5	0.44	0.050	.28	.03	.46

Notes: Mean and pooled SEM.
[a]Arginine supplementation (Arg 1–3): +50%, +75%, and +100% compared to the arginine provision by the basal diet; ornithine supplementation (Orn 1-3): +100%, +150%, and +200% compared to the arginine provision by the basal diet.

4 | DISCUSSION

The dietary supplementation of arginine and ornithine could demonstrate immunomodulating effects in cats. While the impact of the supplements on the proliferative activity of blood leukocytes was less clear, the linear decrease of phagocytic blood granulocytes with increasing amounts of arginine and ornithine in the diet revealed a significant dose-dependent effect.

The results contrast with data previously observed in cats, where a dietary arginine supplementation led to an increase of the

proliferative and phagocytic activity of blood leukocytes.[2] In addition, an in vitro study could also demonstrate stimulating effects of high arginine concentrations in the cell media (up to the 8-fold arginine blood concentration) on the proliferative activity and cytokine secretion of feline T cells.[3] Importantly, in the study of Rutherfurd-Markwick et al,[2] a low-protein diet (22.7% in dry matter) was offered and only a single dose of arginine (1.9% in dry matter) was tested. In the present study, markedly higher protein (and arginine) intakes were evaluated in cats. Therefore, differences in the study design and dietary regimen might explain the different outcomes of the studies. With regard to the potential clinical relevance of the decreased number of phagocytic blood granulocytes observed at high arginine and ornithine supplementation, it should be considered that the effects were relatively small, as the cell numbers were reduced by 3%-4%. In general, it can be concluded that both, arginine and ornithine revealed immunomodulating properties. As arginine is the precursor of ornithine,[8] it is interesting to notice that the effects of both amino acids were comparable, but slightly more pronounced for ornithine. This could be possibly attributed to the higher doses considered for this amino acid.

For the interpretation of the detected effects of dietary arginine and ornithine, it should finally be considered that the protein and therefore amino acid concentration in the basal diet was already high and above maintenance requirements. The effects of an additional supplementation of arginine or ornithine might be more pronounced when feeding a low-protein diet, where the baseline intake of these amino acids is low. As an alternative, it is also possible that a low-protein diet would require even higher doses of arginine or ornithine supplementation to achieve comparable results as observed in the present study. This aspect should be clarified in future investigations.

It is a limitation of the present investigation that no low- or moderate-protein diet has been included in this feeding study as another control diet. This might have had further advantages for data interpretation, particularly for comparing the observed effects of a high-protein intake in principal. However, as especially the effects of a dietary arginine and ornithine supplementation were evaluated in the present study, it was most important to have the same diet, but without the supplements, as a control diet. In addition, the effects of increasing protein levels in a diet for cats have already been described,[5] why the present study did not focus on this aspect in general.

5 | CONCLUSION

The dietary supplementation of arginine and ornithine revealed immunomodulating properties of these amino acids in cats, as the proliferative activity and number of phagocytic blood leukocytes were affected. These findings should be further evaluated, particularly in diseased cats with an impaired or even exaggerated immune response.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The study obtained approval by the Animal Welfare Committee (Landesamt für Gesundheit und Soziales) in Berlin, Germany (G 0120/15).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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