

Supplemental Appendix 1 – Detailed methods description

Preparation of fecal samples

About 100 mg of fecal sample was mixed with 1 mL ice-cold sample diluent (assay buffer, KENO751, IBL International). Samples were homogenized, using a bead beater at medium speed for 2 x 1 minute and then centrifuged for 15 minutes at 2500 x g and 4°C. Supernatants were transferred to a fresh tube and immediately used in ELISAs or frozen at -20°C for short-term storage (used within 5 days).

EED biomarker ELISAs

Myeloperoxidase (R&D Systems, DY3174)

Myeloperoxidase ELISA was used according to manufacturers' instructions. Prior to the procedure, 96-well MaxiSorp plates were coated with 50 µL capture antibody solution (diluted 1:180 in Phosphate Buffered Saline, pH 7.4 [PBS]) and incubated overnight at room temperature. Wells were washed with 3x 400 µL wash buffer (0.05% Tween-20 in PBS). To block potential sites of nonspecific interaction, 200 µL reagent diluent (1% bovine serum albumin in PBS) was added to each well and incubated for 1 to 3 hours at room temperature. A standard dilution series was prepared from recombinant protein standard from 8000 pg/mL to 125 pg/mL standard concentration using reagent diluent. Fecal sample supernatants were diluted 1:500 in reagent diluent (final dilution 1:5000).

For the assay, wells were washed with 3x 400 µL wash buffer, 50 µL of standard or diluted sample was added to the respective well and wells were incubated overnight at 4°C. Wells were emptied and washed with 5x 400 µL Wash buffer. Detection antibody solution (50 µL diluted 1:180 in reagent diluent) was added to the wells and incubated for 1 hour at room temperature. Wells were washed with 3x 400 µL wash buffer and 50 µL Streptavidin-Horseradish Peroxidase (HRP) solution (diluted 1:200 in reagent diluent) was added and incubated for 45 minutes at room temperature. Wells were washed with 3x 400 µL Wash buffer and 100 µL Tetramethylbenzidine (TMB) solution (Thermo Scientific™ Pierce™ TMB Substrate Kit) was added. Wells were incubated for several minutes at room temperature in the dark. The reaction was stopped by adding 50 µL stop solution (1M H₂SO₄) and optical density was measured at 450 nm (reference wavelength at 570 nm). The obtained optical density of the standards were plotted against their concentrations and a standard curve was calculated using a 4 parameter logistic curve fit.

Alpha-1-Antitrypsin (R&D Systems, DY1268)

Alpha-1-Antitrypsin ELISA was used according to manufacturers' instructions, with some adjustments made to the buffer solutions. Prior to the procedure, 96-well MaxiSorp plates were coated with 50 µL capture antibody solution (diluted 1:180 in PBS) and incubated overnight at room temperature. Wells were washed with 3x 400 µL wash buffer (0.05% Tween-20 in PBS). To block potential sites of nonspecific interaction, 200 µL sample diluent (5% Tween-20 3% fetal calf serum in PBS) was added to each well and incubated for 1 to 3 hours at room temperature. A standard dilution series was prepared from recombinant protein standard from 8000 pg/mL to

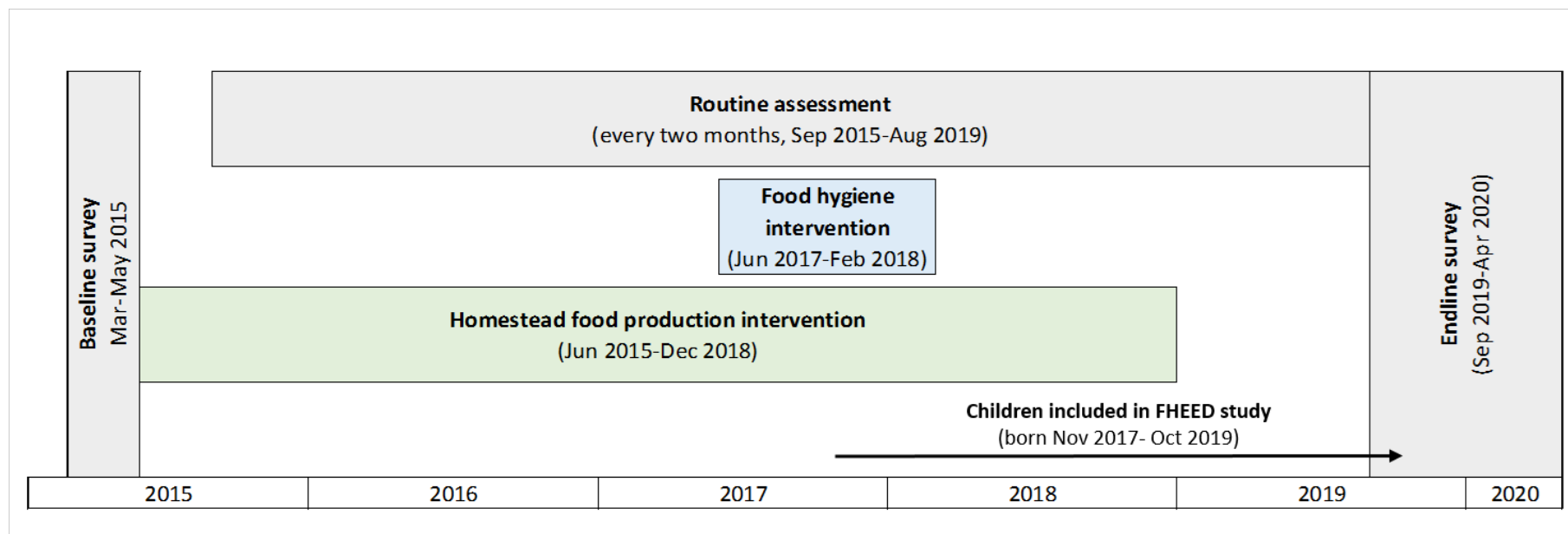
125 pg/mL standard concentration using sample diluent. Fecal sample supernatants were diluted 1:2000 in sample diluent (final dilution 1:20000).

For the assay, wells were washed with 3x 400 μ L wash buffer, 50 μ L of standard or diluted sample was added to the respective well and wells were incubated overnight at 4°C. Wells were emptied and washed with 5x 400 μ L wash buffer. Detection antibody solution (50 μ L diluted 1:180 in reagent diluent, 5% Tween-20 in PBS) was added to the wells and incubated for 1 hour at room temperature. Wells were washed with 3x 400 μ L wash buffer and 50 μ L streptavidin-HRP solution (diluted 1:200 in reagent diluent) was added and incubated for 45 minutes at room temperature. Wells were washed with 3x 400 μ L wash buffer and 100 μ L TMB solution (Thermo Scientific™ Pierce™ TMB Substrate Kit) was added. Wells were incubated for several minutes at room temperature in the dark. The reaction was stopped by adding 50 μ L stop solution (1M H₂SO₄) and optical density was measured at 450 nm (reference wavelength at 570 nm). The obtained optical density of the standards are plotted against their concentrations and a standard curve was calculated using a 4 parameter logistic curve fit.

Neopterin (IBL International, RE59321)

Neopterin ELISA was used according to manufacturers' instructions. Prior to use, the microtiter plate with assay strips was brought to room temperature and wash buffer concentrate was diluted 1:20 with bidistilled water. Fecal sample supernatants were diluted 1:10 with assay buffer (final dilution 1:100).

For the assay, 20 μ L of ready-to-use standard, ready-to-use controls, or diluted sample was added to the respective wells of the microtiter plate. Enzyme conjugate (100 μ L) and neopterin antiserum (50 μ L) was added to each well. The plate was covered with black adhesive foil and incubated for 90 minutes at room temperature (20-25°C) on an orbital shaker at 500 rpm. After 90 minutes, the incubation solution was discarded and the plate was washed 4x 300 μ L with wash buffer. TMB substrate solution (150 μ L) was added to each well and the plate was incubated for 10 minutes at room temperature in the dark. The reaction was stopped by adding 150 μ L TMB stop solution, contents were mixed by gently shaking the plate. Optical density was measured at 450 nm (reference wavelength at 600 nm) within 15 minutes. The obtained optical density of the standards are plotted against their concentrations and a standard curve was calculated using a 4 parameter logistic curve fit.



Supplemental Figure 1: Timeline of the FAARM trial and FHEED sub-study.

Abbreviation: FAARM: Food and Agricultural Approaches to Reducing Malnutrition; FHEED: Food Hygiene to reduce Environmental Enteric Dysfunction.

Supplemental Table 1: Pairwise correlation of EED and systemic inflammation biomarker concentrations

Biomarker	log MPO <i>r</i>	log AAT <i>r</i>	log NEO <i>r</i>	log CRP <i>r</i>	log AGP <i>r</i>
log MPO	1.0				
log AAT	0.40	1.0			
log NEO	0.26	0.25	1.0		
log CRP	0.08	0.04	-0.09	1.0	
log AGP	0.04	0.03	-0.05	0.66	1.0

n=574 for the correlations between MPO, AAT, and NEO; n=428 for all correlations involving CRP and AGP. Abbreviations: EED: Environmental Enteric Dysfunction, MPO: Myeloperoxidase, AAT: Alpha-1-Antitrypsin, NEO: Neopterin, CRP: C-reactive protein, AGP: Alpha-1-acid glycoprotein, *r*: correlation coefficient.

Supplemental Table 2: Diarrhea 7-day period prevalence and severity

Symptom [#]	Overall % <i>mean (se)</i>	Control % <i>mean (se)</i>	Intervention % <i>mean (se)</i>	p-value
Diarrhea	5.6	6.3	4.9	0.46
<i>for children with diarrhea (n=32)</i>				
Times stool passed	4.8 (0.2)	4.9 (0.2)	4.5 (0.3)	0.30
Blood in stool	6.3	5.9	6.7	0.93
Fever	50.0	41.2	60.0	0.24

n= 574, n (control)= 270, n (intervention)= 304, including all children aged 0-24 months who provided a fecal sample, p-value: from Wald test with standard errors adjusted for settlement-level clustering. [#]Diarrhea 7-day period prevalence and severity at the day of stool collection. Abbreviations: se: robust standard error

Supplemental Table 3: EED biomarker concentrations by diarrhea status of children

Inflammatory marker		N	mean	se	coef. (CI)	p-value
log MPO (ng/mL)	No diarrhea	542	4.35	0.03	-0.19 (-0.39 – -0.006)	0.04
	Diarrhea [#]	32	4.18	0.09		
log AAT (µg/mL)	No diarrhea	542	2.62	0.02	-0.15 (-0.28 – -0.02)	0.02
	Diarrhea [#]	32	2.48	0.06		
log NEO (nmol/L)	No diarrhea	542	3.16	0.02	-0.1 (-0.27 – 0.07)	0.25
	Diarrhea [#]	32	3.12	0.12		
log CRP (mg/L)	No diarrhea	384	-0.23	0.04	-0.21 (-0.48 – 0.06)	0.14
	Diarrhea ^{##}	44	-0.42	0.14		
log AGP (g/L)	No diarrhea	384	-0.09	0.01	-0.05 (-0.11 – 0.01)	0.13
	Diarrhea ^{##}	44	-0.14	0.03		

n=574 for log MPO, log AAT, log NEO; n=428 for log CRP, log AGP. [#]for log MPO, log AAT, and log NEO: diarrhea 7-day period prevalence at the day of stool collection; ^{##}for log CRP and log AGP: diarrhea 7-day period prevalence at the day of blood collection; se: robust standard error; coef. (coef.) and p-value from multilevel regression models, adjusted for child age and sex, with settlement random effects. Abbreviations: EED: Environmental Enteric Dysfunction, MPO: Myeloperoxidase, AAT: Alpha-1-Antitrypsin, NEO: Neopterin, CRP: C-reactive protein, AGP: Alpha-1-acid glycoprotein; se: robust standard error.

Supplemental Table 4: Concentration of EED biomarkers by intervention group and child age.

	Control predicted conc. (CI)*	Intervention predicted conc. (CI)*	coefficient (CI)	p-value
log MPO (ng/mL)				
0-5 months	4.63 (4.45 – 4.80)	4.62 (4.44 – 4.79)	-0.01 (-0.25 – 0.24)	0.95
6-11 months	4.36 (4.24 – 4.48)	4.47 (4.35 – 4.59)	0.11 (-0.06 – 0.28)	0.21
12-17 months	4.20 (4.06 – 4.34)	4.32 (4.19 – 4.45)	0.12 (-0.07 – 0.32)	0.22
18-24 months	4.11 (3.99 – 4.24)	4.26 (4.15 – 4.38)	0.15 (-0.02 – 0.32)	0.08
log AAT (ug/mL)				
0-5 months	2.67 (2.55 – 2.79)	2.58 (2.46 – 2.71)	-0.09 (-0.26 – 0.08)	0.31
6-11 months	2.58 (2.49 – 2.67)	2.66 (2.57 – 2.74)	0.07 (-0.05 – 0.19)	0.25
12-17 months	2.56 (2.46 – 2.66)	2.63 (2.53 – 2.72)	0.07 (-0.07 – 0.21)	0.35
18-24 months	2.59 (2.49 – 2.68)	2.66 (2.57 – 2.74)	0.07 (-0.05 – 0.19)	0.28
log NEO (nmol/L)				
0-5 months	3.35 (3.20 – 3.49)	3.26 (3.11 – 3.41)	-0.08 (-0.29 – 0.12)	0.43
6-11 months	3.26 (3.16 – 3.36)	3.40 (3.30 – 3.49)	0.16 (-0.005 – 0.28)	0.06
12-17 months	3.13 (3.01 – 3.26)	3.12 (3.01 – 3.23)	-0.02 (-0.18 – 0.15)	0.84
18-24 months	2.87 (2.76 – 2.98)	2.99 (2.90 – 3.08)	0.12 (-0.02 – 0.26)	0.10
log CRP (mg/L)				
6-11 months	-0.22 (-0.41 – -0.02)	-0.10 (-0.30 – 0.10)	0.11 (-0.16 – 0.39)	0.42
12-17 months	-0.33 (-0.57 – -0.10)	-0.27 (-0.48 – -0.06)	0.06 (-0.25 – 0.38)	0.69
18-24 months	-0.41 (-0.61 – -0.20)	-0.22 (-0.40 – -0.05)	0.18 (-0.09 – 0.45)	0.18
log AGP (g/L)				
6-11 months	-0.08 (-0.12 – -0.03)	-0.06 (-0.11 – -0.01)	0.02 (-0.05 – 0.08)	0.62
12-17 months	-0.09 (-0.14 – -0.03)	-0.13 (-0.18 – -0.09)	-0.05 (-0.12 – 0.03)	0.22
18-24 months	-0.10 (-0.15 – -0.06)	-0.12 (-0.16 – 0.08)	-0.01 (-0.08 – 0.05)	0.66

n= 574 for log MPO, log NEO, log AAT; n= 428 for log CRP and log AGP, excluding all children below 6 months at the day of blood collection as they did not provide a serum sample; *estimated from multilevel regression models using marginal standardization, an interaction term was used to calculate effects by age group, regression models were adjusted for sex of child, with settlement random effects. Abbreviations: EED: Environmental Enteric Dysfunction, CI: 95% Confidence Interval, MPO: Myeloperoxidase, AAT: Alpha-1-Antitrypsin, NEO: Neopterin, CRP: C-reactive protein, AGP: Alpha-1-acid glycoprotein.

Supplemental Table 5: Effect of the intervention on EED biomarker concentrations (subgroup and sensitivity analyses)

Inflammatory marker		age group: <18 months ^a			adjusting for baseline wealth ^b		
		N	coef. (CI)	p-value	N	coef. (CI)	p-value
log MPO (ng/mL)	Control	191	0.09 (-0.03 - 0.21)	0.15	264	0.11 (0.005 – 0.22)	0.04
	Intervention	201			298		
log AAT (µg/mL)	Control	191	0.02 (-0.07 - 0.11)	0.61	264	0.04 (-0.05 – 0.12)	0.42
	Intervention	201			298		
log NEO (nmol/L)	Control	191	0.03 (-0.07 - 0.14)	0.50	264	0.06 (-0.03 – 0.14)	0.19
	Intervention	201			298		
log CRP (mg/L)	Control	128	0.09 (-0.11 - 0.30)	0.38	194	0.13 (-0.04 – 0.29)	0.13
	Intervention	139			224		
log AGP (g/L)	Control	128	-0.01 (-0.06 - 0.04)	0.75	194	-0.02 (-0.06 – 0.02)	0.45
	Intervention	139			224		

Coefficient (coef.) and p-value from multilevel regression models, adjusted for child age and sex, with settlement random effects; ^a this analysis only includes children below 18 months of age, which was the pre-defined age cut-off of the FHEED study, n =392 for log MPO, log NEO, log AAT, n=267 for log CRP and log AGP, excluding all children below 6 months at the day of blood collection as they did not provide a serum sample; ^b sensitivity analysis additionally adjusting for baseline wealth, n=562 for log MPO, log NEO, log AAT (including children 0-24 months), n= 418 for CRP and AGP (including children 6-24 months); Abbreviations: EED: Environmental Enteric Dysfunction, CI: 95% Confidence Interval, MPO: Myeloperoxidase, AAT: Alpha-1-Antitrypsin, NEO: Neopterin, CRP: C-reactive protein, AGP: Alpha-1-acid glycoprotein.

Supplemental Table 6: Household poultry ownership characteristics

Characteristics	Overall % <i>mean(sd)</i>	Control % <i>mean (sd)</i>	Intervention % <i>mean (sd)</i>
Households owning poultry	59	56	62
Number of poultry in owning households [#]	3.6 (3.8)	3.3 (3.3)	3.9 (4.2)
Average number of poultry per household in the settlement ^{##}	2.5 (1.3)	2.0 (1.2)	2.9 (1.3)
Poultry shed			
None	65	94	38
Traditional	6	5.6	6
Modern	29	0.4	56
Poultry location			
No poultry	41	44	38
In shed ^{###}	20	4	34
Never in shed	39	52	28

Overall n=561, missing: 13, n(Intervention): 295, n(Control): 266; data from routine bimonthly survey collected between May and September 2019; [#]among poultry-owning households: n=332, n(Intervention): 184, n(Control): 148; ^{##}in households that are part of the FAARM trial; ^{###}poultry kept in shed for any time of day.

Supplemental Table 7: Effect of poultry ownership on EED biomarker concentrations

Exposure	log MPO (ng/mL)		log AAT (µg/mL)		log NEO (nmol/L)		log CRP (mg/L)		log AGP (g/L)	
	Coef. (CI)	p-val	Coef. (CI)	p-val	Coef. (CI)	p-val	Coef. (CI)	p-val	Coef. (CI)	p-val
Poultry ownership										
No poultry	Ref.		Ref.		Ref.		Ref.		Ref.	
Poultry	0.07 (-0.02 – 0.16)	0.11	-0.04 (-0.1 – 0.02)	0.22	-0.07 (-0.15 – 0.01)	0.08	0.04 (-0.13 – 0.21)	0.63	0.04 (0.002 – 0.08)	0.035
Poultry location										
No poultry	Ref.		Ref.		Ref.		Ref.		Ref.	
In shed [§]	0.04 (- 0.09 – 0.16)	0.56	-0.06 (-0.15 – 0.02)	0.15	-0.06 (-0.17 – 0.05)	0.28	-0.02 (-0.24 – 0.20)	0.85	-0.002 (-0.05 – 0.05)	0.94
Not in shed	0.09 (-0.009 – 0.19)	0.07	-0.03 (-0.1 – 0.04)	0.45	-0.08 (-0.16 – 0.01)	0.09	0.07 (-0.11 – 0.26)	0.44	0.06 (0.02 – 0.11)	0.003
Settlement-level average number of poultry ^{§§}										
Poultry number	0.04 (-0.003 – 0.08)	0.07	0.02 (-0.01 – 0.05)	0.27	0.01 (-0.02 – 0.04)	0.54	0.02 (-0.04 – 0.08)	0.55	0.005 (-0.01 – 0.02)	0.54

Overall n= 561 for log MPO, log AAT, and log NEO, missing: 13; n= 419 for log CRP and log AGP, excluding all children below 6 months at the day of blood collection as they did not provide a serum sample; data on poultry ownership/location from routine surveillance system collected between May and September 2019; Coefficient (coef.) and p-value from multilevel regression models, adjusted for child age and sex, with settlement random effects; [§]poultry kept in a shed for any time a day; ^{§§} average number of poultry in FAARM households in a settlement; Abbreviation: EED: Environmental Enteric Dysfunction, CI: 95% Confidence Interval, p-val: p-value, MPO: Myeloperoxidase, AAT: Alpha-1-Antitrypsin, NEO: Neopterin, CRP: C-reactive protein, AGP: Alpha-1-acid glycoprotein.

Supplemental Table 8: Association of EED biomarker concentrations with poultry ownership or location, and interaction with intervention group

Exposure	log MPO (ng/mL)		log AAT (µg/mL)		log NEO (nmol/L)		log CRP (mg/L)		log AGP (g/L)	
	Coef. (CI)	p-val	Coef. (CI)	p-val	Coef. (CI)	p-val	Coef. (CI)	p-val	Coef. (CI)	p-val
Intervention allocation and poultry ownership										
Intervention	0.1 (-0.01 – 0.21)	0.09	0.04 (-0.05 – 0.13)	0.35	0.08 (-0.009 – 0.16)	0.08	0.13 (-0.04 – 0.30)	0.13	-0.02 (-0.06 – 0.02)	0.37
No poultry	Ref.		Ref.		Ref.		Ref.		Ref.	
Poultry	0.07 (-0.02 – 0.16)	0.13	-0.04 (-0.10 – 0.02)	0.21	-0.07 (-0.15 – 0.006)	0.07	0.03 (-0.13 – 0.20)	0.69	0.04 (0.003 – 0.08)	0.03
Intervention effect by poultry ownership[#]										
Interv*No poultry	0.05 (-0.1 – 0.2)	0.52	0.06 (-0.05 – 0.18)	0.29	0.10 (-0.02 – 0.23)	0.11	0.25 (-0.02 – 0.51)	0.07	0.03 (-0.03 – 0.09)	0.34
Interv*Poultry	0.13 (-0.006 – 0.26)	0.06	0.03 (-0.08 – 0.13)	0.59	0.06 (-0.05 – 0.16)	0.29	0.05 (-0.16 – 0.27)	0.64	-0.05 (-0.1 – 0.001)	0.055
<i>p-value of interaction</i>	0.41		0.58		0.56		0.25		0.04	
Intervention allocation and poultry location										
Intervention	0.12 (0.008 – 0.24)	0.04	0.06 (-0.04 – 0.15)	0.24	0.08 (-0.009 – 0.17)	0.08	0.19 (0.003 – 0.38)	0.05	0.003 (-0.04 – 0.05)	0.90
No poultry	Ref.		Ref.		Ref.		Ref.		Ref.	
In shed [§]	0.004 (-0.12 – 0.13)	0.95	-0.07 (-0.16 – 0.13)	0.09	-0.09 (-0.20 – 0.03)	0.13	-0.09 (-0.33 – 0.14)	0.42	-0.003 (-0.06 – 0.05)	0.91
Not in shed	0.1 (0.002 – 0.20)	0.05	-0.02 (-0.09 – 0.05)	0.50	-0.07 (-0.16 – 0.02)	0.13	0.10 (-0.09 – 0.29)	0.30	0.06 (0.02 – 0.11)	0.003
Intervention effect by poultry location[#]										
Interv*No poultry	0.05 (-0.1 – 0.21)	0.51	0.06 (-0.05 – 0.18)	0.28	0.11 (-0.02 – 0.23)	0.10	0.25 (-0.02 – 0.51)	0.07	0.03 (-0.03 – 0.09)	0.33
Interv*In shed [§]	0.1 (-0.23 – 0.44)	0.54	-0.002 (-0.24 – 0.23)	0.99	-0.25 (-0.54 – 0.05)	0.10	0.30 (-0.36 – 0.97)	0.37	-0.02 (-0.17 – 0.13)	0.80
Interv*Not in shed	0.2 (0.05 – 0.36)	0.01	0.06 (-0.06 – 0.18)	0.32	0.12 (-0.01 – 0.25)	0.07	0.11 (-0.17 – 0.38)	0.45	-0.02 (-0.09 – 0.04)	0.48
<i>p-value of interaction</i>	0.33		0.87		0.07		0.72		0.44	

Overall n=561 for log MPO, log AAT, and log NEO, missing: 13; n= 419 for log CRP and log AGP, excluding all children below 6 months at the day of blood collection as they did not provide a serum sample; exposure data from routine surveillance system collected between May and September 2019; Coefficient (coef.) and p-value from multilevel regression models, adjusted for child age and sex, with settlement random effects; [#] An interaction term was used to calculate effects by poultry ownership or location, [§]poultry kept in a shed for any time a day, Abbreviation: EED: Environmental Enteric Dysfunction, CI: 95% Confidence Interval, p-val: p-value, Interv: Intervention, MPO: Myeloperoxidase, AAT: Alpha-1-Antitrypsin, NEO: Neopterin, CRP: C-reactive protein, AGP: Alpha-1-acid glycoprotein.