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The effect of myo–inositol supplementation on feed physicochemical structure and viral load of dry cat food contaminated with SARS–CoV–2 by simulating sneezing

Efecto de la suplementación con mioinositol en la estructura fisicoquímica de la carga viral en el alimento para gatos contaminados con SARS–CoV–2 mediante la simulación de estornudos

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ABSTRACT

The study was carried to investigate the effect of myo–inositol supplementation on feed physicochemical structure and viral load of dry cat food contaminated with inactive SARS–CoV–2 by simulating sneezing. The most natural infection of severe acute respiratory syndrome coronavirus 2 (SARS–CoV–2) in animals is related to close contact with their owners with COVID–19 which is handling, taking care and feeding them. SARS–CoV–2 can survive on food, fomites and surfaces for extended periods related to environmental conditions. Many natural feed additives and supplements have been a candidate in recent antiviral treatment strategies against COVID–19. In this study, myo–inositol which is permitted in animal nutrition was used at different concentrations (0, 12.5, 25 and 50 mg·100 g^{-1} cat food) and conditions (22°C at room temperature and 4°C in the refrigerator) to investigate its effects on feed physicochemical structure and viral load of dry cat food contaminated with inactive SARS–CoV–2 by simulating sneezing. For the interactions between myo–inositol, feed structure and viral load, dry matter, moisture, water absorption index (WAI), water solubility index (WSI), pH and virus gene copy (GC) by RT–qPCR were measured. As only storage temperature affected both WAI and WSI as expected, myo–inostol supplementation dose–dependently decreased gene copy in dry cat food (IC_{50}:366.4–581.5 mg·100 g^{-1} cat food) at 22°C storage temperature. Virus GC did not correlate with the dry matter, moisture content, pH and WAI after the 30 min contact time (except WSI). In conclusion, myo–inositol as a feed additive might have the potential to control serious viral infections such as COVID–19 for human–animal interactions in a One–Health context.

Key words: Myo–inositol; feed–borne; feed safety; SARS–CoV–2; cat food

RESUMEN

Se llevo a cabo un experimento con el objetivo de estudiar el efecto de la suplementación con mio–inositol en la estructura fisicoquímica del alimento y la carga viral del alimento seco para gatos, contaminado con SARS–CoV–2 inactivo, mediante la simulación de estornudos. La infección más natural del síndrome respiratorio agudo severo coronavirus 2 (SARS–CoV–2) en animales está relacionada con el contacto cercano con sus dueños con el COVID–19 que es el manejo, cuidado y alimentación de los mismos. El SARS–CoV–2 puede sobrevivir en alimentos, fómites y superficies durante períodos prolongados en relación con las condiciones ambientales. Muchos aditivos y suplementos naturales para piensos han sido candidatos en las recientes estrategias de tratamiento antiviral contra el COVID–19. En este estudio, se utilizó mio–inositol, que está permitido en la alimentación animal, en diferentes concentraciones (0; 12,5; 25 y 50 mg·100 g^{-1} de alimento para gatos) y condiciones (22 °C a temperatura ambiente y 4 °C en frigorífico) para investigar sus efectos sobre la estructura fisicoquímica y la carga viral del alimento seco para gatos contaminado con SARS–CoV–2 inactivo mediante la simulación de estornudos. Para las interacciones entre el mioinositol, la estructura del alimento y la carga viral, se midieron la materia seca, la humedad, el índice de absorción de agua (WAI), el índice de solubilidad en agua (WSI), el pH y la copia del gen del virus (GC) por RT–qPCR. Como solo la temperatura de almacenamiento afectó, tanto a WAI como a WSI como se esperaba, La suplementación con mioinositol disminuyó de forma dependiente de la dosis la copia genética en la comida seca para gatos en (IC50: 366,4–581,5 mg·100 g^{-1} de comida para gatos) a una temperatura de almacenamiento de 22 °C. La GC del virus no se correlacionó con la materia seca, el contenido de humedad, el pH y el WAI después del tiempo de contacto de 30 min (excepto WSI). En conclusión, el mioinositol como aditivo para piensos podría tener el potencial de controlar infecciones virales graves como la COVID–19 para las interacciones entre humanos y animales en un contexto de One–Health.

Palabras clave: Alimento de gato; inocuidad de los alimentos; mioinositol; transmisión por alimentos; SARS–CoV–2

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INTRODUCTION

World Health Organization reported the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread and caused 659 million cases and 6.6 million deaths Worldwide [1]. The genomic structure of this zoonotic virus is much closed among humans, wild and domestic animals, however, the transmission routes have not been identified [2, 3]. Also, the natural and experimental infections of SARS-CoV-2 were demonstrated in wild domestic and captive animals such as (companion animals) ferrets (*Mustela putorius furo*), hamsters (*Mesocricetus auratus*) and household or stray cats (*Felis catus*) through replication and shedding of the virus Ribonucleic acid (RNA) [4, 5, 6]. The most natural SARS-CoV-2 infection in cats was related to close contact with their owners infected with COVID-19 while handling, taking care and feeding them. Recent diagnostic, serologic and also phylogenetic studies suggested that household or stray cats can be infected by each other, other animal species and humans [2, 3, 4, 5, 6]. In Thailand, the transmission by sneezing was reported from cat to human, then veterinarian [2]. On the other hand, experimental studies by intranasal inoculation presented the SARS-CoV-2 replication in the gastrointestinal tract, upper and lower respiratory tract, oronasal shedding for up to 9 days, and transmission in cats which were co-housed with the infected cats [4, 7]. In the studies on virus survival and stability in biological secretions (saliva, mucus) and on fomites (plastic, steel, cotton, glass), SARS-CoV-2 can survive for extended periods related to the temperature and humidity of storage conditions [8, 9, 10]. So, it is suspected that shared cages, beds, litter boxes, food and water bowls might cause indirect SARS-CoV-2 transmission from secretion, feed and fomites to household cats or stray cats in shelters in close contact with each other or person with COVID-19 [3, 4, 11, 12].

Recently, many natural compounds and new molecules are addressed and targeted as candidate food/feed additives and supplements in the antiviral treatment strategies and developing therapeutic agents for COVID-19 [13, 14]. Polysaccharides from the plant as a food/feed additive were suggested that they could have potential activities against SARS-CoV-2 and highly contagious viruses of animals including feline coronavirus, feline herpesvirus 1, feline influenza viruses, feline panleukopenia virus and feline calicivirus of domestic cats [13, 15, 16, 17, 18]. Myo-inositol is a natural polysaccharide synthesized by both animal and plant cells and presented in all tissues as an essential component of biological membranes and lung surfactant [19, 20]. Besides its polysaccharide structure, myo-inositol is described as a nutritional additive as vitamins, pro-vitamins and chemically well-defined substances having a similar effect in fish, dog and cat nutrition by authorities [20, 21, 22]. But, there is a limited number of *in vitro* studies on the antiviral activity of myo-inositol and its derivatives against highly contagious and serious viruses with enveloped or non-enveloped Deoxyribonucleic acid (DNA) and RNA genomes such as rhinovirus, norovirus, Coxsackie virus, herpesvirus, HIV and iridovirus [23].

This study aimed to investigate the interaction of myo-inositol content, storage condition, physicochemical structure and SARS-CoV-2 load of dry cat food.

MATERIALS AND METHODS

Sample preparation

Myo-Inositol was in powder form and food grade with purity >0.98% (Aromel Chem. Medical, Turkey) Packed dry cat foods which had the same lot and part number were supplied from the retail market. The manufacturer of the food declared that the food was for 2–12 month aged kittens and did not supplement with inositol or its derivatives.

Whole food was ground and sieved through 600 µm mesh. Myo-inositol powder was added at the concentrations of 0, 12.5, 25 and 50 mg·100 g^{-1} feed and mixed homogenously in an industrial food mixer following each cleaning step.

Feed physicochemical analysis

Ground and sieved (200 µm) feed samples were dried at 60°C for 24 h to determine the dry matter and moisture content of experimental feeds supplemented with myo–inositol. Then, 1 g of dried feed samples were weight in a tared centrifuge tube and 10 mL ultra–pure distilled water was added at room temperature of 22°C and in the refrigerator of 4°C (Antech, MPR–60, China). The tubes were inverted three times in 10 min for 30 min. Then, all tubes were centrifuged (~1500 G, 25 min, Eppendorf 5804 Centrifuge, Germany). Each supernatant was transferred to a tared aluminium foil and dried at 60°C for 12 h. The pellets in the centrifuge tubes were weighted (Isolab, 602.31.001, Germany) to calculate the water absorption index (WAI) and the water solubility index (WSI) as follows;

$$WAI\left(g/g\right) = Weight\ of\ pellet \Big/ Sample\ weight\ (dry-based)$$

$$WSI(\%) = Weight\ of\ dried\ supernatant \Big/ Sample\ weight\ (dry-based)$$

To measure pH of feed, 20 mL of ultra–pure water was added to 20 g of feed sample in a 50 mL beaker. The suspension was stirred for 5 min and kept at 22°C at room temperature and 4°C in the refrigerator for 1 h. After the separation of the solid phase from the suspension, the pH of the aqueous phase was measured by a digital pH meter (MW102, Milwaukee, USA).

Virus inoculation in cat food samples

A stock solution of inactivated SARS–CoV–2 virus was prepared at the concentration of 2.1×10^8 gene copies·μL^{-1} verified by RT–qPCR. The stock solution was separated from the residual cell debris by centrifuging and the supernatant was stored at -80°C (Thermo Scientific, EXF24086A, USA) for further analysis.

A plastic bottle sprayer was used in the BSL–2 cabinet to simulate the virus contamination by sneezing and to spread the inactive virus on feed homogeneously (FIG. 1). The virus stock solution was once sprayed (300 µL) on feed sample surfaces of 100 g in triplicate and thoroughly mixed in capped sterile 500 mL bottles. Each bottle containing the virus–inoculated feed mixture was stored at room temperature (21.8 ± 0.4°C) or 4 ± 0.5°C in a refrigerator for 30 min.

After the storage period at two different temperatures, 400 mL cold Phosphate Buffered Saline (PBS) (pH 7.4) was added to each bottle to make a 20% suspension and the bottles were inverted three times. The suspension was filtered with a sterile 0.45 µm syringe filter and the aliquots were used for SARS–CoV–2 RT–qPCR assay.

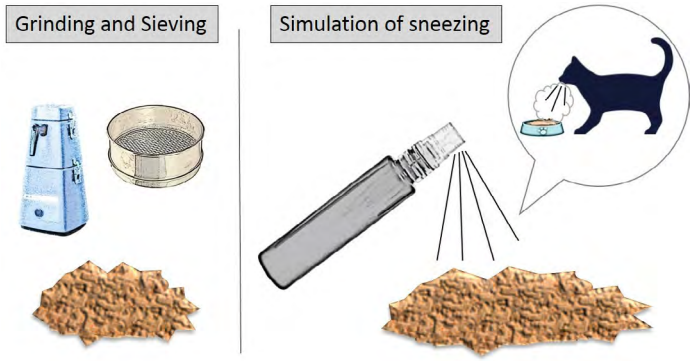

FIGURE 1. Virus inoculation on the ground and sieved cat food by simulating sneezing

Extraction and quantitation of total RNA in cat foods

A total nucleic acid isolation kit (Roche Diagnostics GmbH, Germany) integrated with MagNa Pure LC LCPG 1170 (Roche Diagnostics GmbH, Germany) device was used to isolate total nucleic acid from aliquots (large volume of 1 mL) of virus suspension. Briefly, lysis/binding buffer lysed cells and released nucleic acids. For the digestion of proteins, the proteinase K enzyme was used. Then, released nucleic acids bonded by adding MGP (magnetic glass particles). MGPs with bound nucleic acids are magnetically separated from the residues and washed with wash buffers to remove unbound substances like proteins (nucleases), cell membranes, PCR inhibitors and salt. The wash buffer containing residual debris was discarded. The purified nucleic acids were eluted at +70°C from the MGPs in a final volume of 50 µL and stored at +4°C to quantitate total RNA and gene copies (GC) of SARS-CoV-2 in the extracts on same day.

The UV/VIS spectrophotometer (ND-1000, NanoDrop, Thermo Fisher Scientific, USA) was used to measure the total RNA. Both optical surfaces of the micro-spectrophotometer were cleaned by bathing with sterile deionized water (2 µL) and wiping with a Kimwipe (Kimberly-Clark Professional, USA) following each measurement. One microliter nucleic acid extract (three replicates) was used to measure the total RNA in three replicates. For the blank, sterile DNAse/RNAse-free water was used. The absorption ratios of 260/280 were verified for the quality measurement of the nucleic acid extraction from feed samples.

RT-qPCR protocol and gel electrophoresis

SARS-CoV-2 gene copies were quantified in 20 µL reaction volume (5 µL sample and 15 µL master mix) using SARS-CoV-2 (2019 nCoV) real-time PCR diagnostic kit (Ref: KRM-136-002, V2, KrosQuanT, Turkey) recommended by the World Health Organization (WHO), China-CDC and USA-CDC [24]. Forward primers, reverse primers and probes for two gene regions as N1 and N2 of SARS-CoV-2 were designed as in TABLE I. The human RNAse P gene was used as the internal control. The RT-qPCR assays were performed using the Rotor-Gene Q (QIAGEN, Hilden, Germany). Thermal cycling conditions consisted of RT at 45°C for 10 min, denaturation and Taq polymerase activation at 95°C for 2 min and 45 cycles of 95°C for 10 s followed by 55°C for 30 s (data collection). RT-qPCR reactions were performed with six replicates for each sample. The quantification of virus gene copy in feed extracts was performed with the positive and negative controls in the kit by generating the standard curve.

TABLE I
The primers and TaqMan probes used for the RT-qPCR detection of SARS-CoV-2

Assay	Name	Function	Sequence (5'----3')
2019-nCoV_N1 (72 bp)	CDCN1-F	Forward primer	GACCCCAAAATCAGCGAAAT
	CDCN1-R	Reverse primer	TCTGGTTACTGCCAGTTGAATCTG
	CDCN1-P	TaqMan probe	ACCCCGCATTACGTTTGGTGGACC-
2019-nCoV_N2 (67 bp)	CDCN2-F	Forward primer	TTACAAACATTGGCCGCAAA
	CDCN2-R	Reverse primer	GCGCGACATTCCGAAGAA
	CDCN2-P	TaqMan probe	ACAATTTGCCCCCAGCGCTTCAG-

All probes were labelled with 5'-FAM and Q1 3'

For the confirmation by gel electrophoresis, RT-qPCR amplification products were used with 1.5% (w/v) agarose gel stained by 2 µL ethidium bromide (Invitrogen). The gel on the caster was kept at room temperature for 20 min to be cool and solid. Then, it was transferred to the wide gel electrophoresis system (Wide Mini Sub Gel integrated with PowerPac™, Bio-Rad Laboratories, Inc., USA) filled with TBE buffer (1x). Five microliters of each PCR product, as well as a ladder marker, were loaded into the wells and electrophoresed at 90 V for 30 min. The bands were imaged using a standard cellular phone camera on the purple LED flashlight device (FIG. 2).

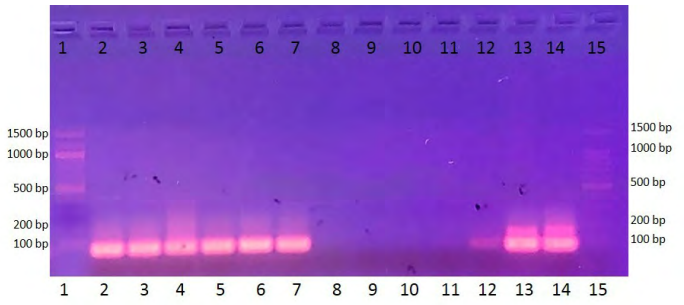

FIGURE 2. The photograph of gel electrophoresis confirmation. Lanes 1 and 15: ladder | Lanes 2, 3, 4: 12.5, 25 and 50 mg·100 g^{-1} feed at 4°C | Lanes 5, 6, 7: 12.5, 25 and 50 mg·100 g^{-1} feed at 22°C | Lanes 8, 9, 10, 11, 12: negative controls | Lanes 13 and 14: positive controls (72-67 bp) at 22°C and 4°C

Statistical analysis

The data analysis was conducted with parametric and non-parametric methods by SPSS software version 15 software (IBM, USA). The normality and homogeneity of variance were controlled by Kolmogorov-Smirnov, Shapiro-Wilk and Levene's Test. The differences between groups were analysed by Kruskal Wallis test and one-way ANOVA with post hoc Tukey. The general linear model (GLM) was used to evaluate the effect of myo-inositol concentration and storage temperature as fixed factors on physicochemical parameters, total RNA content and gene copy of the virus as dependent variables. The interaction between measurement parameters was exhibited by Pearson's two-tailed bivariate correlation test. The significance level was regarded as $P<0.05$. The tables and the graphs were generated in Office Excel 2016 (Microsoft, USA).

RESULTS AND DISCUSSIONS

Physicochemical parameters of experimental cat foods

There was no significant difference in the dry matter, moisture and pH properties of the samples containing different myo-inositol concentrations and stored at both temperatures (TABLE II). While myo-inositol supplementation did not affect WHC, WSI was significantly decreased by decreasing myo-inositol concentration. The increase in storage temperature significantly reduced the WHC and WSI of all experimental foods (P<0.01, TABLE II). So, there was a significant interaction between myo-inositol supplementation and storage temperatures on WHC and WSI as shown in TABLE II (P<0.05).

Dry matter, moisture content and pH of food samples did not significantly correlate with the storage temperature, myo-inositol content, virus gene load and virus reduction rates after the 30 min contact time (P>0.05). WHC and WSI were significantly related to the storage temperature with a negative correlation (P<0.01). And, a positive-moderate correlation was determined between WHC and virus gene load in food samples (P<0.05) independently of myo-inositol content and virus reduction rate (TABLE III).

TABLE II
Physicochemical properties of cat food supplemented with myo-inositol

Myo-inositol (mg·100 g^{-1} feed)	Dry matter		Moisture		WHC		WSI		pH	
	4°C	22°C	4°C	22°C	4°C	22°C	4°C	22°C	4°C	22°C
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
50	94.51±0.06	94.51±0.07	5.49±0.06	5.49±0.07	3.88±0.11	3.37 ± .09	10.75±0.01a	8.62±0.01a	5.59±0.03	5.61±0.05
25	94.46±0.04	94.50±0.08	5.54±0.04	5.50±0.08	3.94±0.10	3.37±0.22	10.12±1.08a	8.42±0.35a	5.59±0.03	5.62±0.05
12.5	94.57±0.05	94.48±0.02	5.43±0.05	5.52±0.02	3.81±0.38	3.68±0.07	8.42±0.35b	6.73±0.22b	5.61±0.06	5.63±0.05
0 (Control)	94.61±0.09	94.46±0.04	5.39±0.09	5.54±0.04	3.76±0.14	3.64±0.14	8.02±0.01b	6.62±0.02b	5.58±0.06	5.63±0.15
	P-value									
Myo-inositol (A)	0.970	0.962	0.097	0.962	0.945	0.197	<0.01	<0.01	0.993	0.999
Storage Temp. (°C) (B)	0.084		0.084		<0.01		0.003		0.312	
(A) × (B)	0.051		0.051		0.02		<0.01		0.911	

TABLE III
The correlation coefficiencies (β) between food parameters and viral load

β Coefficiency	Storage Temp. (°C)	Myo-inositol Conc.	Gene Copy (GC·mL^{-1})	Virus reduction (%)
Dry matter (%)	-0.343	-0.097	0.070	-0.124
Moisture (%)	0.343	0.097	-0.070	0.124
WHC	-0.766**	-0.094	0.422*	0.202
WSI (%)	-0.556**	0.038	0.250	0.318
pH	0.207	0.065	-0.130	0.004

*P<0.05; **P<0.01

The physicochemical properties of food such as nutritional content, dry matter, moisture, humidity, water holding and pH can affect the persistence, transmission and stability of possible feed-borne viruses by influencing their survival and replication in the feed matrix [25, 26, 27, 28]. On complete feed and feedstuff with high moisture ingredients porcine alpa- and delta-coronavriuses (PEDV, TGEV, PDCoV) were longer stable and survived [27, 29]. It was suggested that PEDV survived much better under cooler and high moisture conditions than in warm and drier conditions [30]. Similarly, hepatitis A virus (HAV) and porcine parvovirus (PPV) were tittered greater than 4 and 3.2 log in feed samples containing high moisture [31]. Also, African swine fever virus (with DNA genome) survived at a higher titter in moist cat and dog food than dry dog food and complete porcine feed [25]. In this study, while myo-inositol supplementation did not affect the physicochemical parameters, the storage temperature did WHC and WHC expectedly. But a significant interaction was not determined between the feed structure and SARS-CoV-2 gene load on dry cat food.

The quantification of total RNA

The total RNA content in the isolates was significantly decreased by increasing the myo-inositol concentration in the feed at each storage temperature compared with the control (P<0.01) (TABLE IV). Thus, the storage temperature did not affect the total RNA content in the isolates of the experimental feed (P=0.106). A260/A280 ratio indicates the quality of RNA extraction and protein contamination level in the isolates. Greater than 1.8 is generally considered an acceptable extraction quality with low contamination and low inhibitor [32, 33]. The A260/A280 was calculated between 1.45 and 1.96.

The quantification of viral load by RT-qPCR

The gene copy of SARS-CoV-2 was significantly reduced by increasing the concentration of myo-inositol in cat food (P<0.001) and storage temperature (P=0.003). The highest relative reduction (more than 50%) of virus GC was measured with 25 mg myo-inositol at 22°C and 50 mg myo-inositol at both 22°C and 4°C (P<0.01) (TABLE V). The supplementation of 12.5 mg myo-inositol did not significantly affect the virus gene copy in cat food at both storage conditions compared with the others and the control food. As the virus GC decreased significantly by increasing the storage temperature (P=0.003), no interaction between myo-inositol concentration and storage temperature for the gene copy of SARS-CoV-2 inoculated

TABLE IV
The total RNA measured by the UV/VIS spectrophotometer

Myo–inositol (mg·100 g^{-1} feed)	Storage Temp. (°C)	Total RNA (mean±SD) (ng·µL^{-1})	Abs. 260 (nm)	Abs. 280 (nm)	A260/A280
50	22°C	56.96±2.36^{c}	1.424	0.968	1.47
25		72.25±2.05^{b}	1.218	0.799	1.52
12.5		77.06±4.53^{ab}	1.926	1.327	1.45
0 (Control)		84.41±5.56^{a}	1.522	1.031	1.48
50	4°C	44.42±3.94^{z}	1.111	0.765	1.45
25		81.74±4.06^{y}	2.051	1.374	1.49
12.5		82.04±2.36^{xy}	2.043	1.369	1.49
0 (control)		91.17±3.10^{x}	2.28	1.162	1.96

Myo–inositol: P<0.01, Temp: P=0.106, Myo–inositol*Temp: P=0.077

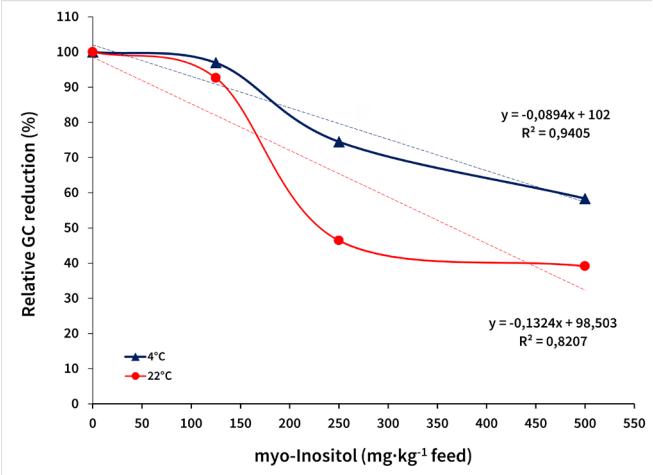

FIGURE 3. The reduction of SARS-CoV-2 gene copies by myo-inositol supplementation

in cat foods (P=0.185) was found. IC_{50} was calculated as 366.4 mg and 581.5 mg myo–inositol/100 g feed for the storage conditions of 22°C and 4°C respectively (FIG. 3).

The high standard deviation increases the coefficient of variation (CV)(TABLE V). The precision (%CV) of the measurements at each level should not exceed 15% [34, 35]. In this study, the calculated CV (%) for each experimental feed was lower than the acceptance criteria of 15%.

Inositol or myo–inositol is an endogenous active substance and is allowed to use as feed supplement. Although there are some limitations in human nutrition, it is permitted in fish, Crustacean, cat and dog nutrition, feed production and foodstuffs of animal origin [20, 22, 36, 37]. European Food Safety Authority categorized inositol as a nutritional additive (functional group: vitamins, pro–vitamins and chemically well–defined substances having a similar effect) in food for dogs (*Canis lupus familiaris*) and cats on panel of additives and products or substances used in animal feed (FEEDAP). The FEEDAP Panel suggested that free inositol could be safe for dogs and cats at the concentration of up to 3,000 mg free inositol·kg^{-1} dry complete feed in commercial diets for pets [36]. In this study, myo–inositol was used at recommended safe concentrations with a maximum 500 mg·kg^{-1} complete cat food (527.5 mg·kg^{-1} based on dry matter of 94.5%). Previous studies have shown the antiviral effects of inositol and its derivatives on mostly high contagious and serious agents with DNA or RNA genomes [23, 39, 40]. Especially, at various concentrations, it was effective in the inhibition of Coxsackie (non–enveloped, RNA), Herpesviruses (enveloped, DNA). HIV (enveloped, RNA), and Iridovirus (enveloped, RNA) threaten human and animal health [23, 38, 39]. Phosphate and sulphate derivatives of myo–inositol had shown antiviral activity against viruses including Coxsackie virus, Herpes Simplex virus and Iridovirus with IC_{50} concentrations of lower than 50 µg·ml^{-1} [39]. Meanwhile, plant based–inositol was not cytotoxic on fish spleen cell culture at the concetrations of up to 2,000 µg·ml^{-1} and also it presented an *in vitro* antiviral activity against Iridovirus at the rate of up to 90% [40]. In this study, myo inositol supplementation dose–depently decreased inactive SARS–CoV–2 in cat food with IC_{50} of 366.4 – 581.5 mg·100 g^{-1} feed. Recent results had shown that myo–inositol supplementation at safe concentration could have the ability of dose–response inhibition of SARS–CoV–2 (enveloped, RNA) contamination or persistency on cat food, independently of the storage temperature and feed physicochemical structure.

Pets, especially cats, are still suspected of Covid–19 transmission routes because of its zoonosis characteristic. Experimental and

TABLE V
The effect of myo–inositol on viral load by RT–qPCR quantification

Myo–inositol (mg·100 g^{-1} feed)	Storage Temp. (°C)	Inoculated (GC·µL^{-1})	Recovered (mean±SD) (GC·µL^{-1})	Ct	CV (%)	Relative Recovered (%)	Relative Reduction (%)	IC50 (mg·100 g^{-1} cat food)
50	22°C	16×10^{5}	$3.8\times10^{4}\pm1.6\times10^{3\,b}$	24.10±0.08	4.1	39.2	60.8	366.4
25		16×10^{5}	$4.5\times10^{4}\pm3.7\times10^{3\,b}$	23.82±0.14	8.3	46.4	53.6	
12.5		16×10^{5}	$8.9\times10^{4}\pm7.6\times10^{3\,a}$	22.81±0.16	8.5	92.6	7.4	
0 (control)		16×10^{5}	$9.6\times10^{4}\pm6.3\times10^{3\,a}$	22.88±0.05	6.6	100.0	0.0	
50	4°C	16×10^{5}	$5.9\times10^{4}\pm5.3\times10^{3\,y}$	23.38±0.14	8.9	58.3	41.7	581.5
25		16×10^{5}	$7.6\times10^{4}\pm7.4\times10^{3\,xy}$	22.98±0.16	9.8	74.5	25.5	
12.5		16×10^{5}	$9.8\times10^{4}\pm6.1\times10^{3\,x}$	22.84±0.12	6.2	96.9	3.1	
0 (control)		16×10^{5}	$10.0\times10^{4}\pm3.4\times10^{3\,x}$	22.78±0.17	3.4	100.0	0.0	

Myo–inositol: P<0.001, Temp: P=0.003, Myo–inositol*Temp: P=0.228

epidemiological studies suggested the symptoms (sneezing, nasal draining), transmission risk and immunity among stray or household cats with COVID-19-positive owners [7, 41, 42]. The frequencies of feeding, contacting such as kissing, grooming, handling of feed and water bowls, surfaces and fomites which animal also has contacted was expected to be significant risk factors of viral gene load and transmission of SAR-CoV-2 [42]. Moreover, the environmental conditions such as temperature, humidity and surface material types were also effective factors in the infectivity and lifespan of the virus. At 20°C and more, the viability and half-life of SARS-CoV-2 sharply decreased on many fomites and surfaces [8, 10, 43]. On surfaces such as glass, stainless steel and both paper and polymer banknotes, the half lives of SARS-CoV-2 were determined between 1.7 and 2.7 days at 20°C, reducing to a few hours at 40°C [8]. In nasopharyngeal and oropharyngeal liquids of the patients, SARS-CoV-2 was invective in 46.2 % of patients. While after coughing, no infectious virus was recovered, Viral recovery was high in intensive moistening with saliva contaminating steel carriers So, its genome could be recovered from high-moistening surfaces contacted with saliva [10]. Similarly, SARS-CoV-2 maintained infectivity in foods with high protein, fat and moisture such as raw meat for up to 14 days, but not in processed food due to food additives and preservative contents [44]. The dry cat food used in the study had very high dry matter (~95%) and low moisture rates (~5%). Likewise the other studies, the viral load significantly decreased by both myo-inositol supplementation and the high temperature condition (at 22°C). This first study represented that both myo-inositol supplementation as feed additive, temperature and also WHC could have a role in the persistence of SARS-CoV-2 load on dry cat food.

CONCLUSION

Despite no difference in the physicochemical properties, myo-inositol supplementation cause dose-response reduction of SARS-CoV-2 gene load on cat food at both storage temperatures. So, while more research is needed to fully understand the antiviral activity of myo-inositol, recent results suggested that it may have the potential as a feed additive to reduce the risk of zoonotic viral infections such as COVID-19 for human-animal interactions in a One-Health context.

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