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Učinci preparata Ekolostrum na salivarni IgA: prethodno istraživanje

A Pilot Study of Effects of Ecolostrum on Salivary IgA

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Sažetak

Svrha rada: Svrha ove studije bila je procijeniti utjecaj oralne primjene *Ekolostruma* (goveđeg kolostruma) na koncentraciju salivarnog IgA (sIgA). **Materijali i metode:** Dvadesetero zdravih ispitanika u dobi između 23 i 54 godine nasumce je podijeljeno na ispitnu i kontrolnu skupinu (n = 10). Svatko u ispitnoj skupini dobio je 60 kapsula *Ekolostruma*. Na početku istraživanja od svih je sudionika bila prikupljena nestimulirana slina. To isto učinjeno je nakon 30 dana, na kraju pokusa. Koncentracija sIgA iz sline određivala se testom ELISA (enzim-linked immunosorbent assay). Usporedbe rezultata unutar skupine obavljene su studentovim t-testom, a usporedbe rezultata između dviju skupina primjenom ANOVE (p=0,05). **Rezultati:** Sudionici ispitivanja koji su uzimali *Ekolostrum* imali su statistički značajno (p = 0,026) niže koncentracije sIgA nakon tretmana negoli prije. Kontrolna skupina nije pokazala statistički značajnu razliku u koncentraciji sIgA na početku i na kraju istraživanja. Na kraju istraživanja nije bilo statistički značajne razlike (p = 0,047) srednjih koncentracija sIgA između ispitne i kontrolne skupine (464 ± 59.89μg/mL i 620,16 ± 42.26μg/mL). **Zaključak:** Rezultati ovog istraživanja pokazuju da je nakon jednomjesečne oralne primjene *Ekolostruma* koncentracija sIgA bila statistički značajno niža nego prije tretmana. Potrebna su daljnja, opsežnija i detaljnija istraživanja kako bismo dobili pouzdanije rezultate.

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Ključne riječi
Kolostrum, sIgA; Slina

Uvod

Kolostrum je tekućina koja se izlučuje iz mliječnih žlijezda kratko nakon rođenja sisavaca. Goveđi kolostrum mlijeko je koje se izlučuje prvih nekoliko dana nakon teljenja, a njegova važnost za zdravlje teladi, ali i ljudi, dobro je poznata (1). Sadržava hranjive tvari poput bjelančevina, ugljikohidrata, masti, vitamina, minerala, čimbenika rasta i antimikrobnih čimbenika koji uključuju imunoglobuline i laktoperoksidaze, lizozim i laktoferin (2,3).

Kako se preparat kolostruma sve češće može naći na tržištu i sve je raširenija njegova uporaba, to zahtijeva nova istraživanja, kao što je njegov utjecaj na koncentraciju salivarnog IgA (sIgA).

Žlijezde slinovnice proizvode IgA antitijela izravnom imunizacijom limfnog tkiva u crijevima (GALT), odakle senzibilizirani B-limfociti dospijevaju u žlijezde slinovnice. Salivarna IgA protutijela imaju izravan pristup površini zuba. Ona mogu spriječiti prianjanje *S. mutans* na površinu cakline ili onemogućiti nastanak dekstrana inhibicijom aktivnosti glukoziltransferaza. Salivarni IgA, kao primarni imunoglobulin sadržan u sekretu mukoznog imunosnog sustava i njegova razina u slini, korelira s povećanim brojem karijesa više nego serumski imunoglobulini ili drugi imunosni parame-

Introduction

Colostrum is a pre-milk fluid secreted only for a short time after birth of any mammal. Bovine colostrum is milk secreted during first few days after calving and its importance for the health of calves has been well known (1). It contains nutrients such as proteins, carbohydrates, fats, vitamins, minerals, growth factors and antimicrobial factors which include immunoglobulins, lactoperoxidase, lysozyme and lactoferrin (2,3).

A more frequent presence of colostrum on the market and its widespread use requires new studies such as the effects colostrum on salivary IgA (sIgA).

The salivary glands produce secretory IgA antibodies by direct immunization of the gut associated lymphoid tissue (GALT), from where sensitized B-cells may be housed in the salivary glands. The salivary IgA antibodies have, of course, direct access to the tooth surface. They may prevent *S. mutans* from adhering to the enamel surface or they may prevent formation of dextran by inhibiting the activity of glucosyltransferase (GTF). Salivary IgA as the primary immunoglobulin contained in the secretion of the mucosal immune system and its levels in salivary fluids correlate more closely with the increased number of dental caries than the serum of im-

tri (4). Salivarni IgA je dimerička molekula – dvije molekule IgA su spojene J lancem. Proizvod je plazma stanica u mukoznom limfoidnom tkivu i izlučuje se kroz epitel u lumen dišnog ili probavnog trakta (5). Nakon što dospije u lumen IgA zaštićuje mehanizme koji uključuju (i) onemogućuju prianjanja mikroorganizama na mukozne površine, (ii) onemogućuju prodor antigena kroz površinu epitela, (iii) stvaranje kompleksa s antigenom na površini epitela sluznice kako bi se olakšala eliminacija egzocitozom (iv) mehanizmi spašavanja na međustaničnoj i unutarstaničnoj razini. Mnogobrojna su istraživanja o učincima goveđeg kolostruma na koncentraciju IGF-I u serumu, o koncentracijama protutijela u goveđem kolostrumu i njihovoj ulozi u oralnoj imunoterapiji te o učincima na *Helicobacter pillori* (7-9), a rezultati su bili dvosmisleni. Samo se nekoliko istraživanja bavilo učincima primjene goveđeg kolostruma na sIgA.

Svrha ovog istraživanja bila je procijeniti učinak jednog od proizvoda s goveđim kolostrumom dostupnih na hrvatskom tržištu – *Ekolostruma* (Medis d.o.o, Ljubljana, Slovenija) na koncentraciju sIgA u slini.

Materijali i metode

Ispitanici

Ukupno trideset i dva dobrovoljca odabrana su za sudjelovanje u ovoj studiji. Prije početka istraživanja svatko je od njih odgovorio uzima li ikakve lijekove i dodatke prehrani te je li nedavno imao simptome nekih bolesti. Samo zdravi ispitanici koji nisu uzimali lijekove ili dodatke prehrani i bez poznatih alergija na komponente *Ekolostruma* uključeni su u istraživanje. Ukupno dvadesetero dobrovoljaca (studenti, profesori, pacijenti i zubni asistenti na Zavodu za endodonciju i restaurativnu stomatologiju Stomatološkog fakulteta Sveučilišta u Zagrebu) odabrano je za ovo istraživanje. Među njima bilo je četrnaest žena i šestorica muškaraca između 23 i 54 godine. Četvero od njih bili su pušači. Nakon informiranja ispitanika o svrsi istraživanja, mogućim rizicima i koristima, svi su potpisali informirani pristanak. Sve dijelove istraživanja odobrilo je Etičko povjerenstvo Stomatološkog fakulteta u Zagrebu.

Plan istraživanja i postupci

Desetero dobrovoljaca nasumce je svrstano u ispitnu skupinu, a ostalih desetero u kontrolnu. Program MedCalc (MedCalc Software Mariakerke, Belgija) odabran je za randomizaciju. Svaki ispitanik iz obje skupine dobio je detaljne upute o oralnoj higijeni i rečeno mu je da nastavi sa svakodnevnim aktivnostima. Također je prije početka istraživanja svaki sudionik dobio upute da pola sata prije prikupljanja sline ne jede i ne pije, ne puši i ne pere zube kako bi se smanjila opasnost od kontaminacije uzoraka. Ispitivanje je provedeno prije početka istraživanja za sve ispitanike isti dan između osam i dvanaest sati te trideseti dan nakon primjene

munoglobulins or other immune parameters (4). sIgA is a dimeric molecule; two IgA molecules are joined together by the J chain. It is made by plasma cells in the mucosal lymphoid tissue and secreted through the epithelium into the lumen of the respiratory or gastrointestinal tract (5). Once in the lumen, IgA affords protection by mechanisms that include (i) interfering with microbial adherence to mucosal surface; (ii) inhibiting penetration of antigens across the epithelial surface; (iii) complexing with antigens at the basolateral surface of the mucosal epithelium to facilitate elimination by exocytose into the mucosal lumen; and (iv) salvage mechanisms at intercellular and interstitial levels (6).

There were many studies on the effects of bovine colostrum supplementation to the concentration of serum IGF-I, concentration of antibodies in bovine colostrum and their role in oral immunotherapy as well as effects against *Helicobacter pillori* (7-9) which have shown equivocal results. Only few studies tested the effects of bovine colostrum on sIgA.

The purpose of this pilot study was to evaluate the effects of one of the bovine colostrum products available on the Croatian market- Ekolostrum (Medis d.o.o, Ljubljana, Slovenija) on concentration of sIgA in a saliva.

Materials and methods:

Subjects

In total thirty-two volunteers were considered for participation in this study. Before experimental testing each participant was questioned about the present use of medications and dietary supplements as well as about the recent signs and symptoms of some diseases. Only healthy participants who were not taking dietary supplements or medications and had no proven allergies on components of Ekolostrum were included. After selection, twenty participants (students, professors, patients and dental assistants at the Department of Endodontics and Restorative Dental Medicine, School of Dental Medicine, University of Zagreb) were included in this study. They were between 23 and 54 years of age, that is, fourteen females and six males. Four of them were smokers and that was noted. After explaining to them the purpose of the study, possible risks and benefits, all participants signed written informed consent. All parts of study were approved by the Ethics Committee of the School of Dental Medicine Zagreb.

Study design and procedures

Ten volunteers were randomly selected for the test group and ten of them for the control group. Program MedCalc (Medcalc Software Mariakerke, Belgium) was used for randomisation. Each subject from both groups was instructed about oral hygiene and was told to continue with everyday activities. Also, before beginning of the study, each subject was instructed not to eat or drink, smoke or brush teeth half an hour before the saliva collection in order to minimize the risk of contamination. Testing was performed for all the subjects on the same day between eight and twelve a.m. before and thirty days after dietary supplementation with Ekolostrum. Subjects were asked to record any strange signs or any

Ekolostruma. Ispitanici su zamoljeni da zabilježe bilo kakve neobične simptome ili neugodne promjene ako se pojave i odmah nam se obrate. Koncentracija sIgA iz sline određena je čitačem mikroploče (Sinergy2 Alpha, Biotech instrumenti, Winooski, Sjedinjene Američke Države), a zatim je izražena u g/mL.

Prikupljanje sline

Nestimulirana slina prikupljala se kontinuirano u volumenu od 1,8 ml. Koristile su se dvije epruvete (Safe-Lock Eppendorf tube, 2,0 ml, Hamburg, Njemačka) i plastične pet centimetara duge sterilne slamke. Uzorci su zatim dostavljeni u laboratorij Odjela za imunologiju Klinike za onkologiju i nuklearnu medicinu Kliničke bolnice Sestara milosrdnica u Zagrebu gdje su deset minuta centrifugirani na 3000 okretaja i zatim je supernatant pohranjen na temperaturi od - 20 ˚C. Isti postupak bio je i nakon 30 dana. Prikupljanje sline obavljeno je u skladu s uputama proizvođača ELISA testa. Za određivanje sIgA koristili smo se ELISA (Enzim-linked immunosorbent assay) testom (Demeditec Dek 8870, Demeditec Diagnostics GmbH, Kiel, Njemačka). Normalni raspon sIgA u slini prema tom testu je 102 - 471µg / ml.

Tretman Ekolostrumom

Ispitanici u ispitnoj skupini dobili su dvije kutije kapsula *Ekolostruma* (30 u svakoj, 60 ukupno) i uzimali su ih 30 dana dva puta na dan po jednu prema uputama proizvođača. Jedna kapsula sadržava 480 mg kvalitativno i kvantitativno standardiziranog kolostruma, a dio IgA je 2,60%. Odlučeno je da se slijede upute proizvođača, što znači 960 mg kolostruma svaki dan. Kontrolna skupina nije uzimala nikakvu terapiju.

Statistička analiza

Parni t-test korišten je za usporedbu koncentracija sIgA prije i poslije tretmana s Ekolostrumom u ispitnoj skupini te u kontrolnoj. ANOVA je korištena za usporedbu početnih i završnih koncentracija sIgA između ispitne i kontrolne skupine. Statistička analiza provedena je s pomoću MS Excela i SPSS ® ver.16.0 (IBM, New Orchard Road, NY, SAD). Razina značajnosti postavljena je na p <0,05. Vrijednosti su izražene kao aritmetička sredina ± SEM (standardna pogreška srednje vrijednosti).

Rezultati

Pregled srednjih vrijednosti koncentracija sIgA prije istraživanja u ispitnoj (698,67 ± 78,38 g / mL) i kontrolnoj (668,56 ± 54,72 g / mL) skupini nije pokazao statistički značajnu razliku. Ipak postoji statistički značajna razlika srednjih vrijednosti koncentracija s IgA između kontrolne i ispitne skupine na kraju istraživanja (ANOVA, F = 4,539, df = 1, p = 0,047) (tablica 1). Utvrđeno je da postoji statistički značajna razlika (t = 2,656 df = 9, p = 0,026) između koncentracija sIgA u ispitnoj skupini prije tretmana (698,67 ± 78,38 g / mL) i nakon njegova završetka (463,99 ± 59,89 g / mL) (slika 1).

unpleasant change they experienced and to contact us immediately. All 20 volunteers initially enrolled in this pilot- study completed basic study requirement. Concentrations of sIgA in saliva were determined by micro plate reader (Sinergy2 Alpha, BioTech instruments, Winooski, United States) and then were expressed in µg/mL.

Saliva collection and analytical assay

Unstimulated content of saliva was collected continuously. Two test tubes (Safe- Lock Eppendorf tubes, 2,0 ml, Hamburg, Germany) and plastic 5 cm long sterile straws were used. The selected volume was 1,8 ml. Samples were then directly transported to the Laboratory of Department of Immunology, Clinic of Oncology and Nuclear Medicine, Clinical hospital 'Sestre milosrdnice', Zagreb where they were centrifuged at 3000 rpm for ten minutes and supernatant stored at the temperature of – 20 ºC. The same procedure was repeated after 30 days. Collecting of saliva was made according to the manufacturer's instructions. For the determination of sIgA, an enzyme- linked immunosorbent assay (ELISA) (Demeditec DEK 8870, Demeditec Diagnostics GmbH, Kiel, Germany) was used. For this assay normal range of sIgA in saliva was 102- 471µg/ ml.

Ekolostrum treatment

The test group participants were given two packages of Ekolostrum (30 capsules in each, 60 in total) and were told, according to the manufacturer's instructions, to take one capsule two times a day for 30 days. One capsule contains 480 mg of qualitatively and quantitatively standardized colostrum and part of IgA is 2.60 %. It was decided to follow the instruction of Ekolostrum's manufacturers which means 960 mg per day. The control group did not get dietary supplementation or placebo capsules.

Statistical analysis

Paired sample t-test was performed to compare the concentration of sIgA before and after treatment with Ekolostrum in the test group and also in the control group. ANOVA was used for comparison of initial and final concentrations of sIgA between the test and control groups. Statistical analysis was performed using MS Excel and SPSS®ver.16.0 (IBM, New Orchard Road, NY, USA). Significance level was set at p< 0.05. Values are reported as mean ± SEM (standard error of the mean).

Results

An examination of mean concentrations of sIgA before experiment in the test (698.67 ± 78.38 µg/mL) and control (668.56 ± 54.72 µg/ mL) group showed no statistically significant difference. However, there was a statistically significant difference of mean concentrations of IgA between the control group and test group at the end of the experiment (ANOVA, F=4.539, df=1, p=0.047) (Table 1). There was a statistically significant difference (paired samples test, t=2.656 df=9, p=0.026) between IgA concentrations in the test group before (698.67± 78.38 µg/ mL) and after (463.99± 59.89 µg/ mL) the treatment (Figure 1). The control group

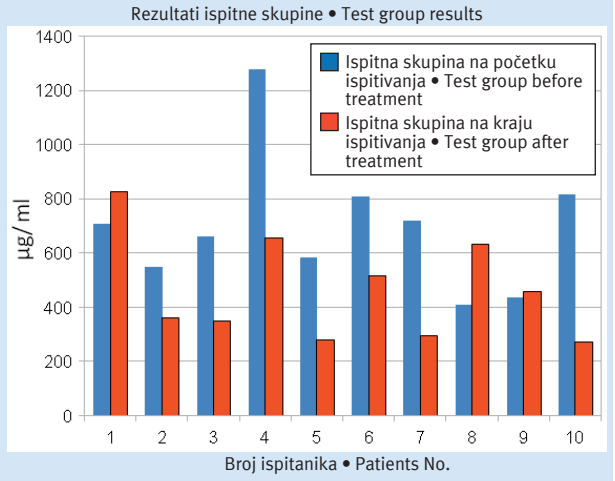
Rezultati kontrolne skupine pokazali su značajne razlike između koncentracija prije (668,56 ± 54.72µg / mL) i poslije (620.16 ± 42,26 g / mL) tretmana *Ekolostrumom* (slika 2).

results showed no significant difference between concentrations before (668.56± 54.72µg/ mL) and after (620.16± 42.26 µg/ mL) treatment (Figure 2).

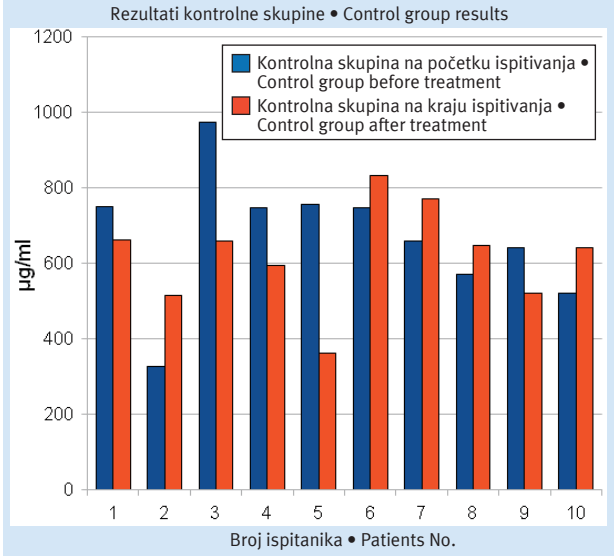
Tablica 1. Koncentracije sIgA u ispitnoj i kontrolnoj skupini na kraju ispitivanja
Table 1 Results of IgA concentrations in the test group and control group after treatment

Skupina • Group	Srednja vrijednost • Mean	N	Standardna devijacija • Std. Deviation	Standardna pogreška srednje vrijednosti • Std. Error of Mean
Ispitna skupina na kraju ispitivanja • Test group after treatment	464,00*	10	189,401	59,894
Kontrolna skupina na kraju ispitivanja • Control after treatment	620,16*	10	133,642	42,261

* µg/ml



Slika 1. Koncentracije sIgA u ispitnoj skupini (µg/ml)
Figure 1 Results of IgA concentrations in the test group (µg/ml)



Slika 2. Koncentracije sIgA u kontrolnoj skupini (µg/ml)
Figure 2 Results of IgA concentrations in the control group (µg/ml)

Rasprava

Svrha naše studije bila je procijeniti učinke preparata *Ecolostrum* na sIgA. Taj se imunoglobulin smatra glavnim nositeljem humoralne imunosti u usnoj šupljini i kao takav je najodgovorniji za smanjenje karijesa. Dosadašnja istraživanja proučavala su vezu između karijesa i koncentracija sIgA i imala su proturječne rezultate. Fontana i suradnici (10), Sepuku i suradnici (11), Sani i suradnici (12) i Cogulu i suradnici (13) pronašli su povećane koncentracije sIgA kod ispitanika bez karijesa, što podupire hipotezu da više koncentracije sIgA mogu rezultirati smanjenjem pojavnosti karijesa. Drugi autori, poput Thaweboona i suradnika (14) te Al Almoundija i suradnika (15), pronašli su veću koncentraciju sIgA u prisutnosti kariogenih mikroorganizama. Bagherian i suradnici (16) smatraju da viša razina sIgA može odražavati prijašnju izloženost domaćina kariogenim mikroorganizmima te da veći broj mikroorganizama rezultira ubrzanom proizvodnjom protutijela. Istraživanje Kuglera i suradnika (17) u kojem je zabilježen pad sIgA nakon uklanjanja karijesa, u skladu je s već navedenim.

Discussion

The aim of this study was to evaluate the effects of Ecolostrum on sIgA. This immunoglobulin is considered to be the main carrier of humoral immunity in the oral cavity and as such is the most responsible for the reduction of dental caries. So far, numerous studies have been performed on the connection between dental caries and the concentration of sIgA with conflicting results. Fontana et al. (10), Sepuku et al. (11), Sani et al. (12) and Cogulu et al. (13) found increased concentrations of sIgA in subjects without dental caries which tends to support the hypothesis that higher levels of sIgA may lead to less dental caries. Other authors such as Thaweboon et al. (14) and Al Almoundi et al. (15) found increased concentration of sIgA in the presence of cariogenic microorganisms. Bagherian et al. (16) suggest that higher levels of sIgA may reflect the past exposure of the host to cariogenic microorganisms and also that the increased load leads to high production of antibodies. Research by Kugler et al. (17) in which they noted the decrease of sIgA after removal of caries is consistent with previous research.

Rezultati našeg istraživanja pokazali su statistički značajan pad koncentracije sIgA nakon mjesec dana oralne primjene *Ekolostruma*, a u kontrolnoj skupini nije bilo statistički značajne razlike. Postoji nekoliko istraživanja o povezanosti prehrambenih dodataka s goveđim kolostrumom i koncentracije sIgA. Mero i suradnici (7,18) obavili su dva istraživanja s kolostrumima različitih proizvođača. Njihovi su rezultati proturječni – u jednom je bila povećana koncentracija sIgA (33%) nakon primjene kolostruma, a u drugom nije bilo značajnijih promjena u koncentraciji sIgA prije i poslije primjene kolostruma. Slične rezultate dobili su u istraživanjima Crooks i suradnici (19, 20). Otkrili su povećane koncentracije sIgA kod maratonaca nakon oralne primjene kolostruma, a u sličnom istraživanju provedenom četiri godine poslije kod trkača na duge staze nije bilo mjerljivih promjena u koncentracijama sIgA. Kao što se vidi, sIgA nije pokazao obrazac povećanja/smanjenja nakon oralne primjene goveđeg kolostruma.

Jedan od razloga za to može biti mnogo parametara koji bi mogli utjecati na koncentraciju sIgA kao što su genetska komponenta, prehrana, stres, pušenje, ...

Neki od naših ispitanika bili su pušači (dvije ispitanice u kontrolnoj skupini i dvojica ispitanika u ispitnoj skupini) te su imali povećani broj sIgA, što je u skladu s rezultatima Usshera i suradnika (21) i Ausara i suradnika (22).

Naša studija korelira s razdobljem završnih ispita kada je najčešće prisutna viša razina stresa kod učenika i profesora. Stres također može utjecati na rezultate pronađene u istraživanjima Matosa Gomeza i suradnika (23), Deinzera i suradnika (24) i Jemmota i suradnika (25).

Ovo istraživanje pokazalo je da se nakon jednomjesečne oralne primjene kapsula *Ekolostruma* od ukupno 960 mg smanjila koncentracija sIgA u slini. Uzimajući u obzir trajanje ispitivanja, broj ispitanika i vanjske čimbenike koji mogu utjecati na rezultate, potrebna su dodatna, opsežnija istraživanja.

Zahvala

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Izjava

Autori negiraju bilo koji sukob interesa.

The results of our pilot study showed a statistically significant decrease in the concentration of sIgA after one month of oral intake of Ekolostrum, while in the control group there were no statistically significant changes. There are few studies on the connection between dietary supplementation with bovine colostrum and concentration of sIgA. Mero et al. (7,18) carried out two studies on colostrum by different manufacturers. Their results were contradictory; in one study, there was an increased concentration of sIgA (33%), while in the other, and there were no significant changes. Similar contradictory results were obtained in studies by Crooks et al. (19, 20). They found increased concentrations of sIgA after colostrum supplementation in marathon runners and four years after, in a similar study on distance runners, there were no measurable changes in sIgA. As seen, sIgA did not show a pattern of increasing/ decreasing after oral intake of bovine colostrum.

One of the reasons for that may be that there are many parameters which could influence the concentration of sIgA, such as genetic component, diet, stress, smoking, etc.

Some of our subjects were smokers (two in the control and two in experimental group) and they had increased amount of sIgA, which is consistent with the results obtained by Ussher et al. (21) and Ausar et al. (22).

Our study correlates with final exams and consequently higher levels of stress in students and professors. Stress could also influence the results, which was found in studies by Matos Gomez et al. (23), Deinzera et al. (24) and Jemmot et al. (25).

This pilot study showed that one month of oral intake of Ekolostrum with 960 mg of colostrum resulted in decreased concentration of sIgA in saliva. Respecting the length of the study, the number of participants and the effect of surrounding factors which may include artefacts to produce the difference, we conclude that more extensive studies are needed in the future.

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Transparency declaration

The authors deny any conflicts of interest.

Abstract
Objective: The aim of the present pilot- study was to evaluate the influence of oral intake of Ekolostrum (bovine colostrum) on concentration of salivary IgA(sIgA) in saliva. **Materials and Methods:** Twenty healthy participants between 23 and 54 years of age were randomly selected for the test and control group (n= 10). The test group took 60 capsules of Ecolostrum. The entire amount of the unstimulated saliva was collected before treatment from all participants. The procedure was repeated after 30 days at the end of the experiment. The concentration of sIgA in saliva was determined using ELISA (enzyme- linked immunosorbent assay). Intra- group comparisons were performed by paired sample T-test and inter- group differences were compared by ANOVA (p= 0.05) **Results:** Participants who were taking Ecolostrum had statistically significant (p=0.026) lower concentrations of sIgA after the treatment than before. The control group showed no statistically significant difference. There were statistically significant differences (p=0.047) of mean concentrations of sIgA between the test group and control group at the end of the experiment(464 ±59.89μg/mL and 620.16± 42.26μg/mL). **Conclusions:** The results of this pilot- study show that after one month of orally administrated Ekolostrum, the amount of sIgA was lower than before treatment. Some more extensive and detailed studies are needed to obtain more reliable results.

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Key words
Colostrum; sIgA; Saliva

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