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Original research

A novel role of probiotics in improving host defence of elite rugby union athlete: A double blind randomised controlled trial

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ABSTRACT

Objective: To examine the effects of a probiotic protocol on the incidence and severity of respiratory and gastrointestinal infections in elite rugby union athletes across an international competition season. Associations were also investigated between salivary biomarkers of stress (cortisol, alpha-amylase) and mucosal immunity (secretory(s)-IgA).
Design: A double-blind RCT was conducted over 27-weeks, divided into three stages: (1) control period; (2) domestic competition; and (3) international competition.
Methods: Athletes were assigned a probiotic (n = 9) or placebo (n = 10) supplement. Ultrabiotic 60™ or placebo was taken with food twice daily for 17 weeks and SB Floractiv™ 250 mg added twice daily during stage three.
Results: Five infections were diagnosed by the team sports physician across the 27-weeks, three within the intervention period in athletes randomised to the placebo group. No significant group x time interaction effects for salivary cortisol, alpha-amylase or s-IgA were identified over the 27-week time period, although a significant main effect for group and time was identified for salivary cortisol, alpha-amylase, and s-IgA ($p < 0.05$ for all). When considering stage, significant differences were identified in stage one with s-IgA lower in the probiotic group ($p = 0.015$). In stage two and three, salivary cortisol was higher in the probiotic group ($p = 0.016$ and $p = 0.001$ respectively), and salivary alpha-amylase was higher in the probiotic group in stage three ($p = 0.007$).
Conclusion: The probiotic protocol used in this study was associated with an increase in salivary alpha-amylase supporting its possible role as a host defence peptide.

Practical implications

- Probiotics may minimise the incidence of upper respiratory infections in elite team sport athletes through enhanced mucosal immunity.
- Probiotics may minimise the incidence of gastrointestinal illness in elite team sport athletes.
- The assessment of salivary alpha amylase may be an appropriate adjunct marker for assessing immune function in athletes.

1. Introduction

Innovative ways to reduce an athlete's risk of illness and develop resistance to infections is fundamental to enhancing an athletes physical and psychological health and wellbeing.[1] Respiratory and gastrointestinal illness are the most common medical presentation to sports medicine clinicians after injury among elite athletes, with the incidence of illness at the last Summer Olympic games reported to be 5.4 illnesses per 100 participating athletes, 47% of these illnesses affecting the respiratory system, and 21% the gastrointestinal system.[2] Increasingly, sports physicians are taking a proactive approach to preventing illness among their athletes to minimise training days lost to illness, and in turn enhance athletic performance. One such approach gaining momentum is the potential role probiotic bacteria and yeasts play in reducing the incidence of upper respiratory tract and gastrointestinal symptoms in athletes.[3] A core group of species including *Bifidobacterium* (*adolescentis, animalis, bifidum, breve* and *longum*) and *Lactobacillus* (*acidophilus, casei, fermentum, gasseri, johnsonii, paracasei, plantarum, rhamnosus* and *salivarius*) are considered likely to confer general health benefits including competitive inhibition of pathogens on mucosal surfaces and secretion of antimicrobial

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proteins.[4] Intervention studies involving athletes indicate that *Lactobacillus rhamnoses*,[5] *Lactobacillus fermentum*[6] *and Lactobacillus acidophilus*[7] reduce the number of days and severity of respiratory illness in endurance athletes, and a multispecies probiotic containing *Lactobacillus gasseri*, *Bifidobacterium bifidum* and *Bifidobacterium longum* reduced the incidence of upper respiratory and gastrointestinal symptoms in elite team sport athletes.[8]

A range of salivary biomarkers including cortisol,[9] salivary alpha amylase (sAA)[9] and salivary immunoglobulin A (s-IgA)[10] have been employed to evaluate the biological effects of probiotics on mucosal immunity and the interaction with the hypothalamic-pituitary-adrenal axis (cortisol) and autonomic nervous system (sAA). The most common biomarker used in studies to measure mucosal immunity is s-IgA, which serves as a first line of defence to repel pathogenic microorganisms thereby playing a major role in host resistance to respiratory infections.[11] In athletes, the relationship between intense exercise and susceptibility to respiratory infections has been correlated with a decrease in s-IgA,[3] although this relationship has recently been questioned.[12] A recent review concluded that monitoring s-IgA may provide a means to identify an athletes risk for developing upper respiratory tract symptoms, however monitoring s-IgA alone cannot be used to predict illness.[13] Salivary alpha-amylase is typically used as a biomarker for evaluating autonomic stress in elite team sport athletes,[14] and is also used in studies exploring dental health and mucosal immune responses in oral health.[15] Given its use in these two fields it may add valuable information in conjunction with s-IgA for predicting illness in athletes.

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Rugby Union is a team sport where the physical and psychological cost of training and competing is high, with athletes competing at the highest level of competition required to travel domestically and internationally for a majority of the year. A higher incidence of illness among elite rugby union athletes following international travel has been reported,[16] with the transmission of illness between athletes more likely as they live, train and eat in close quarters for extended periods of time. The high pressure and stress associated with this level of competition may result in chronic increases in cortisol, which is likely to contribute to reductions in mucosal immunity in these athletes, potentially pre-disposing them to increased risk of illness.[17]

To our knowledge, there are no studies reporting the preventative effects of multispecies probiotic formulations on the incidence and severity of upper respiratory or gastrointestinal infections and the measurement of salivary concentrations of IgA, cortisol, and sAA in elite team sport athletes. Therefore, the aim of this study was to evaluate the effect of a multispecies probiotic protocol on the incidence and severity of upper respiratory and gastrointestinal tract infections in conjunction with immune biomarkers of elite team sport athletes across an international competition season.

2. Methods

In this double-blinded RCT, 19 elite rugby union athletes were stratified based on playing position (forward or back), age and body mass, then randomised into a probiotic or placebo group. The 19 athletes who participated in the study were the entire population available to the research group at this elite, international level of competition who provided written informed consent. The study was completed across a 27-week period encompassing the southern hemisphere winter (domestic competition), and the northern hemispheres autumn (international competition), and encompassed training camps, domestic and international test matches, recovery weeks and training at the athletes' home club. Seventeen weeks of physical data collection took place when the athletes were in camp, which has been divided into three distinct stages: 4-week baseline control period which encompassed a national training camp and 3 domestic games (stage one), 6-weeks of domestic competition (stage two), and 8-weeks of international competition (stage three), see Table 1. For the purposes of this study, domestic games were classified as games that required $\leq$5 h of air travel.[18]

The study was approved by The University of Canberra's Human Research Ethics Committee (16–49). The research was conducted in compliance with Good Clinical Practice and in accordance with the guidelines of the Australian National Health and Medical Research Council and the Declaration of Helsinki (as revised in 2004). The trial was registered with the Australian and New Zealand Clinical Trials Register (ACTRN12616000555459).

The randomisation schedule was prepared by a researcher not involved in the coordination of the study using a computer-generated blocked random sequence. The code was kept by two independent researchers in a locked computer file. The preparations were distributed in numerical order, matching the participants' enrolment number with the number on the intervention label. Once the preparations were labelled, they were sent to a member of the research team working with the athletes, who distributed the bottles to the participants. This member was blinded to the preparation allocation. Opaque code break envelopes were produced to deal with any serious adverse effects and kept by an independent party. The code was not broken until the trial was completed and the database was locked. The code was broken in two steps, firstly allocation to group A or B to allow blinded statistical analysis, and secondly into actual treatment allocation on completion of the analysis. The statistician, study coordinator and the participants were blinded to treatment allocation.

The study preparations included Ultrabiotic 60™ (FIT-BioCeuticals Ltd, Australia), an encapsulated proprietary blend of probiotic bacteria containing 60 billion viable bacteria *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Bifiodbacterium lactis*, *Bifidobacterium bifidum*, *Streptococcus thermophilus* and listed on the Australian Register of Therapeutic Goods (ARTG) AustL# 259813. The second probiotic supplement which was consumed in conjunction with the Ultrabiotic 60™ during the international competition phase was SBFloractiv™ (FIT-BioCeuticals Ltd, Australia) and contained 250 mg *saccharomyces boulardi* and is listed on Australian Register of Therapeutic Goods (ARTG) AustL# 285024.

A placebo was made up with the same excipient base as the active formulations (Ultrabiotic60™ Cellulose – microcrystalline, Iron oxide yellow, Iron oxide red, gelatin capsule; and SBFloractiv™ Cellulose – microcrystalline, Lactose, Calcium hydrogen phosphate dihydrate, Povodine, Silica colloidal anhydrous, Magnesium stearate, gelatin capsule), and was identical in size, weight and packaging to the active medicine. Compliance was measured by calculating the total number of capsules provided to the athletes by the number of capsules left in the bottle at the end of each 30 day period, which was counted by the teams athletic director and entered into a spreadsheet.

Passive drool saliva samples were collected upon waking twice weekly. All athletes were instructed to avoid any food or fluid prior to providing the saliva sample. To maximise compliance and ensure the saliva samples were clear, athletes assembled as a group, rested supine for 10 min, then provided the saliva sample. Saliva was collected in sterilised cryovials via passive drool using saliva collection aids (Salimetrics) and stored in −20 °C freezer. Saliva was assayed for cortisol, s-IgA and sAA concentrations using commercial enzyme immunoassay kits (Salimetrics LLC, State College, PA).

Table 1
Study design by stages including significant events.

Stage 1: Control period[a]	Stage 2: Ultrabiotic 60™ introduced[b]	Stage 3: SBFloractiv™ introduced[c]
Week 1: training camp	Week 11: Training camp	Week 18–19: 2 games, 2 wins
Weeks 2–4: 3 games, 3 losses	Weeks 12–13: 2 games, 2 losses	Week 20: rest week
Weeks 5–10: training at home club	Weeks 14: rest week	Week 21: 1 game, 1 loss
	Week 15–16: 2 games, 2 wins	Week 22: rest week
	Week 17: rest week	Weeks 23–27: 5 games, 3 wins and 2 losses

[a] Timepoints 1–8 (domestic games).
[b] Timepoints 9–19 (domestic games).
[c] Timepoints 20–34 (international games).

Cortisol assay sensitivity was 0.007 μg/dL with intra assay and inter assay variability of 6.6% and < 4.1%, respectively. Salivary alpha-amylase assay sensitivity was <2.000 U/mL with intra assay and inter assay variability of 7.2% and 5.8% respectively, and s-IgA assay sensitivity was 2.500 μg/dL with intra assay and inter assay variability of 8.6% and 5.2% respectively. Each sample was analysed in the same assay to eliminate inter assay variance.

Medical consultations between the athlete and team sports physician that resulted in a diagnosis of a respiratory or gastrointestinal tract infection were recorded in an online database. Details regarding the date, diagnosis, and training status (i.e. full, modified or no training) were extracted by the research team at the completion of the study. Training load was captured through global positioning system (GPS), as per standard practice (15 Hz SPI-HPU; GPSports Systems, Canberra, ACT, Australia). Total weekly distance (meters), training duration (minutes), high speed running (minutes), very high speed running (minutes) and acceleration (count) data was collected for all participants.

Data are expressed as mean ± SD. Standard descriptive statistical analysis were conducted on participant demographics. To determine the effects of the multispecies probiotic on the above mentioned immune biomarkers over time and between groups, linear mixed models were employed including a random intercept for subjects to account for within-subject dependencies. The linear mixed models also allowed us to handle missing data when athletes were not in attendance at a camp due to non-selection or injury. The normality assumption was visually assessed through Q—Q plots and no obvious deviations from normality were detected. Differences between groups from baseline to the completion of the study and within each stage were investigated. Significance was set a $p \leq 0.05$. All statistics were run using IBM SPSS version 23 (IBM, USA).

3. Results

Nineteen athletes completed the study, with nine randomised to the probiotic group (mean age 27.03 ± 3.15 years, body mass 111.47 ± 7.97 kg), and ten to the placebo group (mean age 26.56 ± 2.87 years, body mass 108.73 ± 12.63 kg). Compliance to the Ultrabiotic 60™ was 74.07 ± 12.70% and 60.90 ± 22.95%, and compliance to the SB Floractive™ was 71.43 ± 16.79 and 61.86 ± 14.83 in the probiotic and placebo groups respectively. No adverse events were reported by any participants.

Over the 27-week study period, there was no significant group x time interaction for salivary cortisol ($p = 0.925$), however, there was a significant main effect for group ($F(1, 450) = 14.056$, $p < 0.001$), with salivary cortisol higher in the probiotic group (0.406 ± 0.144 ug/dL) compared with the placebo group (0.361 ± 0.122 ug/dL). There was also a significant time effect ($F(33, 450) = 3.562$, $p < 0.001$), see Fig. 1a.

There was no significant group x time interaction for s-IgA ($p = 0.992$), however a significant main effect for group ($F(1, 428) = 7.556$, $p = 0.006$) was identified with s-IgA higher in the placebo group (489.149 ± 201.121 ug/dL) compared with the probiotic group (445.928 ± 176.824 ug/dL). A significant time effect over the study duration ($F(33, 428) = 2.070$, $p = 0.001$) was also identified for s-IgA, see Fig. 1b. There was no significant group x time interaction effect for sAA ($p = 0.561$), however there was a significant main effect for group ($F(1, 441) = 4.036$, $p = 0.045$) with sAA higher in the probiotic group (51.313 ± 32.290 U/mL) compared with the placebo group (46.542 ± 32.186 U/mL). There was also a significant time effect for sAA ($F(33, 441) = 1.951$, $p = 0.002$), see Fig. 1c. When

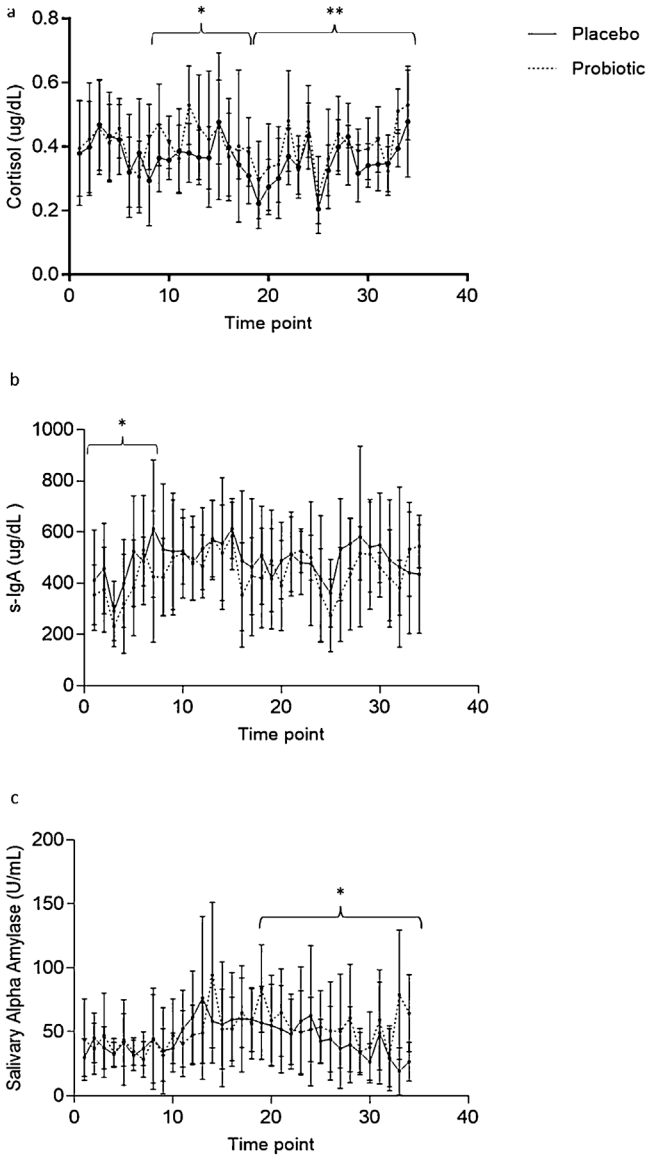

Fig. 1. (a) Saliva cortisol concentration across the intervention period; (b) s-IgA concentration across the intervention period; (c) sAA concentration across the intervention period. * Denotes significant difference between groups ($p < 0.05$), ** Denotes significant difference between groups ($p < 0.001$).

Table 2
Salivary s-IgA, sAA, and Cortisol across the three stages of the study.

Outcome measure	Probiotics			Placebo		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
s-IgA (ug/dL)	380.186 ± 183.876 CI 330.80–430.95 P = 0.015[a]	487.172 ± 173.051 CI 392.875–571.144 P=0.191	452.405 ± 166.320 CI 414.01–487.88 P=0.204	464.559 ± 209.315 CI 420.17–509.34	523.208 ± 192.299 CI 432.776–599.789	480.646 ± 200.622 448.36–520.14
sAA (U/mL)	39.431 ± 23.827 CI 33.32–45.11 P = 0.683	54.644 ± 33.063 CI 34.585–71.783 P = 0.737	55.620 ± 34.435 CI 48.80–61.62 P = 0.007[a]	37.569 ± 20.731 CI 32.32–42.83	55.945 ± 38.108 CI 37.844–72.689	45.712 ± 32.158 CI 36.43–49.02
Cortisol (ug/dL)	0.404 ± 0.139 CI 0.366k0.439 P = 0.526	0.427 ± 0.161 CI 0.655–0.485 P = 0.016[a]	0.390 ± 0.132 CI 0.373–0.413 P = 0.001[a]	0.387 ± 0.145 CI 0.355–0.420	0.375 ± 0.107 CI 0.313–0.435	0.337 ± 0.112 CI 0.326–0.364

All values presented are mean ± SD (with 95% confidence intervals) for each stage. S-IgA = salivary Immunoglobulin A; sAA = salivary alpha-amylase.
[a] Denotes significant difference between groups.

analysing the data by stage, significant differences were identified between groups at different stages for cortisol, s-IgA and sAA. Please see Table 2 for these results.

When reviewing the training load data, there were no significant group x time interaction for distance (p = 0.849), duration (p = 0.823), high speed running (p = 0.431), very high speed running (p = 0.693), and accelerations (p = 0.646). When analysing the data by stage, a significant spike in distance (p = 0.008), duration (p = 0.001), and accelerations (p = 0.04) was identified in stage two when compared to stage one. There was also a significant increase in training load in stage three compared to stage one for distance (p = 0.029), duration (p = 0.006) and high speed running (p = 0.047). No differences were identified for training load between stage two and stage three.

Five incidences of illness and infection were diagnosed and recorded by the team sports physician across the study period. One respiratory tract infection and one gastrointestinal infection were diagnosed in stage one for two individuals randomised to the probiotic group. In stage two, two cases of a respiratory tract infection were diagnosed in participants randomised to the placebo group, and in stage three, one case of tonsillitis was diagnosed in an individual in the placebo group. Training was not modified for any of these participants during their period of illness.

4. Discussion

The main and surprising finding of this study was the significant increase in sAA in the athletes consuming the probiotic compared to the athletes receiving the placebo. Whilst sAA is well accepted as a biomarker of physiological and psychological stress, its role in host defence is largely unexplored in studies evaluating markers of mucosal immunity in athletes. Several in vitro and animal studies have demonstrated that probiotic strains can increase the expression of mucins and host defence (antimicrobial) proteins, which can enhance mucosal barrier function.[19] While the difference in sAA level in this study was noted in stage three only, the accumulative effects on immunity over the second stage of the trial cannot be discounted with most studies suggesting measurable effects in 4–9 weeks.[20] It is possible that the increased sAA in the athletes taking the probiotic is a marker of increased host defence. If the sAA was purely a marker of increased stress in the probiotic group, in conjunction with insignificant changes in s-IgA in this group, we would expect to observe an increase in infections. However, very few respiratory and gastrointestinal infections were diagnosed in this study, and none in participants taking probiotics. These findings are surprising given the known association between the results of the biomarker profile and international travel being associated with an increased susceptibility and risk of infections.[16] It is possible that both the low incidence of respiratory and gastrointestinal infections were simply related to limited exposure of pathogenic organisms during the 6-month competition season, or the low incidence of infection may be related to a 'herd immunity' effect associated with an increase in the host defence protein sAA and or a change in an unknown or unmeasured immune marker/s affected by the probiotic use. Campbell and Turner[12] highlighted the limitations of the commonly assessed biomarker of immune function s-IgA measurement for predicting respiratory infections, and suggest an integrative approach incorporating oral inflammation biomarkers and host mucosal ecology may be appropriate to consider.[12] This study suggests that sAA may be one of these appropriate adjunct markers.

The chronic affect of elevated salivary cortisol on mucosal immunity has been proposed to be associated with the down-regulation of IgA synthesis and expression of the polymeric Ig receptor that helps with the transepithelial transport of IgA into saliva.[21] It is tenable that this may have happened in the probiotic group in the current study however it is unclear why we did not see this same effect in the placebo group. Both the independent and interactive effects of the hypothalamic-pituitary-adrenal axis and autonomic/sympathetic nervous system are important to understanding individuals adaptive and maladaptive responses to stress[22] and were the primary reasons for measuring both salivary cortisol and sAA (surrogate marker of elevated autonomic/sympathetic nervous system activity in response to physical or psychological stress) in the present study. In regards to the asymmetry between salivary cortisol and sAA, this has also been reported in other studies involving both physical and psychological stress, suggesting that the responses to stress can occur independently in these two systems.[23]

It is possible sustained autonomic nervous system activity due to the heavy training and competition requirements explain the elevated sAA.[24] Therefore, the significantly higher sAA level observed in the probiotic group in stage 3 may be associated with maintenance of mucosal immunity, despite no significant change in s-IgA between groups during the probiotic intervention period (stages 2 and 3). It is also conceivable the elevated sAA observed in the probiotic group during the third stage of the study was in direct response to the use of *saccharomyces boulardi*. Alpha-amylase is produced by several bacteria, fungi and yeasts including *saccharomyces boulardi*.[25] Clinically, the majority of research on this probiotic yeast has been related to its role in increasing the secretion of s-IgA in the small intestine and the protection this increased secretion confers against travellers diarrhoea and *clostridia difficille* infections.[26] In this study an increase in s-IgA was not observed which may or may not correlate to increased levels in the intestine.[26]

Whilst stress hormones have been associated with a predisposition to infection, an association between sAA and infection has not

been made prompting a need to understand the likely significance of higher sAA that are often reported during periods of stress.[27] Within the context of this study, the significant increase in sAA in the probiotic group compared to the placebo group is of interest. Given the homogeneity of the study group including training loads and days involved in athletic performance, autonomic nervous system activity cannot be the only explanation. Within the context of this study, we suggest the elevations in sAA may also be a marker of host defence (antimicrobial) protein activity. One of the five infections was diagnosed in stage three in a participant randomised to the placebo group. Whist the low incidence is weak, it could support the role of sAA in conferring protection. A number of animal studies have identified how various probiotic species produce alpha-amylase[28] and modulate s-IgA secretion.[29] A very small double blinded randomised cross over trial involving 8 athletes who were administered *Lactobacillus casei* showed increases in sAA following exertional heat stress, but unlike the present study, this was not superior to placebo.[30]

As with many studies conducted in elite athletes, the sample size of our group is small, and therefore a limitation. However a strength of this study is the long duration and homogenous nature of the participant cohort in terms of their gender, training loads and nutritional intake. Despite the small sample size, this study provides valuable information about elite rugby union athletes response to a specific probiotic supplement. We did not assess the oral health status of the participants, which is known to influence s-IgA levels due to its role in host-bacterial ecology and mucosal homeostasis,[31] and we did not regularly asses the individuals psychological status as part of this study, therefore cannot discount psychological stress as a confounding factor in the s-IgA levels we observed. Finally, we cannot confidently say whether it was the Ultrabiotic 60™ or the SBFloractiv ™ formulations or indeed both that contributed to the altered levels of biomarkers reported in this study.

5. Conclusion

In summary, this study suggests that the probiotic protocol evaluated was associated with an increase in sAA secretion and the low incidence of gastrointestinal and respiratory infections in elite team sport athletes across an international competition season. The association between probiotics, sAA and immunity warrants further research to establish if this association was causal.

Author disclosures

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