Improved Appetite of Pregnant Rats and Increased Birth Weight of Newborns Following Feeding with Probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14

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ABSTRACT

Background: Malnutrition and pathogenic colonization of the vagina are two major contributors to preterm labor, low newborn survival rates, low birth weight, and in surviving infants, a high risk of long term poor quality of life. It was hypothesized that use of probiotics as a food supplement would improve the appetite and health of the mother and ultimately the health of newborns.

Methods: Two probiotic strains, *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 were tested in a Sprague-Dawley albino rat model. The probiotic preparations (1×10^9 cfu/mL) were administered to 8 rats in group A; the 8 rats in group B, the control group, received 1 mL of sterile bicarbonate buffer containing 2% glucose. The probiotic preparations and glucose were supplemented in drinking water for 30 days and feed intake and the birth weight of the newborns were measured.

Results: There was a significant improvement in appetite for the lactobacilli treated animals with a mean weight of 16.81 g of feed consumed per day, compared with 13.16 g of feed consumed for the controls ($P = 0.006$). The mortality rate for the control animals was 18%, while only 2.7% in the probiotic supplemented fed group. There was a significant increase in birth weight among the 37 newborns whose mothers had been fed probiotics (6.5 g), compared to controls (4.5 g) ($P = 0.01$). There was a two-log increase in total lactobacilli recovered from the stool of the probiotic treated animals with significant presence of the two lactobacilli treatment strains. No adverse effects were noted in the animals.

Conclusions: This is the first report of nutritional appetite benefits of probiotics during pregnancy and of improvements in the weight of newborns. Considering the fact that well-nourished newborns, born within the normal weight range, have a considerably better long term prognosis, if the present findings were to be duplicated in pregnant women, the implications for the health of mothers and newborns, especially in developing countries could be very significant.

INTRODUCTION

In many parts of the world, not the least sub-Saharan Africa, nutritional intake, appetite, and the reproductive health of premenopausal women are far from optimal. Often pregnant women are malnourished due to inadequate food production, inadequate food intake, poverty, uneven distribution of food, and food restrictions.[1] In addition, many pregnant women are prone to an abnormal vaginal microflora that increases the risk of miscarriage or preterm delivery of low birth weight infants.[2] A strong link also exists between black race, low socio-economic status, older maternal age, and previous preterm delivery, corresponding to greater risk of preterm delivery and low-birth-weight newborns.[3] These factors and the rapid spread of HIV/AIDS amongst reproductive age women are putting at risk the very survival of nations. In Botswana for example, half of the pregnant women are HIV positive. Practical and effective management systems are required to control and eventually reverse this devastating trend.

The use of probiotics offers one potential intervention for several reasons. Strains of probiotics (defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host"[4]) taken orally on a daily basis, have been shown to improve the urogenital health of premenopausal women,[5] including the reversal of a bacterial vaginosis (BV) flora to a normal one dominated by lactobacilli.[6] Two strains, *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 have been shown to colonize the gut as well as the vagina, thereby potentially also offering benefits to gut health. Previous studies with *L rhamnosus* GG have shown that daily intake during pregnancy was safe and could benefit the newborns who were otherwise at risk of severe allergic reactions.[7] Thus, it was hypothesized that consumption of probiotic strains GR-1 and RC-14 could increase the appetite of pregnant mothers and improve the health of newborns. The objective of the present study was to determine, in a rat model, if daily intake of these organisms influenced appetite during pregnancy, and evaluate their effect on the birth weight of the newborns.

The health of the mother has been shown to be important in the well-being of the fetus and the newborn as well as playing a role in the subsequent long term risk of type 2 diabetes and coronary heart disease.[8] Although it would have been interesting to study the effect of bacterial vaginosis (BV) on premature birth and newborn health; the rat model is not conducive to this testing. Nevertheless, the model was used to examine two important pregnancy outcomes, namely newborn survival and birth weight.

MATERIALS AND METHODS

Probiotic Strains

L rhamnosus GR-1 and *L fermentum* RC-14 in capsular form were provided by Urex Biotech Inc., Canada. The strains originated from the female urogenital tract, and both have been shown to colonize the vagina and gut as well as inhibit the growth and adhesion of urogenital and intestinal pathogens.[9,10]

Laboratory Rats

Sixteen female adult age-matched Sprague-Dawley albino rats weighing between 200 and 250 g were obtained from Chidak Medical Diagnostic Laboratories, Benin City. They were housed in two large cages with 2 male rats weighing 270 g in each cage and fed on rat chow Guinea feed growers (Flour Mills Nig. PLC, Benin, Nigeria) and water ad libitum. The animals were exposed to 12-hour light-dark cycle throughout the experimental period, and were handled according to the standard guidelines for the use of animals for experiments.[11]

Probiotic Feeding

At 65 days post delivery, 16 females rats were randomly selected into two groups (A and B) of 8 in each group. They were housed one rat per cage; group A was fed with probiotics and group B was the control group. Briefly, the probiotic capsule (45 mg) was re-constituted in 10 mL of MRS (de Man Rogosa Sharpe) broth (Lab M, IDG, Manchester, UK), and incubated microaerophically at 37˚C for 48 hours. This served as probiotic stock culture, for 3 days. The stock culture was briefly checked for purity by Gram's stain and negative catalase test, while lactic acid and hydrogen peroxide (H_20_2) production were checked by titrimetric methods.[12] From the stock culture, 0.1 mL was added to 9.9 mL of MRS broth and incubated microaerophically at 37˚C for 24 hours. Appropriate dilutions were made with sterile bicarbonate buffer (0.2 M $NaHC0_3$), containing 2% glucose as previously described by Pavan et al,[13] to get 1×10^9 CFU/mL. One mL of 1×10^9 CFU/mL were added to the nipple drinking system daily for 30 days for the rats in group A, while 1 mL of sterile bicarbonate buffer containing 2% glucose only, was given to control rats in group B.

Each rat was fed with Guinea feed growers 70 g ad libitum and every morning the total feed consumed per day was measured by weighing the remaining content and the difference noted while the consumed quantity was replaced to make up 70 g. The study was closed at 30 days.

The female rats delivered in succession within 6 days and newborn birth weights were measured within 6 hours of delivery in both groups.

Recovery of Probiotic Strains from Fecal Samples

Fresh fecal samples were collected from both groups. Weighed fecal samples (0.5 g) were homogenized in 10 mL of sterile phosphate buffered saline (pH 7.1), and appropriate serial 10-fold dilutions were made. 0.1 mL of appropriate dilutions were spread plated onto MRS agar in duplicates. Also, 0.1 mL was spread plated in MRS agar containing 50 ug/mL of tetracycline (Pfizer Nig. PLC) to aid in selection of GR-1 and RC-14.

RESULTS

The appetite of the pregnant animals was significant (*t* test; 16.81 g ± 5.6 versus 13.16 ± 4.0; $P < 0.01$) with probiotic intake than controls for the duration of pregnancy as assessed in 8 rats per group (Figure 1). This represented 12% to 14% over the 30-day period.

The death rate for newborns from the 8 mothers in the control group up to 21 days post-weaning was 18% (7/38). Only one death (2.7%) occurred in 37 newborns of 8 females fed with probiotics up to 21 days post-weaning. Chi squared analysis was done, showing a positive association between reduced mortality following probiotic supplemented feeding versus controls without probiotic supplementation (2.7% vs 18%, χ^2 [2] = 4.84, $P = 0.05$) (Figure 3).

The birth weight of the 38 offspring from 8 control rats was 4.58 ± 0.6 g (range, 3.6-5.9 g), significantly lower (*P*

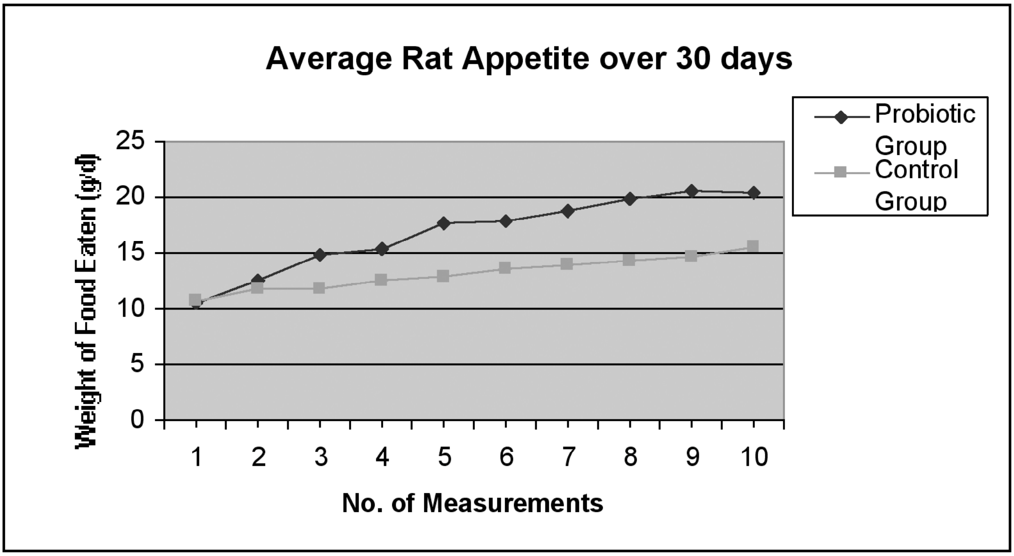

Figure 1. Results show a consistent 12% to 14% stimulation of appetite in probiotic fed animals compared to controls.

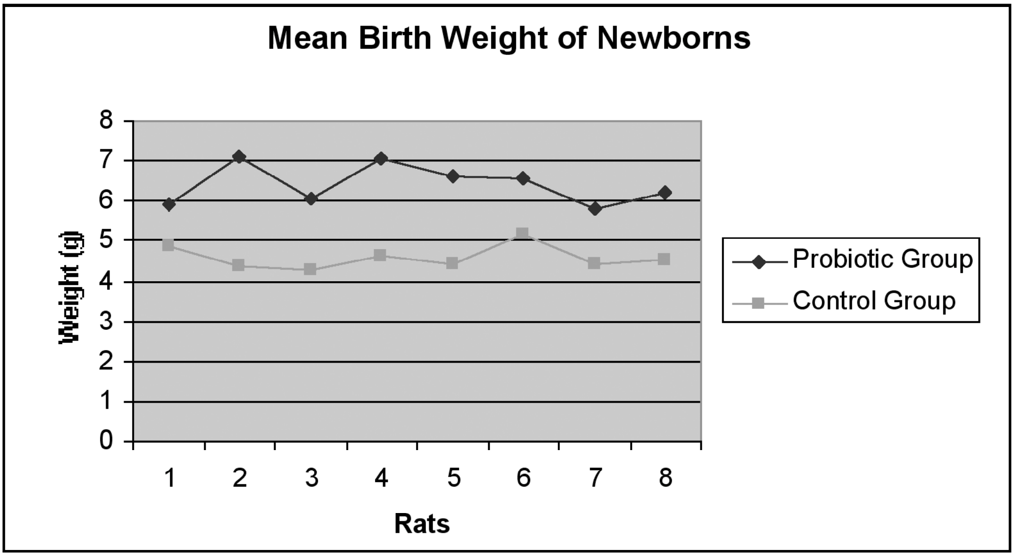

Figure 2. Showing the mean weight (g) of the newborns from probiotic fed rats and controls.

< 0.01) than the birth weight of the 37 newborns from 8 rats fed probiotics during pregnancy (6.5 ± 0.7 g, range 5.2-7.8 g) (Figure 2).

Significantly more lactobacilli was recovered from the stools of the probiotic fed animals (mean $4.5 \pm 0.7 \times 10^8$ CFU/g) than the controls ($5.4 \pm 2.9 \times 10^6$ CFU/g), at $P < 0.001$. Of the lactobacilli in the probiotic group, many ($1.7 \pm 2.9 \times 10^7$ CFU/g) were detected on agar known to support the growth of the two probiotic strains, *L rhamnosus* GR-1 and *L fermentum* RC-14, suggesting that they were indeed present.

DISCUSSION

This is the first study to show that probiotic lactobacilli can have a positive impact on appetite in pregnant animals,

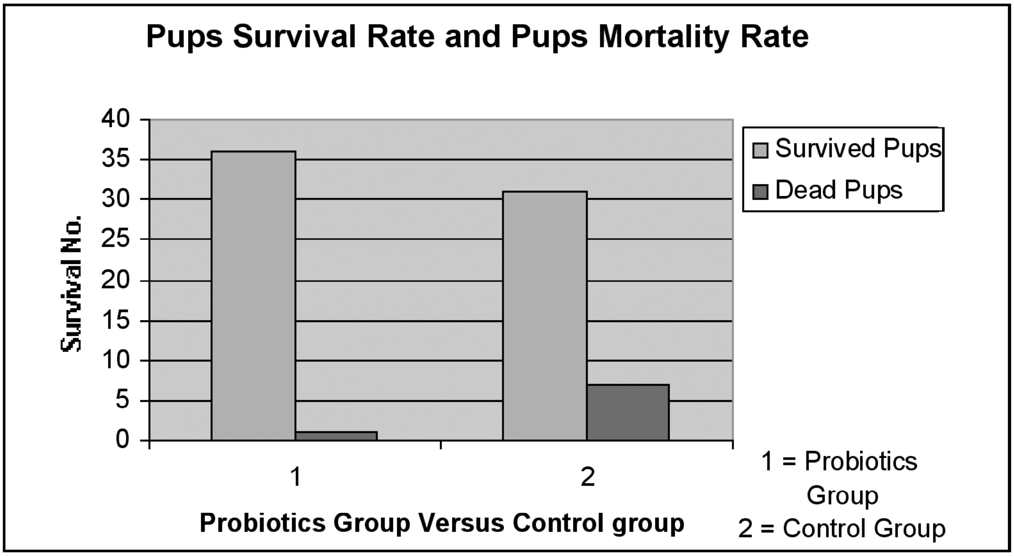

Figure 3. Survival rate of rat pups from mothers fed with probiotics versus control, up to 21 days post weaning.

increasing food intake by 12% to 14% over a 30-day period. The only other appetite stimulation study, assessed in Mongrel pups, also showed a comparable increase (10-15%) in appetite stimulation following lactobacilli intake.[14] Other studies related to substitution of antibiotics used for livestock weight gain rather than well-being of mother and newborns, show that weight gain is possible with probiotic use. For example, one study in chickens showed that 40 days feeding of *Lactobacillus agilis* JCM 1048 and *Lactobacillus salivarius subsp. salicinius* JCM 1230 resulted in a 10.7% increase in weight gain compared to controls ($P < 0.01$).[15] Yet another study, showed weight gain in post-weaning Swiss mice.[16]

The second important finding of the present study was a 30% improvement in the birth weight of newborn Sprague-Dawley albino pups, whose mothers were fed probiotic *Lactobacillus* strains GR-1 and RC-14, when compared to controls. Sprague-Dawley albino rats are rapid growing rodents that reproduce rapidly, as demonstrated in this study, where each mother gave birth to an average 4 to 5 pups. The Sprague-Dawley albino rats have longer, narrower heads and longer tails, which can equal body length. Not all pups survived, and on average, the mortality rate was 18% for the control rats, and only 2.7% for probiotic fed rats, suggesting that probiotic intake may reduce the rate of infant mortality. Studies clearly show that nutrition plays an important role in fetal development, influencing the future health of the newborn.[8] Although the present study did not measure maternal well-being per se, the fact that appetite was stimulated in the pregnant animals and less mortalities occurred in the newborns of probiotic fed rats, indicates the positive impact of probiotic feeding. In terms of health, the prognosis is poor in newborns born prematurely and with low birth weight. Thus, the present findings indicate that probiotics can influence the birth weight of newborns and may be considered during human pregnancy, where daily intake of *Lactobacillus rhamnosus* GG and GR-1/RC-14 can be safely consumed, respectively, although human clinical trials will be needed to ascertain the impact of probiotics on birth weight and well being of newborns.[7,20]

The newborns in the probiotic group maintained a high post-weaning food intake, similar to a mouse study with dietary *Bifidobacterium lactis* HN019.[17] Another mouse study has shown that feeding with 10^7 viable *Lactobacillus rhamnosus* HN001/day for 14 days enhanced food consumption, weight gain, and phagocytic capacity of blood leucocytes.[18]

The recovery of substantially more lactobacilli in the stools of probiotic treated animals compared to controls (4.5×10^8 versus 5.45×10^6 cfu/g) and the high numbers of isolates with similar antibiotic and morphologic profiles to *L rhamnosus* GR-1 and *L fermentum* RC-14, supported the conclusion that these organisms colonized the animals and influenced their well being. A previous study in rats fed propionobacteria also showed an increase in their recovery from stool of 10^8 cfu/g after 36 days of treatment.[19]

In conclusion, daily feeding of probiotic strains, *L rhamnosus* GR-1 and *L fermentum* RC-14, increases the birth weight of Sprague-Dawley albino rats. If similar findings were found in humans, particularly women from relatively low socio-economic countries, those at risk of bacterial vaginosis and preterm labor, or with a history of low-birth-weight newborns, the impact on human health could be significant.

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