Received: 10 April 2023, Accepted: 13 May 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 4, 631-636

Effects of Rumen Juice Transfaunation, *Bacillus subtilis* natto and Premix on the General Health Condition, Productivity, and Some Ruminal and Biochemical Parameters Upon Holstein Heifers after Weaning

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Abstract

This study aimed to investigation of the rumen juice transfounation either alone, or in combination with *Bacillus subtilis* natto, or premix upon the general health condition, productivity as well as upon some ruminal and biochemical parameters in after weaning Holstein heifers. The obtained results revealed significant increase in the body weight in the experimental group receiving ruminal juice plus premix, followed by the group received ruminal juice plus *Bacillus subtilis* natto, and ruminal juice alone compared with the control group. The immune responses, in terms of high leukocyte counts, lymphocytes, and granulocytes, were also significantly upregulated in the group that received ruminal juice plus *Bacillus subtilis* natto, followed by the group that received ruminal juice plus premix, and ruminal juice, respectively. Interestingly, groups treated with ruminal juice plus *Bacillus subtilis* natto, and ruminal juice plus premix had a significant improvement in the hemoglobin level, and the antioxidant capacity. Likely, such treatment groups had significant improvements in both total protozoal count, and eligibility for insemination. In conclusion, the obtained results of the present study strongly recommend the use of ruminal juice in a combination with *Bacillus subtilis* natto, or premix to improve the fertility indices, and immune responses in Holstein heifers.

KEYWORDS
Holstein heifers, Ruminal juice, *Bacillus subtilis* natto; premix; Immunity; Fertility

INTRODUCTION

The efficiency of cow production today is more than it was 60 years ago, especially when considering the number of resources utilized to produce each unit of milk and meat (Capper *et al*., 2009). There is growing evidence that there are differences in feed utilization and production efficiency even among groups of productive cattle fed the same diet (Arthur *et al*., 2001). Nutritionists, who normally create whole-herd nutritional plans for cattle production, may view the variation among cattle in terms of their responses to diets as a difficulty. On the other side, the inherent fluctuation in feed efficiency provides chances to identify indicators that distinguish efficient from inefficient animals and choose animals accordingly. One can anticipate long-term and sustainable solutions for enhancing cattle efficiency as the discovery of such markers may result in new genetic selection targets (Richardson *et al*., 2004). Recent developments, such as the creation of more practical and high-throughput analytical techniques (Ametaj *et al*., 2010; Penner *et al*., 2011; Steele *et al*., 2011; Hollmann *et al*., 2013), have improved our understanding of the molecular interactions between rumen microbiota and epithelia as well as the role of nutrition in rumen physiology. As a result, in recent years, the understanding of the biological factors contributing to variations in feed efficiency has improved, particularly as they relate to rumen health and digesting effectiveness (Khiaosa-Ard and Zebeli, 2014).

In the fermentation process in the rumen, the microflora and microfauna are crucial. Its intricate microbial ecosystem's primary function is to hydrolyze and ferment cell wall carbohydrates into nutrients that may be used by the hosts that it inhabits (Eugène *et al*., 2004). After being hydrolyzed and fermented in the rumen, most soluble carbohydrates, including starch and cell wall polysaccharides, can be used by protozoa as sources of carbon and energy (Fonty *et al*., 1995). The primary protein sources for protozoa are dietary protein and bacteria, which are partially digested into amino acids and ammonia, which is released into urea through urine and then absorbed into their own proteins (Eugène *et al*., 2004).

Live microbial supplements known as probiotics have the power to change the microbiota in the digestive tract for the better (Rook and Brunet, 2005). Improved formation of good gut flora and competitive exclusion of harmful microbes are some of the benefits (Fujiwara *et al*., 2009). Several Bacillus sp. bacteria, including Bacillus licheniformis and *Bacillus subtilis*, have recently attracted attention for their involvement in preventing and treating infectious disorders, which has improved animal productivity (Alexopoulos *et al*., 2004; Chen *et al*., 2009). Additionally, these bacteria's spore forms can endure in the gastrointestinal system (Casula and Cutting, 2002).

A Gram-positive spore-forming bacterium is *B. subtilis* natto. One of the bacterium's mechanisms for survival is the production of spores, and *B. subtilis* spores may endure hostile conditions.

Although the gastrointestinal tract does not often include Bacillus species, the spores that can withstand digestion by digestive enzymes can get through to the intestinal tract and develop there. Thus, spores are generally better, or at least have effects similar to those of bacterial growth. The bacterial strain derived from natto, a fermented soybean product with numerous health benefits, is known as *B. subtilis* natto (Samanya and Yamauchi, 2002). *B. subtilis* has also proven to have probiotic properties because it has been shown to suppress pathogens including *Escherichia coli*, *Campylobacter* species, *Streptococcus* species, and *Clostridium* species (Guo *et al*., 2006; Teo and Tan, 2006). Just a little amount of research on the dietary use of *B. subtilis* in ruminants has been done to date (Jenny *et al*., 1991; Kritas *et al*., 2006), and studies have hardly ever examined the impact of feeding *B. subtilis* natto on ruminal microbial fermentation in dairy cows. The findings of available in vitro research have demonstrated that *B. subtilis* natto has favorable effects on the growth of various anaerobic bacteria, including *Lactobacillus* sp. and *Bifidobacterium*.

This experimental trial aimed at evaluation of the effects of the oral administration of rumen juice, rumen juice with *Bacillus subtilis* natto and rumen juice with premix® on the growth performance, total weight gain, ruminal constituents, eligibility for insemination, fertility index and some blood parameters of Holstein heifers at a private farm in Fayoum Governorate, Egypt. Besides, connection with immune system and incidence of some health problems was also suggested.

MATERIALS AND METHODS

Animals and diets

Forty Holstein heifers in a private dairy farm located in Fayoum governorate were subjected to a three-month experiment during December 2021 to March 2022. These heifers were weaned and aged 3-6 months. These heifers were managed in the same yard, and they received total mixed ration (TMR) for growing heifers (Table 1). The heifers were healthy and weighed 105-126 Kg at the beginning of the experiment. Heifers were classified into four groups (n.= 10 animals/group) and separated into 4 yards.

All experiments using animals were done according to Zagazig University guidelines.

The contents of the premix were described in Table 2.

Table 1. The composition of total mixed ration (TMR) introduced the heifers.

Ingredients	Amounts
Yellow corn	320 kg
Coarse wheat bran	370 kg
Soya bean meal 46%	250 kg
Limestone	20 kg
NaCl	12 kg
Sodium bicarb	12 kg
Antifungal toxin	1 kg
Premix	15 kg
Silage / head	3 kg
Alfa alfa / head	1 kg
Hay / head	1 kg

Sampling

Blood samples

Two blood samples were collected from each heifer seven times (each sample after 3 days from each administration). Blood was collected from jugular vein after shaving the hair and wiping the exposed skin with alcohol 70%.

Table 2. The composition of premix used in the experiment.

Ingredients	Active principles
VIT A	10000000 IU
VIT D3	2000000 IU
VIT E	15000 mg
Manganese	60000 mg
Zinc	50000 mg
Iron	30000 mg
Copper	10000 mg
Iodine	1000 mg
Cobalt	100 mg
Selenium	100 mg
Ca carbonate	3 Kg

The first samples (non-coagulated blood samples): Almost 10 ml blood collected in a dry, clean and sterile labeled glass tube with rubber stopper, containing Ethylene Diamine Tetra Acetate (EDTA) as an anticoagulant and thoroughly mixed with blood by gentile inversion of the tube. These samples were used for the measurement of the hematological parameters (erythrocytic count, leukocytic count, hemoglobin, packed cell volume %) and used for plasma separation and determination of plasma lactate.

The second blood samples (Coagulated blood samples): About 10 ml blood was collected in a clean and dry centrifuge tubes and lifted at room temperature till retraction of the clot, then sera obtained and removed by pipette and clarified by centrifugation at 3000 rpm for 15 minutes to remove the residual blood cells. The obtained clear sera were transferred to dry, clean, sterile, and labeled vials and stored protected from light at -20oC for determination of β-hydroxy butyric acid (βHBA), plasma lactate, liver enzymes (AST, and ALT), and Total antioxidant capacity (TAC) using commercial kits following the manufacturer instructions.

Ruminal juice samples

About 20 ml of ruminal fluid were collected before the morning diet by a stomach tube seven times, every 15 days (Khaled and Baraka, 2011), to determine pH, and for other physical characteristics of the ruminal juice including smell, color, consistency, viability, and potentiality of ruminal protozoa according to Kleen *et al*. (2009).

Clinical examination

Thorough clinical examination, particularly, the assessments of the vital parameters as well as some clinical cores were done for all heifers under experiment before and during periods of experiment, as following:

Assessment of the vital parameter

Temperature, heart rate, mucous membrane, respiratory rate, ruminal contractions were assessed according to the method described previously by Edward (2012).

Assessment of clinical scores

Fecal score

Once daily fecal score of heifers is recorded according to Larson *et al* (1977), scoring was based on the fecal fluidity as following 0=Normal, 1=Soft, 2=Runny, and 3=Watery.

Respiratory score was done according to Constable *et al*. (2017).

Locomotor score according to Constable *et al*. (2017).

Eligibility for insemination

Observation of udder development, body weight (350 kg) and heifers tall (135 cm) at the age of 12,13,14, and 15 months.

Weighting

Heifers subjected to the experiment were weighted (using Digital Balance) at the beginning of the experiment then every 15 days to evaluate the daily weight gain among the different experimental groups.

Hematological Examination

Hematological parameters including hemoglobin, total erythrocytic count, total leukocytic count, packed cell volume %, and blood platelets were measured by the automatic cell counter Sysmex (XN-2000) (Coles, 1986).

Biochemical analysis

Beta- Hydroxybutyrate acid (BHHBA

Serum was analyzed for βHBA using commercial spectrophotometric Kits produced by Pointe Scientific, Inc. USA, according to Koch and Fledbruegge (1987).

Liver enzymes

Aspartate Aminotransferase (AST), and Alanine Aminotransferase (ALT), were measured calorimetrically using kits according to Kelly (1984).

Ruminal Juice analysis for total protozoal count (TPC)

From each heifer, 50 ml of rumen fluid was collected from each heifer on days 0, 15, 30, 45, 60, 75, and 90 of experiment using a rubber stomach tube. Then samples were sieved, divided, and stored for determination of total protozoal count consistent with the tactic described by Dehority (1984).

All previous groups were reevaluated at 12 months for the following parameters: tall, body weight, and udder development.

Statistical analysis

Results were reported as mean ± SEM (Standard Error of Mean). In order to assess the influence of the four treatment groups on the different biochemical parameters, one-way and two-way analysis of variance (ANOVA) followed by Duncan multiple tests as post hoc test were used. The value of $P < 0.05$ was used to indicate statistical significance. ALL Analyses and charts were done using Statistical Package for Social Sciences version 24.0 (SPSS, IBM Corp., Armonk, NY) and Graph Pad prism 8.0.2 (GraphPad Software, Inc).

RESULTS AND DISCUSSION

Intensive livestock rearing systems for high production of milk, and meat is increasing worldwide. Searching for natural ecofriendly feed additives for increasing weight gain, immune responses, and animal productivity is a major purpose for the animal production, and veterinary medicine sectors. Here, this research was done to evaluate the use of ruminal juice in combination with *B. subtilis* as probiotic bacteria, or with premix in achieving a significant improvement in animal body gain, productivity, and immune responses. The obtained results in Fig. 1 showed significant increase in the body gain in the experimental groups receiving ruminal juice plus premix > ruminal juice plus *B. subtilis* > ruminal juice > control, respectively. The body gain increase in the group that received ruminal juice plus premix could be attributed to the high content of minerals and vitamins in the ration introduced to this group compared to other groups. In agreement with this assumption, almost all biochemical processes in the body depend on minerals to operate normally.

Table 3. Effect of rumen juice, rumen juice with *B. subtilis*, and rumen juice with premix on some blood parameters

	G1	G2	G3	G4
RBCs (count x 10^6/ mm^3)				
0	6.50±0.41^b	8.32±0.38^a	8.63±0.25^a	6.88±0.35^b
1	6.51±0.41^c	8.96±0.50^a	8.66±0.22^{ab}	7.70±0.42^{bc}
2	6.52±0.40^a	8.11±0.53^a	8.87±1.38^a	6.64±0.34^a
3	6.52±0.40^b	7.83±0.39^a	7.24±0.31^{ab}	6.61±0.23^b
4	6.49±0.41^a	6.90±0.22^a	6.88±0.21^a	6.45±0.27^a
5	6.50±0.41^a	7.02±0.23^a	6.90±0.20^a	6.48±0.16^a
6	6.51±0.41^b	7.22±0.22^{ab}	7.61±0.47^a	6.99±0.13^{ab}
HCT (%)				
0	26.48±1.19^c	30.34±1.05^b	33.74±0.59^a	28.56±1.18^{bc}
1	26.50±1.17^b	33.54±2.14^a	34.44±0.38^a	31.14±1.42^a
2	26.48±1.18^a	27.80±1.23^a	30.52±2.59^a	29.68±0.74^a
3	26.44±1.18^a	28.34±1.35^a	27.94±0.82^a	26.54±0.93^a
4	26.78±1.02^a	25.82±1.29^a	26.24±0.56^a	27.16±0.37^a
5	26.76±1.01^a	26.56±1.21^a	28.84±0.89^a	28.64±0.61^a
6	26.82±0.98^b	27.86±1.24^b	31.36±1.37^a	29.96±0.73^{ab}
Hb (g/dL)				
0	9.38±0.34^c	11.00±0.38^{ab}	11.96±0.27^a	10.06±0.44^{bc}
1	9.42±0.33^b	11.72±0.67^a	11.96±0.19^a	11.62±0.72^a
2	9.44±0.30^c	11.10±0.06^b	12.70±0.38^a	10.42±0.20^b
3	9.54±0.25^a	10.02±0.40^a	9.96±0.27^a	10.08±0.30^a
4	9.52±0.26^a	9.32±0.09^a	9.44±0.23^a	9.68±0.13^a
5	9.60±0.27^a	9.38±0.07^a	9.78±0.35^a	9.74±0.19^a
6	9.58±0.27^b	9.82±0.32^{ab}	10.54±0.44^{ab}	10.62±0.15^a
WBCs (count x 10^3/ mm^3)				
0	3.84±0.16^a	4.86±0.91^a	5.22±0.41^a	4.10±0.64^a
1	3.88±0.17^b	5.36±0.83^{ab}	6.50±0.75^a	4.80±0.53^{ab}
2	3.97±0.15^b	5.04±0.67^{ab}	6.26±0.52^a	5.32±0.82^{ab}
3	4.14±0.14^b	4.22±0.27^b	6.62±0.42^a	6.36±0.89^a
4	4.23±0.14^{ab}	4.00±0.61^b	5.40±0.30^a	4.72±0.45^{ab}
5	4.36±0.15^{ab}	4.10±0.26^b	5.22±0.35^a	5.02±0.36^{ab}
6	4.50±0.08^{ab}	3.86±0.51^b	5.38±0.37^a	5.42±0.21^a

^{abc} Means within the same row carrying different superscripts are significantly different at $P < 0.05$.

It has been established that a number of macro and microminerals are crucial for mammals. To maximize productivity and health of cattle, it is necessary to provide sufficient amounts of essential minerals to satisfy animal needs (Spears 2002; Darwish *et al.*, 2014).

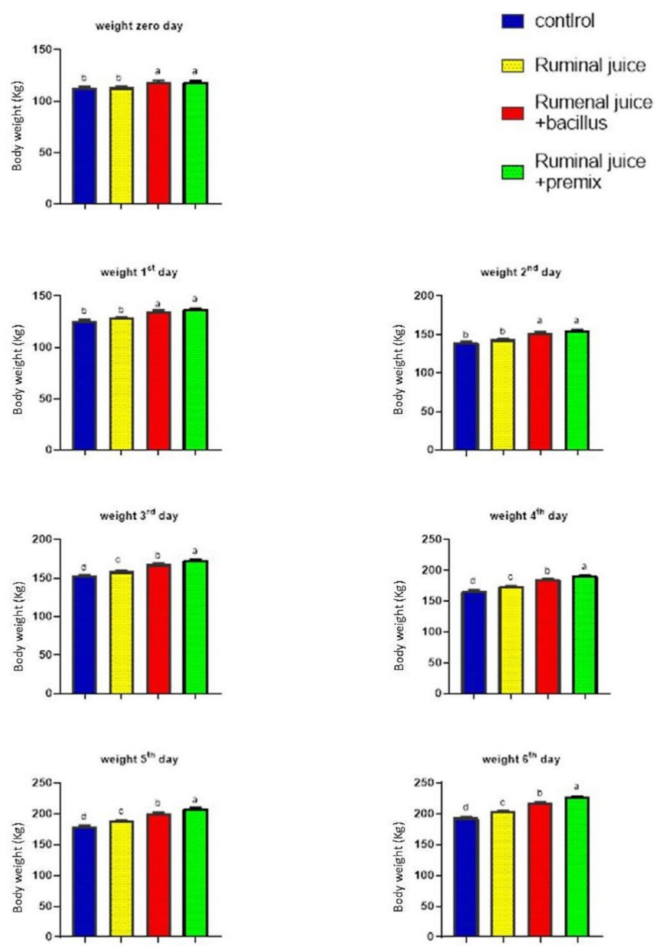

Fig. 1. Effect of rumen juice, rumen juice with *B. subtilis*, and rumen juice with premix on the body gain among treatment groups.

Likely, administration of ruminal juice plus premix could also improve the blood picture of the treated group, particularly a significant increase in the hemoglobin level was recorded followed by the group that received ruminal juice and *B. subtilis*, ruminal juice alone compared with the control (Table 4). However, ruminal juice plus *B. subtilis* could significantly improve the immune response in the treated group followed by the group that received ruminal juice plus premix, ruminal juice alone compared with the control, in terms of significant ($P< 0.05$) increases in total WBCs (Table 4), lymphocytes (Fig. 2), and granulocytes (Fig. 3). In agreement with the obtained results of the present study Raabis *et al.* (2019) reported that probiotics supplementation in the ruminants improve their immune responses via stimulation of the ruminal microbiota, and improvement of the gut colonization. Furthermore, according to Kober *et al.* (2022), ruminants' health, immunity, growth performance, nutritional digestibility, and intestinal microbial balance are all substantially improved by probiotic supplementation. Additionally, it was noted that probiotic use in animals assisted in balancing their beneficial microbial population and microbial turnover by enhancing the host immune response through particular secretions and competitive exclusion of potentially pathogenic bacteria in the gastrointestinal tract.

Interestingly, co-administration of ruminal juice plus premix

Table 4. Effect of rumen juice, rumen juice with *B. subtilis*, and rumen juice with premix on plasma lactate, BHBA, ALT, AST, and TAC

	G1	G2	G3	G4
	Plasma lactate (nmol/L)			
0	0.68±0.02^{c}	5.82±0.89^{a}	4.97±0.52^{ab}	3.65±0.83^{b}
1	0.83±0.02^{c}	4.75±0.76^{a}	3.08±0.45^{b}	3.47±0.46^{ab}
2	0.93±0.02^{b}	4.00±0.82^{a}	1.71±0.38^{b}	3.93±1.11^{a}
3	1.15±0.05^{c}	1.76±0.11^{b}	2.45±0.28^{a}	2.15±0.20^{ab}
4	1.45±0.15^{b}	1.09±0.21^{b}	3.50±0.51^{ab}	4.37±1.48^{a}
5	2.60±0.09^{a}	1.27±0.39^{b}	1.64±0.08^{b}	1.81±0.42^{ab}
6	3.45±0.07^{a}	0.79±0.07^{c}	1.12±0.13^{b}	0.49±0.06^{d}
	BHBA (mmol/L)			
0	0.66±0.02^{ab}	0.67±0.03^{a}	0.65±0.02^{ab}	0.60±0.00^{b}
1	0.66±0.02^{a}	0.67±0.03^{a}	0.66±0.03^{a}	0.67±0.01^{a}
2	0.66±0.02^{a}	0.67±0.03^{a}	0.66±0.03^{a}	0.70±0.00^{a}
3	0.66±0.02^{b}	0.67±0.03^{b}	0.66±0.03^{b}	0.80±0.00^{a}
4	0.66±0.02^{b}	0.67±0.03^{b}	0.66±0.03^{b}	0.89±0.00^{a}
5	0.66±0.02^{b}	0.67±0.03^{b}	0.66±0.03^{b}	0.91±0.01^{a}
6	0.66±0.02^{c}	0.77±0.03^{b}	0.66±0.03^{c}	1.00±0.00^{a}
	ALT (UL)			
0	13.72±0.43^{b}	36.20±6.07^{a}	19.92±1.15^{b}	31.57±4.18^{a}
1	16.67±0.46^{b}	35.03±5.03^{a}	21.17±1.11^{b}	35.47±4.17^{a}
2	18.43±0.52^{b}	29.95±3.53^{ab}	16.76±3.77^{b}	37.06±9.14^{a}
3	30.25±0.71^{a}	38.22±2.14^{a}	38.46±3.21^{a}	37.24±5.48^{a}
4	41.99±0.58^{b}	43.89±2.78^{ab}	55.47±4.62^{a}	41.66±5.94^{b}
5	38.29±1.06^{b}	45.23±3.35^{a}	32.95±1.11^{bc}	28.52±1.37^{c}
6	26.76±0.26^{bc}	45.79±3.68^{a}	36.02±7.01^{ab}	21.14±3.75^{c}
	AST (UL)			
0	43.13±0.82^{b}	97.49±14.77^{a}	42.10±9.34^{b}	18.65±3.45^{b}
1	37.06±0.87^{b}	95.31±16.14^{a}	38.45±4.88^{b}	26.67±3.04^{b}
2	30.97±0.40^{b}	93.58±15.89^{a}	29.98±7.58^{b}	22.62±6.53^{b}
3	26.70±0.65^{bc}	87.25±15.17^{a}	45.43±7.53^{b}	17.93±2.78^{c}
4	23.43±1.09^{b}	79.42±12.33^{a}	59.72±12.04^{a}	17.08±1.96^{b}
5	32.65±0.78^{ab}	38.99±5.10^{a}	28.35±2.83^{b}	18.62±1.95^{c}
6	43.25±0.88^{a}	26.62±0.46^{b}	25.30±2.20^{b}	21.11±3.26^{b}
	Total antioxidant capacity (TAC) (mmol/L)			
0	2.53±0.08^{a}	0.80±0.17^{b}	1.02±0.07^{b}	1.04±0.07^{b}
1	2.41±0.04^{a}	1.01±0.13^{c}	1.35±0.16^{b}	1.45±0.04^{b}
2	2.22±0.03^{a}	1.28±0.34^{b}	2.34±0.10^{a}	1.81±0.03^{ab}
3	1.86±0.04^{a}	1.52±0.33^{a}	1.38±0.12^{a}	1.95±0.02^{a}
4	1.56±0.09^{b}	1.58±0.26^{b}	0.77±0.07^{c}	2.29±0.09^{a}
5	1.19±0.06^{b}	1.64±0.18^{b}	1.29±0.18^{b}	2.82±0.15^{a}
6	0.95±0.01^{c}	1.72±0.12^{b}	1.78±0.19^{b}	3.47±0.26^{a}

^{abc} Means within the same row carrying different superscripts are significantly different at $P < 0.05$.

Table 5. Effect of rumen juice, rumen juice with *B. subtilis*, and rumen juice with premix on the total protozoal count

	G1	G2	G3	G4
0	29.28±0.04^{a}	25.95±0.09^{b}	25.76±0.18^{bc}	25.50±0.15^{c}
1	30.51±0.49^{b}	27.32±0.21^{c}	34.36±0.66^{a}	29.92±0.31^{b}
2	33.04±0.26^{b}	28.98±0.48^{c}	42.86±0.51^{a}	33.83±0.49^{b}
3	35.22±0.16^{c}	34.35±0.93^{c}	52.18±1.38^{a}	39.66±0.61^{b}
4	39.10±0.51^{c}	39.19±0.40^{c}	63.83±1.33^{a}	44.39±0.65^{b}
5	43.84±0.49^{c}	43.00±0.36^{c}	76.18±0.94^{a}	54.79±0.50^{b}
6	49.66±0.39^{d}	51.02±0.18^{c}	92.25±0.71^{a}	65.24±0.09^{b}

^{abc} Means within the same row carrying different superscripts are significantly different at $P < 0.05$.

could also reduce plasma lactate level followed by the group that received ruminal juice and *B. subtilis* indicating their beneficial effects in reducing ruminal acidosis which has several adverse effects on the ruminal health. In addition, such treatments could also improve the liver health of the treated groups as documented by the significant reduction in the liver enzymes, ALT, and AST which tend to increase when the liver is significantly harmed.

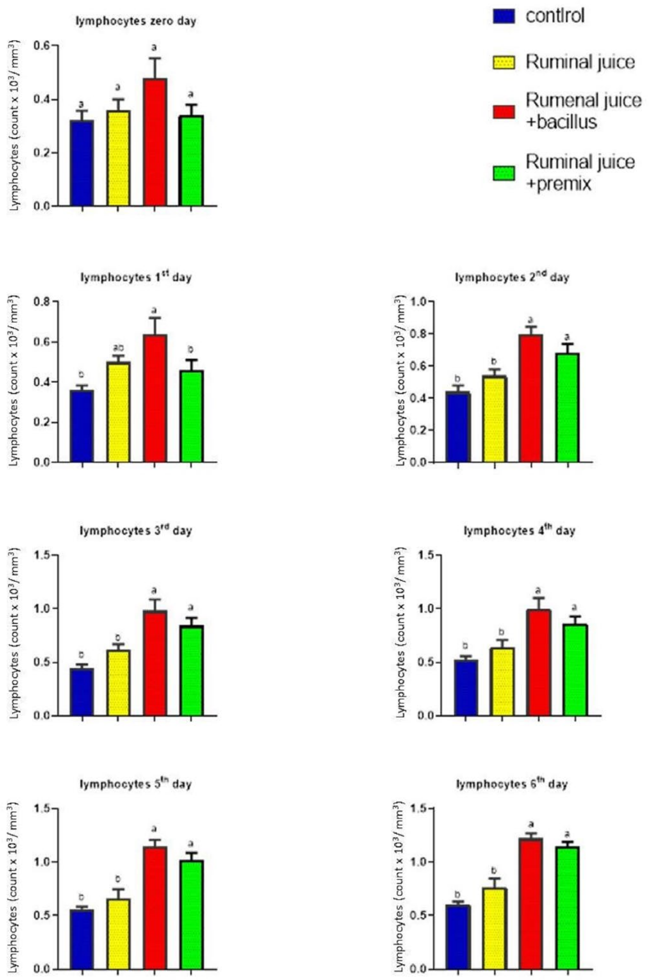

Fig. 2. Effect of rumen juice, rumen juice with *B. subtilis*, and rumen juice with premix on the body lymphocyte counts among treatment groups.

Table 6. Effect of rumen juice, rumen juice with *B. subtilis*, and rumen juice with premix on the different parameters used to assess the eligibility for insemination

	G1	G2	G3	G4
Month	Body weight (reaching 350 Kg)			
12 M	0	0	3	6
13 M	0	2	3	3
14 M	5	4	2	1
15 M	5	4	2	0
Tall reaching 135 Cm				
12 M	0	0	3	6
13 M	0	2	3	3
14 M	5	4	2	1
15 M	5	4	2	0
Udder development				
12 M	0	0	3	6
13 M	0	2	3	3
14 M	5	4	2	1
15 M	5	4	2	0

This protection could be attributed to the high antioxidant activities of the premix constituents, as confirmed by the significant increase in the antioxidant capacities in the groups receiving premix and probiotics (Table 5). The improvement in the ruminal health via administration of the ruminal juice plus premix and ruminal juice plus *B. subtilis* was clearly observed when total protozoal counts were estimated as such treatments could significantly ($P < 0.05$) increase the total protozoal counts (Table 6).

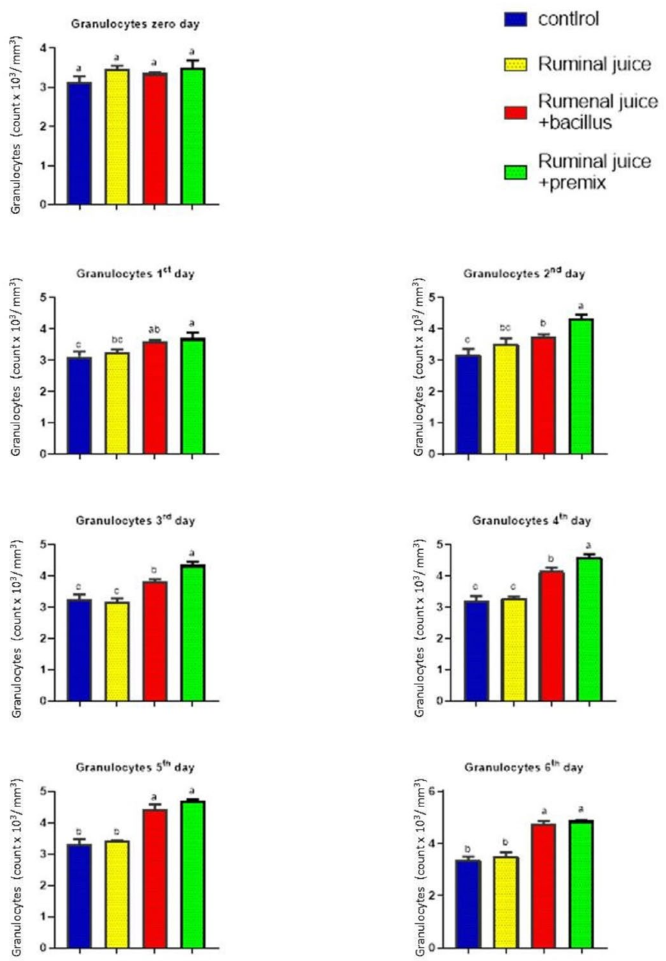

Fig. 3. Effect of rumen juice, rumen juice with *B. subtilis*, and rumen juice with premix on the granulocyte counts among treatment groups.

Likely, regular supplementation with ruminal juice and premix, or ruminal juice with *B. subtilis* could improve the animal reproductivity as confirmed via reducing the time required for being eligible for insemination as assessed via reaching certain body weight (350 Kg), certain length (135 cm), and udder development (Table 7). In line with the findings of the current investigation, probiotic microorganisms such as *B. subtilis* natto act as natural modulators of ruminal health, gut microbiota, general health status, and production efficiency (Bąkowski and Kiczorowska, 2021). Besides, premixes are rich in fat soluble vitamins which are essential as natural antioxidants and stimulators for high reproductivity (Weiss, 2017). In addition, are rich in polyphenols which positively affect animal reproduction and its antioxidant capacity (Bešlo *et al.*, 2022).

CONCLUSION

The obtained results of the present study indicated that the rumen juice transfounation either alone, or in a combination with *Bacillus subtilis*, or premix could improve the animal body gain, ruminal health, liver functions, immune responses, and animal reproductivity. Therefore, it is highly recommended to use such ecofriendly additives during intensive livestock production.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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