ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Turning nervous ewes to calm ones "behavioral and biochemical indicators" by use of Saccharomyces cerevisiae probiotic

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Manuscript Info

Manuscript History:

Received: 15 August 2014
Final Accepted: 26 September 2014
Published Online: October 2014

Key words:

Temperament; probiotics; cortisol; lactate

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Abstract

The ewe's temperament is considered one of the most important factors that affect their lamb survival; recent studies demonstrate that temperament may also have productive, reproductive and economic implications to animal operations. Temperament often regarded as innate rather than learned, but many factors can affect it, such as social environment, habituation and experience. Including temperament in culling, selection scheme may improve the overall temperament and consequent improve performance but this need more effort and we can lose some benefits of temperamental breeds, so the alternate option is to turn the nervous sheep to calm one. Recent studies on probiotics reported their effect on the animal emotional behavior. A growing body of evidence suggests that the host-microbial interaction may result in dysregulation of neuro-immune functions; impacting behavior. The research idea is based on the investigation of the effect of probiotics administration on ewe's temperament. Twelve ewes were sampled for blood and tested for their temperament on a 5 point scale and divided accordingly to nervous and calm ewes, the nervous ones were orally administered the probiotic Actisaf® daily for one month, after which they scored again. Results showed significant ($p<0.05$) improve in their temperament toward calm line. That is confirmed biochemically by a significant ($p<0.05$) decrease in serum cortisol and serum lactate concentration in probiotic administered nervous ewes.

Introduction

Temperament is defined as the animal's behavioral response to handling by humans (Burrow 1997).Maternal behavior is an important determinant of lamb survival but difficult to measure commercially; however temperament could potentially be used as an indicator trait. Studies found that ewes from the 'calm' flocks had a 10% higher lamb survival rate in twins compared with ewes from the 'nervous' flocks (Murphy 1999; Martin and Kadokawa 2006). The evidence indicates an important potential link between temperament and maternal behavior in the immediate post natal period. Such behaviors have already been shown to significantly influence the formation of the ewe lamb bond, and subsequently lamb survival (Lennon et al. 2009).

Nervous temperament has an impact on how well sheep reproduce. Temperament may influence animal reproduction either indirectly by decreasing nutritional status, or directly by altering the physiological mechanism required for ovulation and conception. In addition, the nervous animals have changes in their body physiology, and the hormones produced during fear related stress reaction influence several aspects, such as growth, health, and reproduction (Cooke 2011).That was supported by the study of (Ivanov and Djorbineva 2003) on three years old ewes. They found that the fertility between calm and nervous ewes was significantly ($p<0.05$) different.

On the other hand; one of items that has received a considerable attention in recent period researches is a "probiotics" which are defined by the Food and Agriculture Organization (FAO/WHO, 2002) as live microorganisms, upon administration in adequate amounts improve peripheral (gastrointestinal) and central (psychological) symptoms of the animal. Probiotics improve microbial ecosystem (Musa et al., 2009), nutrient synthesis and their bio-availability resulting in better growth performance in farm animals (Oyetayo and Oyetayo, 2005). A positive impact of probiotics supplementation on nutrient intake, weight gain and feed conversion ratio (FCR) in ruminants has been reported by many workers (Antunovic et al., 2006; Whitley et al., 2009).

The CNS consists of the brain and the spinal cord, with more than four hundred million neurons. Behavior is an excellent read-out of CNS function. Studies have been conducted with a view to establishing a behavioral phenotype and neuro-chemical profile that might be associated with the gut microbiota. That was proved by (Bravo et al., 2011) as probiotics found to affect the animal emotional behavior. Moreover, Sudo et al. (2004), Sudo (2006) and Messaoudi et al. (2011) found that the exposure to probiotic bacteria can reduce stress and depression related behaviors as there is a brain-gut axis bidirectional communication between brain and gastrointestinal tract. Yong (2011) and Emily (2012) who said that probiotics may modulate the activity of brain structures involved in the processing of emotions related to anxiety, mood and aggression. Other probiotic administration studies also support a role for the microbiota in alleviating anxiety-like behaviors. Administration of L. helveticus R0052 and B. longumR0175 taken in combination reduced anxiolytic-like activity in rats (Messaoudi et al., 2011)

From this concept our research is made to investigate whether the live yeast probiotics affect the temperament of animal and consequently improve its performance.

2. MATERIALS AND METHODS

2.1. Accommodation and management of animals

This study was conducted in faculty of veterinary medicine farm in Beni-Suef Governorate. A total number of 12 ossimi ewes aged 15-21 months old with an average weight of 22.6 kg. They were housed in well ventilated sheltered pens on earthy floor. Ewes were fed about 0.5 kg of processed feed. Water was available all the day from a common water trough. All animal studies were conducted in accordance with the criteria of the investigations and Ethics Committee of the Community Laws governing the use of animals in faculty of veterinary medicine, Beni-suef University.

2.2. Temperament test and probiotic administration

Ewe temperament was measured according to the temperament score test by Trillat et al. (2000). Ewes behavior was assessed at weighting (spending 30 second on the scale) in a 5-score system, where:

1- Calm, no movement.
2- Calm with occasional movements.
3- Calm with some more movements but without shaking the scale.
4- Abrupt episodic movements without shaking the scale.
5- Permanent episodic movements and shaking the scale.

The last two scores were categorized as nervous temperament.

According to the temperament test, ewes were divided into two equal groups, calm (n=6) and nervous (n=6) ewes, the nervous ones were identified by colored paint on their head.

The probiotic Actisaf® SC 47(*Saccharomyces cerevisiae* NCYC Sc 47) with a minimum concentration of 5×10^9 CFU/g was administered orally to the nervous group daily for one month.

The temperament score test was done twice again after probiotic administration period (one month) and then 15 days after to investigate its effect on the temperament of the animal.

2.3. Sampling and biochemical analysis:

Blood samples were obtained from ewes by simple jugular vein puncture on three times intervals. The first one was obtained before probiotic administration, the second was obtained 15 days after probiotic administration and the third was obtained at 30 days after probiotic administration. Serum was separated by centrifugation and divided to several aliquots and stored at -20C° for biochemical analysis of the following.

Fasted serum glucose was measured spectrophotometrically directly after serum separation according to Trinder (1969) by using of Diamond commercial kit. Serum lactate was measured according to Young (1995) by using of Spin react commercial kit. The competitive immunoluminometric assay was used for quantitative determination of serum cortisol level according to Canalis et al. (1979) and by using of Magliumi cortisol kit.

2.4. Statistical analysis

Results were statistically analyzed by the use of Wilcoxon signed ranks (a non -parametric) test for temperament score results analysis. However paired sample t- test and independent t- test were used for the rest of results, using Statistical Package for Social Sciences (SPSS) 20 together with least square analysis procedure.

3. Results

The obtained results revealed that probiotic administration to nervous ewes had significant effect on their temperament score and variable effects on the different biochemical parameters that were analyzed.

It was observed from Fig (1) that probiotic Actisaf administration had prominent effect on the temperament score of nervous ewes since the score was (4.3) before the treatment and reduced to (2.6) after one month of administration showing significant ($p<0.05$) difference between the two records. The same figure showed a non-significant increase in the score 2 weeks after stoppage of probiotic administration.

Concerning the effect of probiotics on nervous ewes biochemical blood parameters, Table (1) revealed primarily non-significant increase in cortisol concentration (53.4 to 63.8 ng/ml) at 15 days of administration followed by significant ($p<0.05$) decrease in concentration from (63.8 to 34.7ng/ml) after one month of probiotic administration. Also lactate concentration was significantly ($p<0.01$) decreased after probiotic administration; the concentration was decreased gradually from (25.1 to 20.3mg/dl) at 15 days then to (11.6 mg/dl) after one month of administration.

On the other hand the glucose concentration was increased from (39.1 to 55.6 mg/dl) at 15 days and (58.7 mg/dl) after one month of probiotic administration which was statistically non-significant.

Figures (2,3,4) showed comparison between calm ewes and the probiotic administered nervous ones.

Fig (2) revealed an increase in serum cortisol level in nervous ewes than calm ones at 0 and 15 days which was statistically non- significant, then the cortisol concentration was decreased to become nearly at the same level of that of calm ewes after one month of probiotic administration.

Fig (3) showed increase in serum lactate concentration for nervous ewes than calm ones before administration, this concentration was decreased at 15, 30 days after probiotic administration than that of calm ewes which was statistically non-significant.

Fig (4) revealed decrease in fasted serum glucose concentration in nervous ewes compared to calm ones before probiotic administration. This concentration was increased at 15 days and significantly ($p<0.05$) differ than that of calm ewes after one month of probiotic administration.

Table 1: Effect of Saccharomyces cerevisiae probiotic administration on serum biochemical blood parameters of nervous ewes

Blood parameters	0 day	15 day	30 day
Cortisol (ng/ml)	**53.4±3.5**	**63.8 ± 4.8**	**34.7 ± 8.7***
Lactate (mg/dl)	**25.1 ± 2.2**	**20.3 ± 3.4**	**11.6 ± 3.6****
Glucose (mg/dl)	**39.1 ± 9.4**	**55.6 ± 10.9**	**58.7 ± 11.7**

*Superscript means significance between columns at $p<0.05$

**Superscript means significance between columns at $p<0.01$

Fig. (1): Effect of Saccharomyces cerevisiae probiotic administration on temperament score for nervous ewes

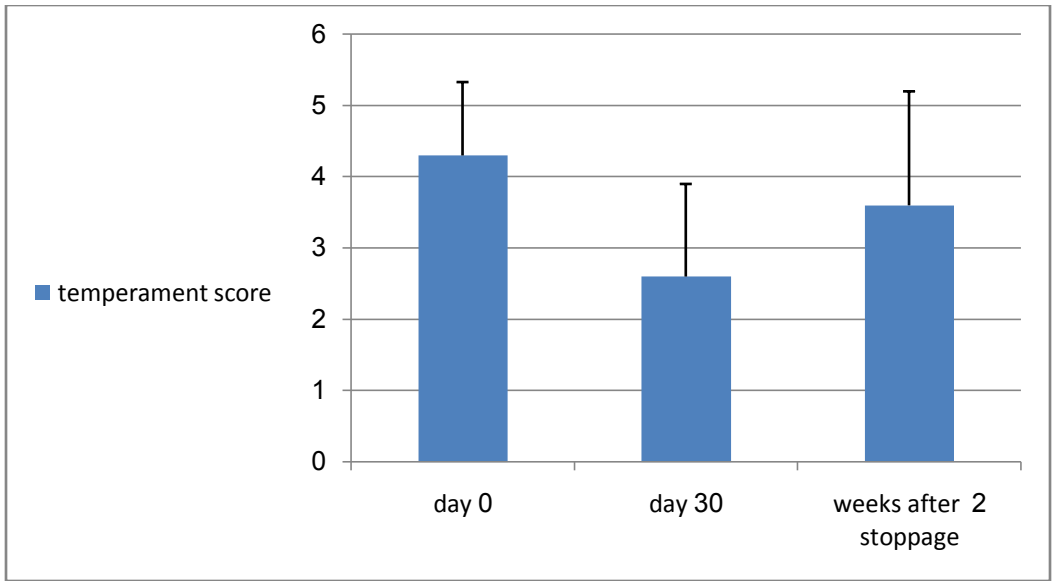
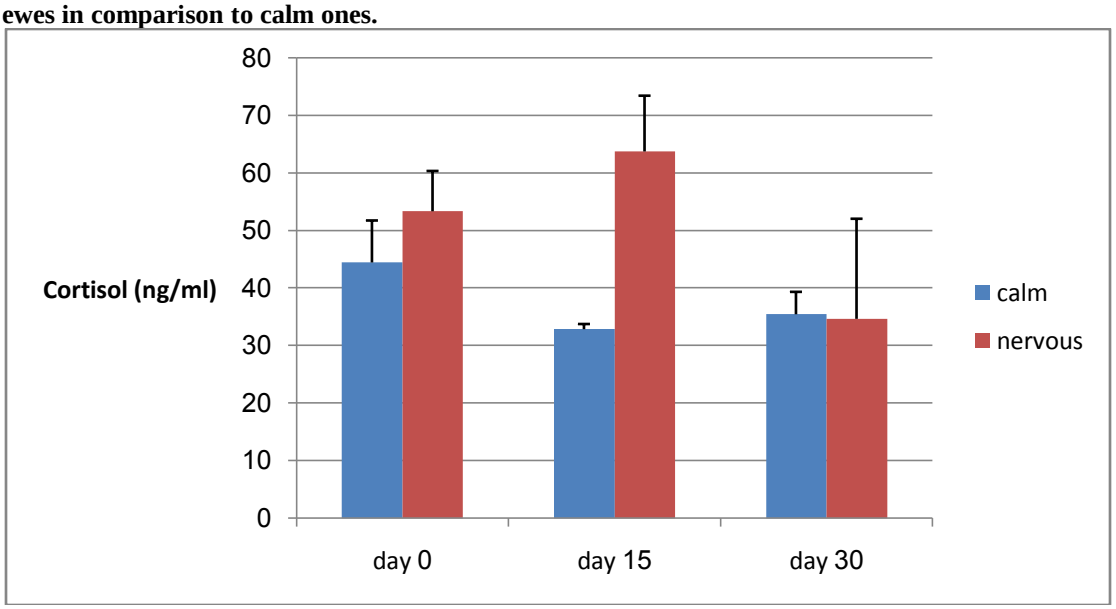
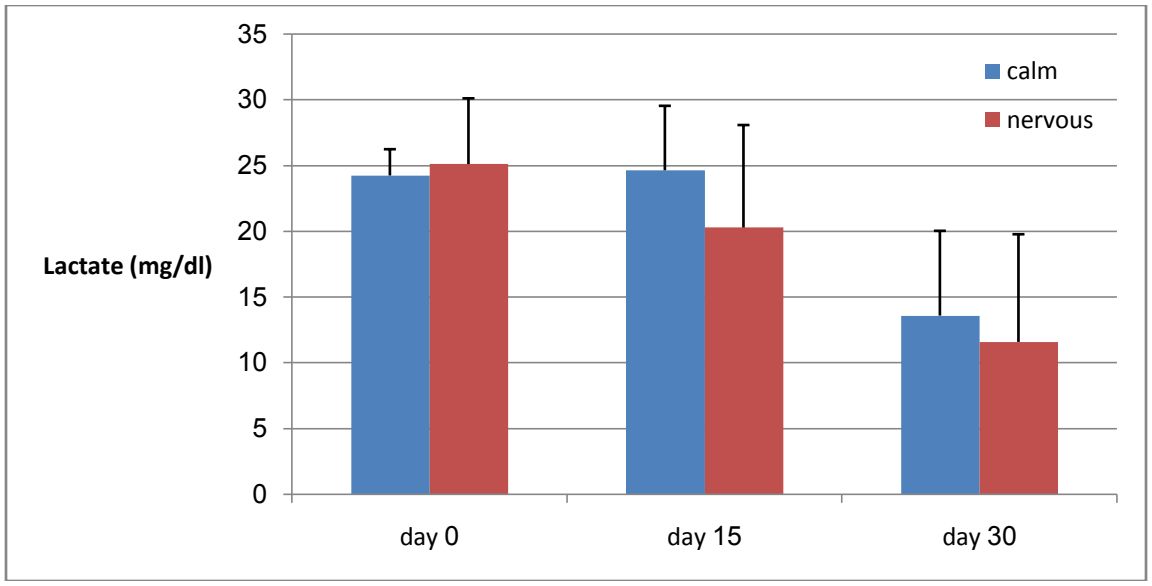


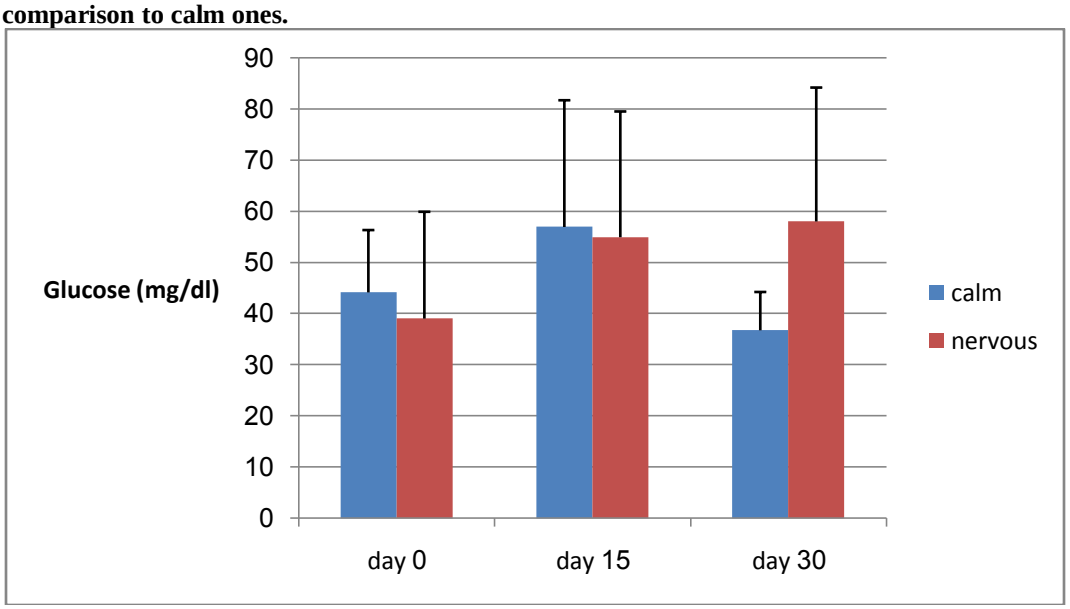
Fig (2): Effect of Saccharomyces cerevisiae probiotic administration on serum cortisol concentration of nervous ewes in comparison to calm ones.



Fig(3):Effect of Saccharomyces cerevisiae probiotic administration on nervous ewes serum lactate concentration compared to calm ones.



Fig(4):Effect of Saccharomyces cerevisiae probiotic administration on serum glucose level of nervous ewes in comparison to calm ones.



4. Discussion:

The aim of this research is to investigate the effect of Saccharomyces cerevisiae probiotic administration on the ewe's temperament which indicated by temperament score and analysis of some biochemical blood parameters. Increasing evidence is mounting that microorganisms may directly interact with elements of the host's neuro-physiological system in a noninvasive manner that ultimately results in modification of host behavior. This ability of microorganisms contained within the microbiome to influence behavior through a non-infectious and possibly non-immune mediated route may be due to their ability to produce and recognize neuro-chemicals that are exactly analogous in structure to those produced by the host nervous system. This is based on bi directional neuro-chemical interactions between the host's neuro-physiological system and the microbiome and has been termed microbial endocrinology (Lyte, 1993).

Among the yeasts belonging to the phylum Ascomycota, the most significant species is certainly S. cerevisiae. Many of the approximately 20 species of this genus are of great biotechnological significance due to applications including alcoholic fermentation, bread making, single cell protein, vitamin production, synthesis of recombinant proteins, and

biological control (Webster and Weber, 2007).One of the most significant non-process uses of yeasts is as probiotic microorganisms.

The results showed a significant effect of yeast probiotic administration on temperament of nervous ewes; such findings coincides with that reported by Jenkins(2014), who observed improved temperament of rumen-zyme treated animals, and with that recorded in a project of Ministry for Primary Industries (2013), which noted that the probiotic supplemented animals were easier to handle than control group. Our results could be explained in the light of published articles (Sudo et al 2004; sudo 2006) who demonstrated that manipulations of bacteria found in the stomach and intestine can modify neural function and affect mood and behavior as there is an important link and interaction between gut microbes and the brain (Lee and Chua 2011) and it is agreed that probiotics improve microbial ecosystem (Musa et al 2009). These results also supported with the data shown in figures 2,3,4 that revealed converge between cortisol and lactate values in nervous ewes that administered probiotic yeast from that of calm ones.

About glucose concentration in nervous ewes that administered probiotic yeast, it found to be significantly increased than that of calm ewes after one month of administration. This increase was in agreement with the research published by Abo El-Nor and Kholif (1998) which may be related to a temperate improvement in gluconeogenesis and increase in lactose absorption (De valdes et al 1997). However it was not agreed with that of Antunovic et al (2005) who found lower blood glucose in weaned lambs supplemented with 0.1% probiotics. And with that of Ding et al. (2008) who reported no difference in blood glucose concentration in lambs fed diets with or without probiotics.

Regarding the effect of yeast probiotics on the cortisol level in nervous ewes, the significant decrease in cortisol concentration in nervous ewes may be attributed to the modification of the mood and behavior of ewes towards the calm line, as the calm ewes had a lower cortisol level compared to nervous animals which exhibit increased responsiveness to handling increased the adrenal function and so increased baseline cortisol concentration (Pajor et al 2010; Cooke 2011). Our results was supported by Bravo et al., (2011) as they found that chronic treatment with the probiotic Lactobacillus rhamnosus over 28 days produced animals with lower levels of corticosterone and reduced depressive behaviors in the forced swim test.

The decreased lactate concentration in nervous ewes after yeast probiotic administration may be attributed also to the change of ewe's temperament, as the nervous ewes had a higher serum lactic acid level compared to calm animals because the increased locomotors activity and increase muscle contraction. Higher muscle contraction leads to higher serum lactic acid contraction (Pajor et al 2010). Chaucheyras et al. (1995) had another explanation as he reported that Saccharomyces cerevisiae also was able to prevent the accumulation of lactic acid production by competing with Streptococcus bovis for glucose and by stimulating the uptake of lactic acid by Megasphaeraelsdenii possibly by supplying amino acids and vitamins.

5. Conclusion:

Saccharomyces cerevisiae probiotic play various roles in livestock feeding and veterinary practices as well as in medicine and the biomedical and pharmaceutical industries. They can exert numerous effects on the intestinal neuro-immune system and influence a variety of animal functions such as metabolic activity, immune response and physiological function. In our experiment administration of Saccharomyces cerevisiae probiotic for ewes for one month improving nervous behavior which detected by temperament score test and decreased serum cortisol and lactic acid concentration and increased serum glucose concentration.

Acknowledgement:

We would like to express our gratitude to Norvet Misr Company who providing us with Actisaf® product to make the necessary research work.

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