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Augmenting milk production in buffaloes by combating heat stress through strategic supplementation of drug based probiotics and immunomodulators

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Abstract
The field trial was aimed to evaluate the efficacy of probiotic and immunomodulator in alleviating the heat stress in buffaloes during summer season and to subsequently augment milk production. Sixty multifarious buffaloes were divided into three uniform groups (T1, T2 and T3) of 20 each. Group T1 served as control, group T2 was supplemented with probiotic (10 gm/day/buffalo) and group T3 were fed with probiotic (10 gm/day/buffalo) along with herbal immunomodulator (50ml/day/buffalo) for a period of five months, March to July where the ambient temperature is relatively high. With increase in temperature, T1 group of buffaloes manifested a reduction in milk yield. In group T2, supplementation of probiotics alone resulted in maintenance of milk yield with a slight increase pattern. In group T3, supplementation of both probiotics and immunomodulators proved to be effective in increasing the milk production of the lactating buffaloes. The average milk production in group T3 was significantly higher than T2 and T1. The results showed a significant effect of heat stress on daily milk yield. This field study clearly indicated that the supplementation of both probiotic and immunomodulator during summer season was effective in alleviation of heat stress and enhancement of milk yield in lactating buffaloes.

Keywords: Buffalo, probiotics, immunomodulator, milk yield, heat stress

Introduction
Reduction in milk yield due to heat stress is more prominent problem encountered in buffaloes. Decreased synthesis of hepatic glucose and lower non esterified and fatty acids (NEFA) level in blood during heat stress causes reduced glucose supply to mammary glands resulting in low glucose and low lactose synthesis which in turn ensues low milk yield. Reduction in milk yield is further intensified by decrease in feed consumption by animals to compensate high environmental temperature. Heat stress negatively affects the productive performance of dairy cattle (Das *et al*., 2016) [8]. Reduced milk production due to stress is attributable only partly to decrease in feed intake. Metchnikoff (1908) [16] found that consumption of yoghurt resulted in life-longevity of Bulgarians. He concluded that lactic acid producing bacteria present in the yoghurt improved the intestinal environment. Thus the concept of live microbial cells as feed additive was born. The term 'Probiotics' derived from the Greek word meaning 'for life' and was first introduced in 1953 by Kollath (Hamilton-Miller, 2003) [12] while term 'Probiotic' was coined by Parker in1974.

Fuller (1989) [10] defined probiotics as ' a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance'. As per the recent definition by FAO and WHO, probiotics are live micro organisms which when administered in adequate amount confers a health benefit on the host'. The most commonly used organisms in probiotic preparation for the lactic acid bacteria (*Lactobacillus*, *Streptococci*, *Bifidobacteria*).These are found in large numbers in the gut of healthy animals and don't appear to affect them adversely. Certain strains of yeast (*Saccharomyces cerevisiae*) and fungi (*Aspergillus oryzae*) are also used for probiotic preparation. Several studies have been conducted using different potential microbial strains as probiotic with various degree of success is different species. On the basis of these studies, probiotics had been grouped into two broad categories probiotics for monogastric animals and probiotic for ruminant animals.

Alshaikh *et al*. (2007) [1] indicated that the cows consuming those diets supplemented with yeast culture began to decrease their dry matter intake and to enhance their milk production. Kudrna *et al*. (2007) [14] concluded that yeast supplementation significantly improved the milk yield in spite of reducing the dry matter intake.

Chandrasekhariah *et al*. (2007) [6] interpreted that characteristic, sources, production of probiotics as well as the influence of prebiotics on animal production have potential to increase milk yield and composition under Indian condition. Extensive studies have been conducted in cattle and poultry by supplementation of probiotics whereas there is a dearth in buffaloes. In addition, Immunomodulatory agents of plant and animal origin enhance the immune responsiveness of an organism against a pathogen by activating the immune system (Sarma and Khosa, 1994) [20]. There are several herbs used in the indigenous systems of medicine that may enhance the body's immune system. A variety of plant derived materials such as polysaccharides, tannins, flavonoids, peptides and lectins have been reported to potentiate the immune system (Ielpo *et al*., 2000; Kuttan, 2000) [13, 15]. Indian medicinal plants are a rich source of substances which are claimed to induce paraimmunity, the non-specific immunomodulation of essentially granulocytes, macrophages, natural killer cells and complement functions (Sainis *et al*., 1997) [19]. Milk production per cow has steadily increased through the combined improvement of management, nutrition, and genetic selection actions (Crowe *et al*., 2018) [7]. However, buffaloes are often blamed for poor productivity due to heat stress during summer season. Even with this limitation, buffaloes contribute more than 55% of total milk production of India. Further, the literature is scanty in studies related to supplementation of probiotics and immunomodulator for ruminant animals such as buffaloes. Thus keeping the aforementioned scenario, the present study was designed to evaluate the effect of supplementation of drug based probiotic and immunomodulator in alleviation of heat stress and to increase the milk production in buffaloes.

Materials and methods

The present study was conducted in an organized buffalo farm at Tindivanam Thaluk, Villupuram District, Tamil Nadu. In this study, sixty (60) milking multifarious buffaloes were used with a mean body weight of 400kg in summer season from March to July, 2018. Before the study, the buffalo's average milk yield was 5 litres and it decreased to 4 litres during the summer period owing to heat stress. It is for this reason the investigation is done purposively in these months (Mar-July) where the average temp increased to 33˚C and the average milk yield was also lowered. The study was conducted in 60 buffaloes that were divided into three uniform groups (n=20) as T1, T2 and T3 in such a way that the order of lactation and average milk yield of two groups were more or less similar. The treatments T1 served as the control (without probiotic and immunomodulator supplementation) and T2, T3 served as the recommended practices. All the buffaloes in T2 were supplemented with Probiotics (10g/day/buffalo) and T3 were supplemented with Probiotics 10g and Herbal immunomodulator (50ml/day/buffalo). Multi-strain probiotic containing *Saccharomyces cerevisiae, Lactobacillus sporongenes* and *Aspergillus oryzae* marketed by Intas Pharmaceuticals ltd. namely Ecotas(3 bolus/day) was used in this study. Herbal immunomodulator and antistressor drug, Restobal liquid containing *Ocimum sanctum, Withania somnifera, Tribulus terrestris, Mangifera indiaca, Asparagus racemosus and Phyllanthus emblica* marketed by Ayurvet Limited was used for the study. Same amount of concentrates (@1 kg/2 litres milk produced), dry fodders and Mineral mixture were fed to all the three groups. Milk production was recorded daily both in the morning and evening and monthly average yield was calculated for the entire investigation period. The data was analysed using ANOVA with computer software, Minitab18. Briefly, the standard error of the mean is calculated and this value along with the confidence estimates based on a t distribution was utilized to determine confidence interval (CI) and an interval plot is constructed to study the variation in different treatments.

Results and Discussion

Effect of Probiotics and Immunomodulator supplementation on milk production in buffaloes: Table 1 shows mean value of average milk production /day/buffalo in control (T1), Probiotics alone(T2) and Probiotics and Immunomodulators(T3) in March, April, May, June and July.

Table 1: Effect of Probiotics and Immunomodulators in milk production during summer season in buffaloes.

Months	Number of milking Buffaloes			Average milk production (liters/day/buffalo)		
	T1	T2	T3	T1	T2	T3
March	20	20	20	5.50	6.00	6.50
April	20	20	20	5.00	6.25	7.25
May	20	20	20	4.50	6.00	7.00
June	20	20	20	4.75	6.00	7.25
July	20	20	20	5.25	5.5	7.5
Average	20	20	20	$5.00^{*}\pm 0.19$	$5.95^{*}\pm 0.12$	$7.1^{*}\pm 0.16$

*: Values bearing superscripts differ significantly ($P \leq 0.05$ percent)

It is observed from the above table that average milk production/ buffalo was 5, 5.95 and 7.1 for treatments T1, T2 and T3 respectively. In T1 group, the average milk production was observed as 5.50, 5.00, 4.50, 4.75 and 5.25 litres in March, April, May, June and July, respectively. This shows there is substantial decrease in daily and monthly milk production especially in summer months. Pawar *et al*., 2013 [18] reported that rise in temperature significantly decreased milk yield and milk fat, protein and SNF in lactating Murrah buffaloes. This was similar with the present control's yield (T1). The study revealed a gradual decrease in milk yield in T2 group in the months of May and June but the yield obtained was significantly higher than the control group. The obvious reason for the decrement in the milk yield in these months is the high ambient temperature. Heat stress negatively affects the milk production performance in dairy cattle (Sirohi and Michaelowa, 2007) [21]. However, the milk yield in T2 group is higher than the T1 group which might be due to regular supplementation of probiotics. This concurred with the findings of Azzaz *et al*. (2015) [2]. Results clearly stated that T3 group is significantly different from T2 and T1 with relatively high lactation. This can be strongly attributed to the supplementation of probiotics and immunomodulators and their synergistic effect. The present results are in accordance with the findings of Mostafa *et al*. (2014) [17]. The data revealed a direct correlation between supplemented diet

with probiotics and immunomodulators in overcoming heat stress and the problem of decrement of milk yield in buffaloes. The minimal milk yield obtained in summer season endorse the well known fact of the adverse effect of hot and humid weather conditions prevailing in summer months which create an economical loss to the livestock keepers.

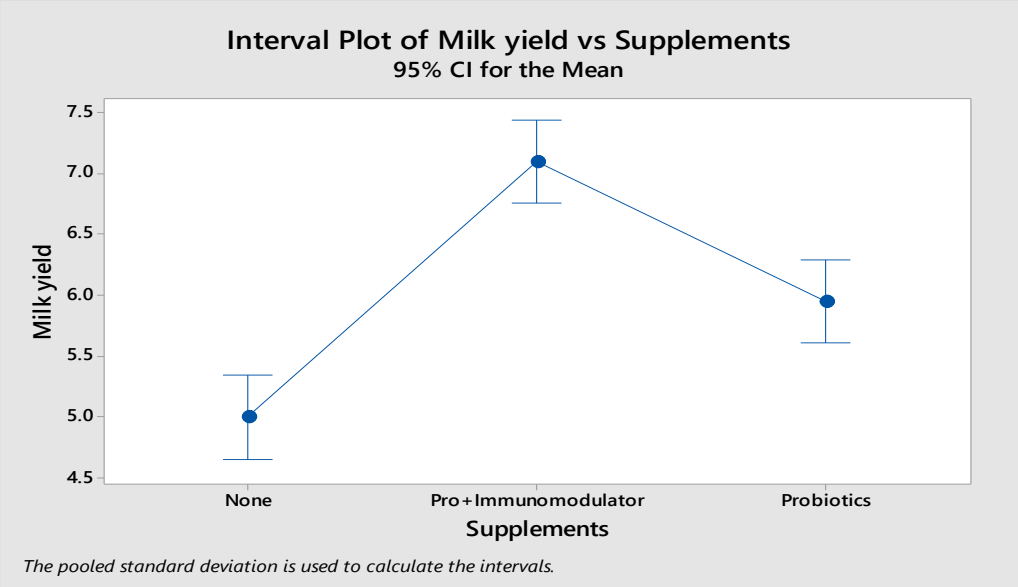

Fig 1: Interval plot showing increment in milk production after supplementation with probiotics alone and both probiotics and immunomodulator

An interval plot of milk yield Vs supplements is constructed (Fig 1) to elucidate the importance of probiotics and immunomodulators in enhancing the milk production of buffaloes in summer months. The width of the interval plot provides an indication of the amount of variation that is present in the data. The width between the T1 to T3 is higher than T3 to T1 which clearly indicates the ameliorative role of probiotics and immunomodulators against the adverse increase in temperature during summer months. The reason attributable for this condition is the summer-induced oxidative stress. The fungal culture like *Saccharomyces cerevisiae* and *Aspergillus oryzae* has proven to improve performance of dairy cattle by alleviating heat stress effect. In dairy animals, supplementation of live yeast improves nutrient digestibility and feed efficiency (Ferraretto *et al*., 2012) [9] and also maintain ruminal pH (Bach *et al*., 2007) [3] and increase milk yield (Bruno *et al*, 2009) [5]. It has been established that oxidative stress is associated with high milk yield. The increase in milk yield may be attributed to the anti-oxidant property of *Withania somnifera* (Bhatnagar *et al*., 2005) [4]. Likewise, the anti-oxidant property was also found to be present in *Ocimum sanctum* (Hakkim *et al*., 2007) [11], a constituent ingredient of herbal immunomodulator which might be the cause of increased milk production in buffaloes at T3 group.

Conclusion

The present study concludes that oral supplementation of Probiotics and Herbal immunomodulators had substantially enhanced the productive performance of the lactating buffaloes in high temperature prevailing during summer season. Diligent use of feed additives like probiotics and immunomodulators will prevent heat stress effects and maximize the buffaloes' performance and eventually increase the herd profitability. On the basis of the present study, it is recommended that buffalo rearers should regularly supplement probiotics and immunomodulators especially during summer season to achieve optimal milk production.

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