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## Assessment of stress and immunoglobulin levels of early lactating murrah buffaloes on supplementation of bypass fatty acids and *Tinospora cordifolia*

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### Abstract

The present investigation was undertaken to assess the effect of supplementation of bypass fatty acids and *Tinospora cordifolia* on stress and immunoglobulin values in early lactating Murrah buffaloes. Twenty freshly calved and healthy buffaloes were selected from institutional herd and divided into four groups having five buffaloes each on the basis of their previous milk yield, body weight and parity. Four groups were assigned as T0 (control), T1 (fatty acid), T2 (*Tinospora*) and T3 (mix) and were fed with specific amount of supplements for 90 days. In T1 group 150gm of bypass fatty acids, in T2 group 150 gm of *Tinospora cordifolia* stem powder and in T3 group 150 gm of bypass fatty acids and 150 gm *Tinospora cordifolia* powder was supplemented over and above the normal ration whereas no supplementation was done in T0 group. Cortisol and IgG levels was analyzed in blood at fortnightly intervals to assess the stress and immunoglobulin levels. Results showed *Tinospora* supplementation also significantly improved IgG concentrations in T2 and T3 in comparison to T0 and T1. It was concluded that bypass fatty acids and *Tinospora cordifolia* supplementation positively reduces stress and increases immunoglobulin concentration in early lactating Murrah buffaloes.

**Keywords:** buffaloes, bypass fatty acid, immunoglobulin, stress, supplementation, *tinospora*

### Introduction

For successful working of dairy farm business, the time period during lactation is critical which highly influence production and health of an animal. It is the transition period which is considered most crucial affecting the production and health performance of an animal and it is considered as the most stressful period for an animal (Abuelo *et al.*, 2019) <sup>[1]</sup>. Various disturbances during this period from severe negative energy balance (NEB) and low serum or plasma concentrations of several minerals and vitamins affect the animal which overall lowers the health status (Goff, 2006; Rollin *et al.*, 2010) <sup>[4, 14]</sup> and otherwise too during the early stage of lactation, the health and immune system of animals are most likely to gets compromised (Sordillo, 2016) <sup>[18]</sup>. It is the difference between the energy requirements of animal and availability that mainly is cause for development of negative energy balance (NEB) (Sirohi *et al.*, 2010) <sup>[17]</sup>. This imbalance in energy during this period attributes to stress and reduced immunity in animals. Directly or indirectly stress leads to decrease in animal's production and compromised immunity along with stress make animal prone to different diseases. Cortisol has long been used as a marker for determining the stress response in dairy animals (Burnett *et al.*, 2014) <sup>[3]</sup> and IgG gives the idea about antibodies level and immunity status of dairy animals (Ulfman *et al.*, 2018) <sup>[20]</sup>. When the maintenance and production requirements of high producing animals are not fulfilled with the regular dietary sources and are compromised there arises a need for extra energy supplementation to fulfil the maintenance and production demand of the lactating animals. A source for high energy is supplementation of bypass fat (Jenkins and Palmquist, 1984; Scott *et al.*, 1995; Sirohi *et al.*, 2010; Mudgal *et al.*, 2012; Tyagi *et al.*, 2010; Rajesh, 2013) <sup>[6, 9, 15-17, 19]</sup>, whereas, *Tinospora cordifolia* serves as immunity booster in animals (Aranha *et al.*, 2012; Mukherjee *et al.*, 2010) <sup>[2, 10]</sup>.

To mark the health status various blood metabolites of animals have been used to evaluate the ongoing changes inside the animal's body (Payne *et al.*, 1970) <sup>[11]</sup>. Analysis of specific blood metabolites like cortisol and IgG gives the picture of undergoing stress on animal and immunoglobulin levels. This can be employed to optimize the production and health management of dairy animals. Taking this into consideration the study was conducted to

evaluate the stress and immunoglobulin levels of lactating Murrah buffaloes on supplementation with bypass fatty acid and *Tinospora cordifolia*.

## Material and Methods

### Description of the study area

The following experimental trial was carried out at Livestock Research Centre of National Dairy Research Institute (N.D.R.I.), Karnal, Haryana, India that is located on 29°42" N and 79°54" E longitudes at an altitude of 245 meters above the mean sea level in the beds of Indo-Gangetic alluvial plain. The minimum temperature of the area falls to near freezing point in winter and maximum goes approximately up to 45°C in May/June months of summer. The annual rainfall is close to 700 mm, most of which is received from July to September. A subtropical climate prevails in the area.

### Selection of animals and design of experiment

Twenty freshly calved Murrah buffaloes were selected from Livestock Research Center of NDRI, Karnal and further divided into four groups of 5 buffaloes each on the basis of previous milk yield, parity, and body weight. It was ensured that the selected animals for study were free from any anatomical, physiological and infectious disorders. The experiment was conducted as per the guidelines of institutional ethical committee. Four groups of animals were assigned as T0, T1, T2 and T3. T0 was taken as control whereas T1, T2 and T3 were given supplementations. T0 was kept without any supplementation and given standard feed (ICAR-2013 standards) whereas T1, T2 and T3 were supplemented with 150gm of bypass fatty acids per animal per day, 150gm of *Tinospora* stem powder per animal per day and combination of 150gm bypass fatty acids and 150gm *Tinospora* per animal per day, respectively. Supplementations were given for a period of 90 days after calving in buffaloes and each buffalo was kept under observations. Buffaloes were kept in loose housing system and given enough space as per BIS requirements but they were tied for 1 hour for individual feeding of supplements. The daily feeds of buffaloes include green fodders like oats, maize, jowar, sugar graze and berseem depending on their availability and mixture of maize silage and wheat straw. Supply of clean and fresh drinking water was available to buffaloes for whole day.

### Parameters observed

Stress of the animal is assessed through the change in cortisol level in blood plasma and immunoglobulin level is measured by IgG concentrations in blood plasma.

### Collection of blood

Blood samples were drawn from individual buffalo in sterile heparinized vacutainer tubes from jugular vein posing minimum disturbances to the animal. Blood samples were collected on day 15th, 30th, 45th, 60th, 75<sup>th</sup> and 90<sup>th</sup> postpartum. Samples were taken to the laboratory in chilled iceboxes soon after collection and was centrifuged at 1200×g at 4°C for 20 minutes to separate out the plasma. Plasma samples were stored at -20°C for further examination. Plasma samples were used to determine cortisol and IgG concentrations.

### Cortisol and IgG estimation

Specific ELISA kits were used to estimate the levels of cortisol and IgG levels in blood plasma. ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying soluble substances

such as peptides, proteins, antibodies, and hormones. Cortisol was determined in plasma of buffalo by "Bovine Cortisol ELISA Kit" (Cat. No E0110Bo) from Life science Inc. Ltd. Whereas, Immunoglobulin G was determined in plasma of buffalo by "Bovine Immunoglobulin G1 ELISA Kit" (Cat. No E0385Bo) from Life science Inc. Ltd.

### Statistical analysis

Data was analyzed using the statistical software IBM(r) SPSS(r) Statistics (version 22, IBM SPSS Inc., Chicago, USA) by applying one-way ANOVA. The pairwise comparison between treatments was performed using duncan test with a significance level of 95% ( $\alpha < 0.05$ ).

## Results

### IgG (Immunoglobulin G)

The differences in mean values of IgG in blood plasma of buffaloes differ significantly the ( $P < 0.05$ ) between the groups and days. During experimental period the value of IgG ranges between  $18.81 \pm 0.89$  to  $19.64 \pm 0.60$  mg/ml for control (T0) group,  $18.63 \pm 0.61$  to  $19.69 \pm 0.78$  mg/ml for fatty acid (T1) group,  $21.27 \pm 1.44$  to  $24.00 \pm 0.70$  mg/ml in *tinospora* (T2) group and  $22.05 \pm 1.25$  to  $24.47 \pm 0.94$  mg/ml in mix (T3) group. The overall mean plasma IgG for 90 days was highest for mix (T3) group ( $23.38 \pm 0.92$ ), followed by *Tinospora* (T2) group ( $22.93 \pm 0.74$ ), then fatty acid (T1) group ( $19.15 \pm 0.33$ ) and least for the control (T0) group ( $19.11 \pm 0.71$ ). There was a significant ( $P < 0.05$ ) increase in the immunoglobulin levels of *Tinospora* supplemented groups (T2 and T3) as compared to control (T0) and bypass fatty acid (T1) supplemented groups. The values of IgG in blood plasma throughout the experimental period were depicted in Table 1 and graphically represented in Figure 1.

### Plasma cortisol

The fortnightly mean ( $\pm$ SEM) plasma cortisol was significantly different between the groups ( $P < 0.05$ ) and between days ( $P < 0.01$ ). In the beginning of experiment cortisol levels were on the higher side in all the groups but as the lactation progresses, significant ( $P < 0.05$ ) decrease was found in the supplemented groups as compared to the control group. During 90 days of experimental period the range of plasma cortisol was  $14.76 \pm 1.71$  to  $7.69 \pm 0.58$  ng/ml for control (T0) group,  $12.92 \pm 1.23$  to  $6.11 \pm 1.09$  ng/ml for fatty acid supplemented (T1) group,  $11.81 \pm 0.26$  to  $6.61 \pm 0.52$  ng/ml for *Tinospora* supplemented (T2) group and  $11.94 \pm 0.42$  to  $6.03 \pm 0.41$  ng/ml for both fatty acid and *tinospora* supplemented (T3) group. The overall mean plasma cortisol for 90 days was least for mix (T3) group ( $8.68 \pm 0.18$ ), followed by *Tinospora* (T2) group ( $9.48 \pm 0.14$ ), then fatty acids (T1) group ( $9.51 \pm 0.81$ ) and highest for the control (T0) group ( $11.27 \pm 0.93$ ). The change in values of cortisol level in blood plasma throughout the experimental period were given in Table 2 and graphically shown in Figure 2.

## Discussion

Stress and immunoglobulin concentration in different groups was analyzed through cortisol and IgG concentrations respectively. Analysis of IgG values showed significant increase in *Tinospora* supplemented groups in comparison to control and fatty acids group. This proved the immunostimulant effect of *Tinospora cordifolia* in animals (Kapil and Sharma, 1997; Manjrekar *et al.*, 2000) [7, 8]. These results were in accordance with reports of Gupta *et al.* (2016) [5] who showed increase in IgG values on supplementation of *T. cordifolia* in dairy cows. Cortisol hormone was estimated

to assess the stress level in buffaloes and it was significantly reduced in supplemented groups in comparison to the control group. The reduced levels of cortisol were attributed to improved energy balance and immune status of the treatment group buffaloes. Similar reports of decrease in cortisol level on supplementation of bypass fat were given by Purwar *et al.* (2017) [12] whereas in contrary to this some reports shows no significant difference in cortisol levels between control and prilled fat supplemented animals in early (Yadav *et al.*, 2015) [21] and mid-lactation (Singh *et al.*, 2014) [16, 21].

### Conclusion

Increased levels of immunoglobulins were found in tinospora supplemented groups shows Tinospora supplementation helps in improving immunity of buffaloes whereas reduction in

cortisol levels with the advancement of lactation showed decreased stress and improved energy balance in supplemented buffaloes. Hence, supplementation of bypass fatty acids and Tinospora cordifolia can be done to ameliorate stress and improvising the immunoglobulin concentration in early lactating Murrah buffaloes.

### Acknowledgements

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**Table 1:** Effect of bypass fatty acid and *Tinospora* supplementation on IgG (mg/ml)

Days	Control (t0)	Fatty acids (t1)	Tinospora (t2)	Mix (t3)	P value
15	18.92 <sup>ax</sup> ± 1.41	18.63 <sup>ax</sup> ± 1.06	21.27 <sup>axy</sup> ± 1.44	22.05 <sup>axy</sup> ± 1.25	0.205
30	18.81 <sup>ax</sup> ± 0.89	18.63 <sup>ax</sup> ± 0.61	22.14 <sup>bxy</sup> ± 0.79	23.34 <sup>bxy</sup> ± 0.67	0.001
45	19.29 <sup>ax</sup> ± 0.89	19.32 <sup>ax</sup> ± 0.49	23.00 <sup>by</sup> ± 0.95	22.56 <sup>bxy</sup> ± 1.34	0.020
60	18.97 <sup>ax</sup> ± 0.95	19.01 <sup>ax</sup> ± 0.34	23.30 <sup>by</sup> ± 0.71	23.75 <sup>by</sup> ± 0.95	0.000
75	19.64 <sup>ax</sup> ± 0.60	19.69 <sup>ax</sup> ± 0.78	23.89 <sup>by</sup> ± 0.71	24.11 <sup>by</sup> ± 1.17	0.001
90	19.01 <sup>ax</sup> ± 0.46	19.63 <sup>ax</sup> ± 1.42	24.00 <sup>by</sup> ± 0.70	24.47 <sup>by</sup> ± 0.94	0.001
Mean ± SE	19.11 <sup>ax</sup> ± 0.71	19.15 <sup>ax</sup> ± 0.33	22.93 <sup>by</sup> ± 0.74	23.38 <sup>bxy</sup> ± 0.92	0.450
P value	0.998	0.966	0.043	0.125	

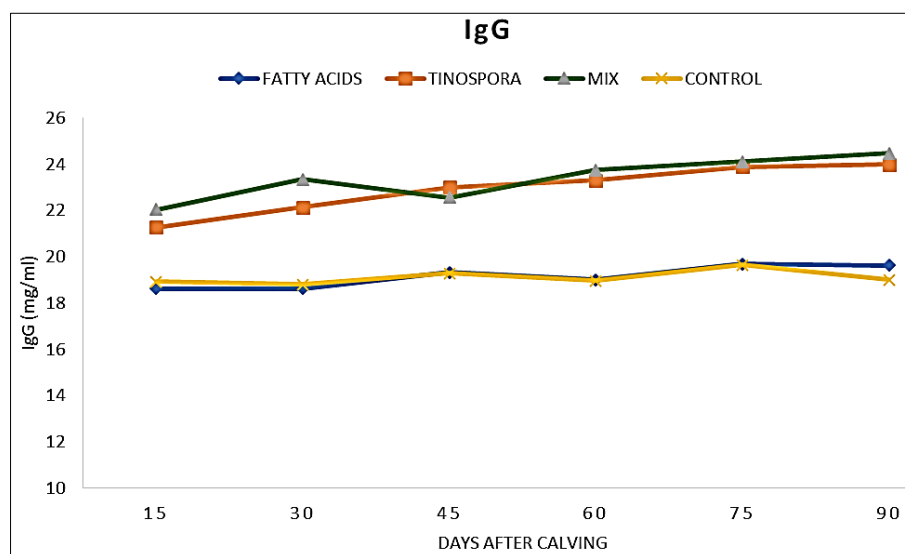
The values are Mean ± SE of six observations on five animals in each group. Values with different superscripts a, b, c and x, y differ significantly ( $P < 0.05$ ) in a row and column respectively.

**Table 2:** Effect of bypass fatty acid and *Tinospora* supplementation on cortisol (ng/ml)

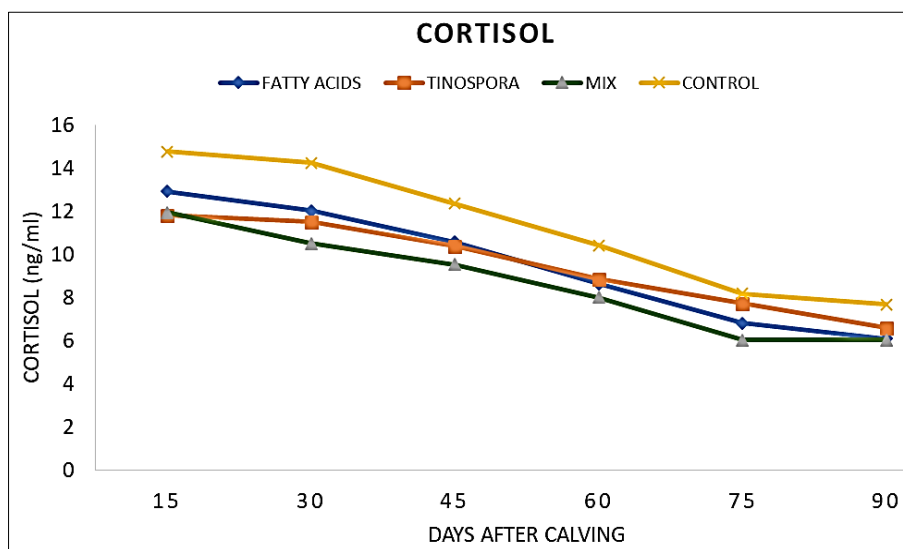
Days	Control (t0)	Fatty acids (t1)	Tinospora (t2)	Mix (t3)	P value
15	14.76 <sup>ay</sup> ± 1.71	12.92 <sup>az</sup> ± 1.23	11.81 <sup>az</sup> ± 0.26	11.94 <sup>az</sup> ± 0.42	0.235
30	14.24 <sup>by</sup> ± 1.47	12.03 <sup>abyz</sup> ± 0.86	11.50 <sup>abz</sup> ± 0.73	10.51 <sup>ayz</sup> ± 0.29	0.071
45	12.35 <sup>bxy</sup> ± 1.128	10.57 <sup>abxyz</sup> ± 0.70	10.37 <sup>abyz</sup> ± 0.27	9.53 <sup>axy</sup> ± 0.23	0.063
60	10.43 <sup>bwx</sup> ± 0.88	8.63 <sup>abvwx</sup> ± 0.73	8.86 <sup>abwx</sup> ± 0.50	8.01 <sup>awx</sup> ± 0.64	0.136
75	8.19 <sup>bvw</sup> ± 0.62	6.81 <sup>abvw</sup> ± 0.51	7.72 <sup>abvw</sup> ± 0.47	6.04 <sup>av</sup> ± 0.68	0.079
90	7.69 <sup>avw</sup> ± 0.58	6.11 <sup>av</sup> ± 1.09	6.61 <sup>av</sup> ± 0.52	6.03 <sup>av</sup> ± 0.41	0.345
Mean ± se	11.27 <sup>bwxy</sup> ± 0.93	9.51 <sup>abwxy</sup> ± 0.81	9.48 <sup>abxy</sup> ± 0.14	8.68 <sup>ax</sup> ± 0.18	0.777
P value	<0.001	<0.001	<0.001	<0.001	

The values are Mean ± SE of six observations on five animals in each. Values with different superscripts a, b, c and u, v, w, x, y, z differ significantly ( $P < 0.05$ ) in a row and column respectively.

### Figure legends



**Fig 1:** Mean plasma IgG (mg/ml) during different fortnights of experimental period in buffaloes



**Fig 2:** Mean plasma cortisol (ng/ml) during different fortnights of experimental period in buffaloes

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