

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.908.261>

Effect of Certain Plasma Hormones, Metabolites, Milk α -lactalbumin and Lactoferrin on the Persistency of Lactation in Sahiwal Cattle

S. M. Mishra^{1*}, A. K. Roy¹, Singh Mahendra¹ and I. D. Gupta²

¹Animal Physiology Division, ²Animal Genetics & Breeding Division, ICAR-National Dairy Research Institute, Karnal-132001, India

*Corresponding author

ABSTRACT

Keywords

Hormones,
Metabolites, Milk
 α -LA and
lactoferrin,
Persistency,
Sahiwal cattle

Article Info

Accepted:
20 July 2020
Available Online:
10 August 2020

A study was conducted on twelve apparently healthy Sahiwal cows selected from National Dairy Research institute's livestock herd. The cows were divided into two groups i.e. persistent and truncated based on their records of milking. The blood and milk samples were collected on 15, 30, 45, 60, 75, 90, 105, 120 days postpartum. Plasma glucose concentration was higher ($P<0.05$) in truncated than persistent cows on 60th and 120th days postpartum as well as between the days within same group. Plasma NEFA concentration was significantly higher ($P<0.05$) in truncated than persistent cows as well as between the days. Plasma cortisol was significantly higher ($P<0.05$) in truncated than Persistent cows. Plasma cortisol also varied ($P<0.05$) between the periods. Plasma GH was higher ($P<0.05$) in persistent than truncated cows but did not vary between periods. Plasma Oxytocin concentration did not vary significantly between animals as well as periods. Milk α -LA was higher ($P<0.05$) in persistent than truncated cows. Milk α -LA did not vary significantly between periods. Milk lactoferrin was higher ($P<0.05$) in persistent than truncated cows and also varied significantly between periods ($P<0.05$). It is concluded that the hormones such as GH and cortisol may have an effect on the persistency of lactation. Plasma metabolites like glucose, NEFA and milk proteins such as α -LA and lactoferrin could also be associated with the persistency of lactation which is not merely a function of livestock management.

Introduction

India has achieved the top-most position in milk production mainly due to the largest number of bovines. The estimated milk production in the country is 187.7 MT (Anonymous. 2019). However, milk production per animal per day is very low especially in our local cattle breeds. It is estimated that 80% of our cattle population is

non-descript. The Sahiwal breed is utilized widely for the improvement of local stock or for initial crossbreeding of indigenous stock with European breeds in many hot-humid countries of the world because of its well-known resistance to tropical diseases, endurance to hot climate of tropics, low cost of maintenance and higher percentage of milk constituents. The term persistency of lactation is used to refer to the degree with which the

rate of milk secretion is maintained as lactation advances. The persistency of lactation is strongly influenced by the rate of apoptosis in the lactating glands (Gaines, 1987). Mammary apoptosis has been detected during lactation, in mammary tissue of lactating mice, goats and cattle (Stefanon *et al.*, 2002). During lactation only 0.3% of mammary cells proliferate in a 24-h period (Capuco *et al.*, 2003). Yet this proliferative rate is sufficient to replace most mammary epithelial cells by the end of lactation. The pattern of the lactation curve is influenced by the number of secretory cells in the mammary gland and the synthetic activity of each secretory cell. Growth and differentiation of the glandular epithelium during puberty and pregnancy are important determinants of the total area of secretory epithelium and consequently of milk yield (Robinson *et al.*, 1995). As lactation progresses the secretory cells gradually regress from a state of active synthesis and secretion to a non-secretory state through a process called "involution". The gradual involution starts around peak lactation and continues up to the time when the animals are dried off (Cannas *et al.*, 2002). Maintenance of milk synthesis and secretion persistency is controlled by both systemic and local regulatory factors.

Effect of plasma hormones on persistency of lactation

The systemic factors involve hormones such as prolactin and growth hormone (GH). As lactation progresses the level of growth hormone and prolactin decrease causing a reduction of milk synthesis (Barber *et al.*, 1992). The influence of these hormones in reducing involution is mediated by the insulin-like growth factor-1, a hormone that is known to increase milk yield (Flint *et al.*, 2000). Plasmin is the predominant protease in milk. In ruminants the effects of high doses of glucocorticoid are inhibitory but the effects of

lower doses are ambiguous (Sjaastad *et al.*, 2010). Cortisol is the main glucocorticoid in cattle and it is indicative of chronic stress in animals (Mostl & Palme, 2002). The reduction in milk production due to dehydration stress or glucocorticoid was correlated with the activities of plasmin and channel blocking activity in the milk of the cows (Silanikove *et al.*, 2000). Growth hormone (GH), plays a key role in the control of postpartum development and growth in mammals (Butler and Le Roith, 2001). GH has an important role in maintaining the lactation. As lactation progresses the levels of GH decrease causing a reduction of milk synthesis (Akers, 2002). The influence of GH in reducing involution is mediated by the insulin-like growth factor-1 (Tonner *et al.*, 2000), a hormone which increases milk yield. The GH whose receptors are not present in the mammary gland exerts its positive effects on milk yield indirectly by stimulating IGF-I synthesis. The gluconeogenesis is highly prioritized by the cow and is under hormonal regulation mainly by insulin, glucagon and growth hormone (Aschenbach *et al.*, 2010). GH plays important role in this process, inhibiting lipid storage and increasing blood flow to the mammary gland (McMahon *et al.*, 2001). Oxytocin influenced both the protein secretion process and the transfer of fat globules on the apical part of the epithelial cells. Prolactin is released at milking along with adrenocorticotrophic hormone (ACTH) and oxytocin (Gorewit, 1988). Oxytocin does not increase milk production, but it will stimulate contraction leading to milk ejection (Papich, 2015).

Effect of Blood metabolites and milk proteins on persistency of lactation

Mammary gland requires approximately 80% of the glucose supply to support the high milk production (Drackley *et al.*, 2001). Glucose uptake by the mammary gland is essential for

milk lactose synthesis (Rigout *et al.*, 2003; Lemosquet *et al.*, 2004). Glucose concentration sharply increases by 1 day prior to calving reflecting hormonal change that promotes gluconeogenesis and glycogenolysis (Knight *et al.*, 2004). Blood NEFA concentrations are strong indicator of disease, reproductive performance and milk production than blood BHBA concentration (Huzzey *et al.*, 2011). Cows in severe negative energy balance have a higher concentration of NEFA and a plasma concentration of > 600 $\mu\text{mol/L}$ indicates severe negative energy balance. The NEFA are utilized for about 40% of milk fat during the first days of lactation (Adewuyi, 2005). Lactose synthetase is composed of galactosyl-transferase and the milk protein α -Lactalbumin (Gradinaru, 2015). Folding variants of α -lactalbumin (α -LA) are known to induce cell death in a number of cell types, including mammary epithelial cells (MEC). Bovine α -LA has an important role in involution and/or maintaining the luminal space in mammary alveoli during lactation. It could be possible to enhance milk protein expression and/or improve lactation persistency by influencing the balance between proliferation and apoptosis of BMEC (Riley *et al.*, 2008). Presence of α -LA is indicator of responsiveness of mammary gland to the lactogenic hormonal complex (Husveth, 2011). Prolactin induces the secretion of α -lactalbumin, which helps in maintaining persistency of lactation. Lactoferrin has a variety of functions viz. antibacterial, antiviral, antifungal, antiparasitic, anti-inflammatory, anticarcinogenic and antitumor activities (Tomita *et al.*, 2009). Lactoferrin may also serve as a modulator of immune and inflammatory responses. Although bovine colostrum contains up to 5 mg/ml lactoferrin, levels drop very rapidly as lactation proceeds, so that mature bovine milk normally contains only 20-200 $\mu\text{g/ml}$ lactoferrin. The direct

effect of LTF on persistency of lactation is not yet identified but its effect on cell differentiation, proliferation and its cytokine like activities with respect to immun-epithelial cells are well documented.

Materials and Methods

The geographical location of NDRI livestock farm is at an altitude of 250 meters above the mean sea level (MSL) in the Indo-gangetic alluvial plains on 29042' N latitude and 72002' E longitude. The climate of the farm is subtropical in nature. The minimum temperature falls to 20C in winter months, whereas the maximum temperature goes up to 45C in summer. The annual rainfall is about 760 to 960 mm out of which most of the rainfall is received during the months of July and August. The relative humidity ranges from 41 to 85%. The experiment was performed in compliance with the regulations of the Institutional Animal Ethics Committee under the approved research program. Sahiwal cows (n=12) at 15 days postpartum were selected from the herd of National Dairy Research Institute, Karnal and divided into 2 groups of 6 animals each. Six cows were kept as control on the basis of excellent persistency of their milk yield and six test animals chosen showing truncation in their milk production or prior history of truncation after attending peak milk yield. The plasma samples were collected at fortnightly intervals starting 15 days after parturition i.e. on 15, 30, 45, 60, 75, 90, and 105 days from all cows for analysis of blood metabolites (Glucose, NEFA) and hormones (Cortisol, GH, Oxytocin). Milk samples were also collected fortnightly starting 15 days postpartum i.e. on 15, 30, 45, 60, 75, 90, and 105 days from all cows for the estimation of whey proteins (α -LA and Lactoferrin). Blood samples were drawn in sterile heparinized tubes from jugular vein puncture posing minimum disturbances at 15 days interval during

experimental period. Blood (8 ml) was collected on days 15, 30, 45, 60, 75, 90, 105 with respect to the day of parturition from each animal. Samples were brought to the laboratory in chilled ice boxes after collection and centrifuged at 1200*g at 4⁰C for 20 minutes to separate plasma which was stored at -20⁰C until analyzed. Milk samples were collected once in a day during evening (6.00 pm). The milk fat was separated by centrifugation at 5⁰C at 3800 R.P.M. for 20 minutes. The skimmed milk was stored at -20⁰C for whey protein estimation. Milkα-Lactalbumin, lactoferrin and NEFA were determined by ELISA kits from SunRed Biotechnological Company whereas Glucose was estimated in plasma samples by GOD-PAP TRINDER's kit from Recombigen Pvt. Ltd., China. Oxytocin, GH and Cortisol was determined in blood plasma by ELISA kits from Bioassay Technology Laboratory, China.

Results and Discussion

Plasma glucose

Glucose is used by mammary gland cells to form lactose (Mcmanaman & Neville, 2003). The blood glucose concentration during different periods varied from 63.85±5.7 to 77.02±3.45 mg/dl and 57.92±4.39 to 76.65±5.14 mg/dl in persistent and truncated cows respectively (Table-1). The result shows significant difference (P<0.05) between persistent and truncated cows in glucose levels on 60th day and 120th day postpartum (Fig.-1). Mammary gland requires approximately 80% of the glucose supply to support the high milk production (Drackley *et al.*, 2001). Glucose uptake by the mammary gland is essential for milk synthesis (Rigout *et al.*, 2003; Lemosquet *et al.*, 2004). Glucose concentration is sharply increased by 1 day prior to calving reflecting hormonal change that promotes gluconeogenesis and

glycogenolysis (Knight *et al.*, 2004). The significant difference of glucose may be due to less utilization of glucose by mammary gland to synthesize lactose. It could also be attributed to certain genetic factors. Insulin stimulates glucose uptake via phosphatidylinositide 3-kinase-linked signaling pathway in bovine mammary epithelial cells (Zhao *et al.*, 2014).

Plasma NEFA

The plasma NEFA concentration during different periods varied from 223.20±9.51 to 401.14±8.13 μM/L and 265.67±5.04 to 427.33±8.90 μM/L in persistent and truncated cows respectively (Table-1). The result shows significant difference (P<0.05) between persistent and truncated cows in NEFA levels on 15th, 30th, 45th, 60th, 75th, 90th and 105th day postpartum (Fig.-2). It shows the negative energy balance in truncated Sahiwal cows as the level of NEFA are elevated in comparison to the persistent Sahiwal cows on the same days. The significant higher levels of NEFA in truncated Sahiwal cows indicate effective mobilization of adipose tissue in truncated lactation group (Drackley, 1999). Cows in negative energy balance mobilize more body fat and produce glycerol for energy which leads to increased NEFA concentrations in blood (DeVries and Veerkamp, 2000). Plasma NEFA is an important attribute to help evaluate energy balance of the cow because they are elevated in energetic stress (Burke and Roche, 2007) and stored fat is mobilized (Holcomb *et al.*, 2001).

Plasma cortisol

The plasma cortisol concentration during different periods varied from 2.42 ± 0.08 to 6.32 ± 0.18 ng/mL and 2.83 ± 0.15 to 7.02 ± 0.12 ng/mL in persistent and truncated cows respectively (Table-2). The result shows significant difference (P<0.05) between

persistent and truncated cows in cortisol levels on 15th, 30th, 45th, 60th, 75th and 105th day postpartum (Fig.-3). Cortisol is indicative of chronic stress in animals (Mostl & Palme, 2002). The stress is often linked with plasminogen-plasmin system (PPS) which is an enzymatic mechanism in milk, leading to the breakdown of the major milk protein casein.

The reduction in milk production due to dehydration stress or glucocorticoid was correlated with the activities of plasmin and channel blocking activity in the milk of the cows (Silanikove *et al.*, 2000). Some workers indicate that a change in mammary cell number is due to stress imposed imbalance between cell proliferation and cell removal and a principal cause of declining milk production (Stefanon *et al.*, 2002).

Cortisol has got a major function in enhancing the action of PRL in stimulating differentiation of the epithelium and milk protein gene expression in the mammary gland during lactogenesis (Akers, 2002).

Plasma GH

The plasma GH concentration during different periods varied from 1.84 ± 0.06 to 2.18 ± 0.06 ng/mL and 1.58 ± 0.10 to 2.11 ± 0.06 ng/mL in persistent and truncated cows respectively (Table 2). The result shows significant difference ($P < 0.05$) between persistent and truncated cows in GH levels on 30th, 45th, 60th and 90th day postpartum (Fig.-4). The significant role of GH on persistency of lactation in Sahiwal cows may be due to lactopoietic effect of GH through IGF-1 (Purup *et al.*, 1993) which acts directly on the mammary gland. GH was associated with an increase in mammary cell proliferation in cows (Capuco *et al.*, 2001) Although the action of GH is mediated mainly through the IGF axis, there is evidence that GH may also act without IGF-I to stimulate milk production (Hadsell *et al.*, 2008). The influence of GH in reducing involution is mediated by the insulin-like growth factor-I (Tonner *et al.*, 2000). The GH whose receptors are not present in the mammary gland exerts its positive effects on milk yield indirectly by stimulating IGF-I synthesis.

Table.1 Mean values of plasma metabolites in Persistent and Truncated cows

Group	Glucose (mg/dl)							
	Postpartum							
	15	30	45	60	75	90	105	120
Persistent	70.45 ^{ab} ±4.45	77.02 ^b ±3.45	68.17 ^a ±4.09	64.52 ^{aX} ±3.07	63.85 ^a ±5.7	71.41 ^{ab} ±3.02	75.43 ^b ±2.22	75.26 ^b ±1.86
Truncated	67.46 ^b ±1.49	70.17 ^{bc} ±3.69	70.18 ^{bc} ±1.03	73.09 ^{bcY} ±4.43	57.92 ^a ±4.39	71.95 ^{bc} ±3.49	76.65 ^b ±5.14	74.32 ^c ±1.75
	NEFA (µmol/L)							
Persistent	401.14 ^{eX} ±8.13	321.28 ^{dX} ±6.30	286.34 ^{cX} ±6.36	264.45 ^{bX} ±6.62	255.5 ^{bX} ±9.98	239.12 ^{aX} ±4.29	223.20 ^{aX} ±9.51	
Truncated	427.33 ^{fY} ±8.90	348.83 ^{eY} ±6.18	318.33 ^{dY} ±6.62	301.50 ^{cY} ±9.15	290.17 ^{bcY} ±6.03	280.18 ^{bY} ±6.03	265.67 ^{aY} ±5.04	
Values with different superscript ^{a,b,c,d,e} and ^{X,Y} in a row and column respectively differ significantly ($P < 0.05$).								

Table.2 Mean values of plasma hormones in persistent and truncated cows

Group	Cortisol (ng/ml)						
	Postpartum						
	15	30	45	60	75	90	105
Persistent	6.32 ^{fX} ±0.18	5.86 ^{eX} ±0.12	5.14 ^{dX} ±0.14	4.21 ^{cX} ±0.11	3.76 ^{bX} ±0.31	2.86 ^a ±0.10	2.42 ^{aX} ±0.08
Truncated	7.02 ^{fY} ±0.12	6.65 ^{eY} ±0.18	6.09 ^{dY} ±0.23	4.96 ^{cY} ±0.09	4.46 ^{bY} ±0.09	3.18 ^a ±0.17	2.83 ^{aY} ±0.15
Group	Growth Hormone (ng/ml)						
	Postpartum						
	15	30	45	60	75	90	105
Persistent	1.92 ^a ±0.09	1.89 ^{aX} ±0.09	1.84 ^{aX} ±0.06	1.88 ^{aX} ±0.03	1.99 ^a ±0.07	2.10 ^{bX} ±0.01	2.18 ^b ±0.06
Truncated	1.78 ^b ±0.06	1.66 ^{abY} ±0.09	1.58 ^{aY} ±0.10	1.79 ^{bY} ±0.04	1.87 ^{bc} ±0.05	2.02 ^{cdY} ±0.04	2.11 ^d ±0.06
Group	Oxytocin (pmol/L)						
	Postpartum						
	15	30	45	60	75	90	105
Persistent	17.06 ±4.24	17.01 ±4.22	18.31 ±4.09	16.05 ±4.97	17.41 ±3.74	16.07 ±3.60	15.57 ±4.78
Truncated	17.21 ±4.27	19.79 ±4.84	17.59 ±4.10	18.90 ±4.90	20.17 ±4.58	16.88 ±4.20	15.93 ±4.86

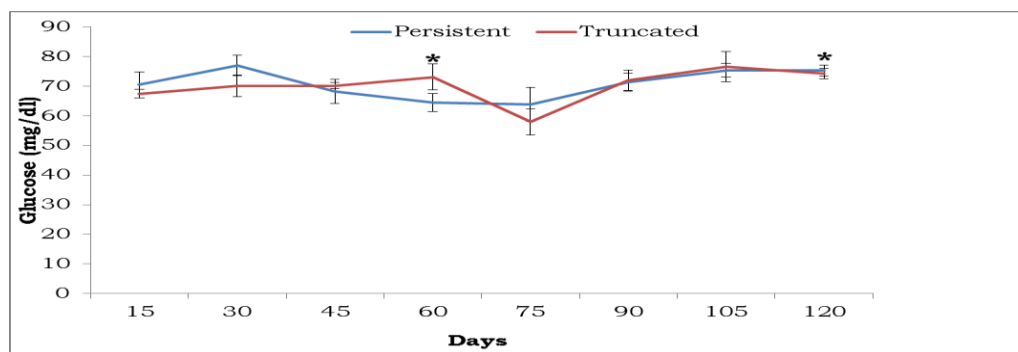
Values with different superscript ^{a,b,c,d,e,f} and ^{X,Y} in a row and column differ significantly (P<0.05)

Table.3 Mean values of milk proteins in persistent and truncated cows

Group	α -Lactalbumin (μ g/dl)							
	Postpartum							
	15	30	45	60	75	90	105	120
Persistent	1.48 ^{cX} ±0.007	1.43 ^{cX} ±0.01	1.37 ^{bX} ±0.03	1.34 ^{bX} ±0.01	1.28 ^{aX} ±0.02	1.24 ^{aX} ±0.01	1.22 ^{aX} ±0.01	1.21 ^{aX} ±0.006
Truncated	0.96 ^Y ±0.06	0.91 ^Y ±0.05	0.86 ^Y ±0.03	0.88 ^Y ±0.008	0.86 ^Y ±0.01	0.82 ^Y ±0.02	0.82 ^Y ±0.02	0.88 ^Y ±0.02
Group	Lactoferrin(μ g/ml)							
	Postpartum							
	15	30	45	60	75	90	105	120
Persistent	51 ^{cX} ±3	47 ^{bcX} ±4	44 ^{bX} ±3	41 ^{abX} ±4	36 ^{aX} ±4	35 ^{aX} ±4	37 ^{aX} ±4	37 ^{aX} ±6
Truncated	37 ^{cY} ±1	29 ^{bcY} ±1	25 ^{bcY} ±0.8	23 ^{bY} ±0.8	19 ^{abY} ±5	22 ^{bY} ±3	21 ^{bY} ±3	13 ^{aY} ±2

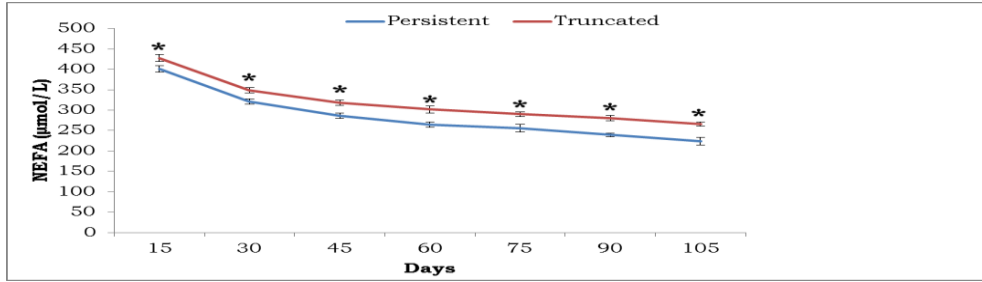
Values with different superscript ^{a,b,c} and ^{X,Y} in a row and column differ significantly (P<0.05).

Fig.1 Plasma glucose profile in experimental Sahiwal cows



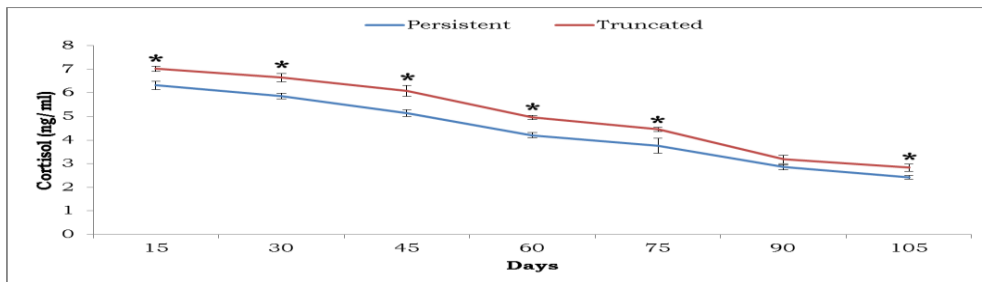
* indicates the significant difference (p<0.05) within same day between the groups

Fig.2 Plasma NEFA profile in experimental Sahiwal cows



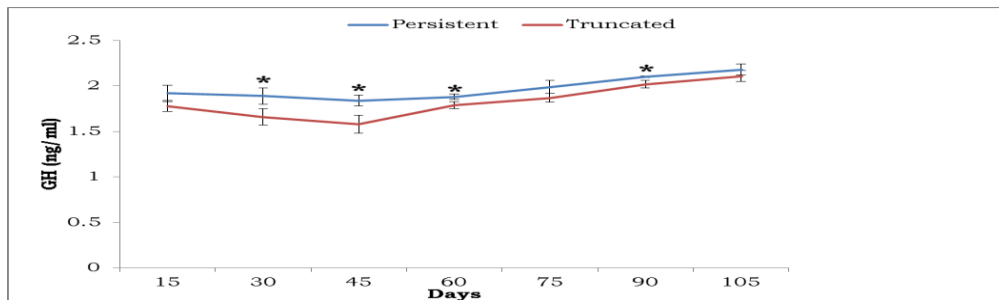
* indicates the significant difference ($p<0.05$) within same day between the groups

Fig.3 Plasma cortisol profile in experimental Sahiwal cows



* indicates the significant difference ($p<0.05$) within same day between the groups

Fig.4 Plasma GH profile in Experimental Sahiwal Cows



* indicates the significant difference ($p<0.05$) within same day between the groups

Fig.5 Plasma oxytocin profile in experimental Sahiwal cows

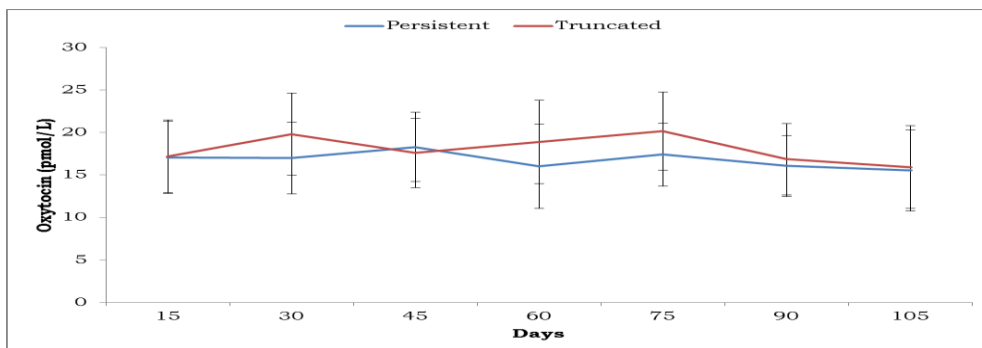
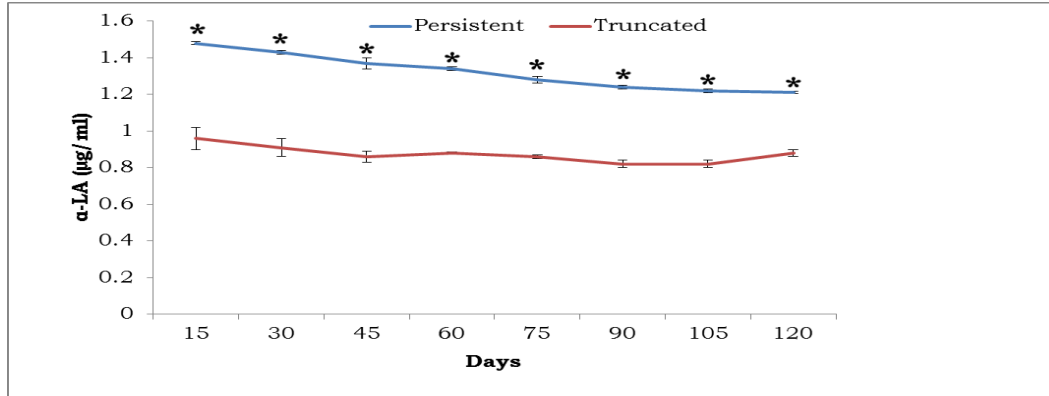
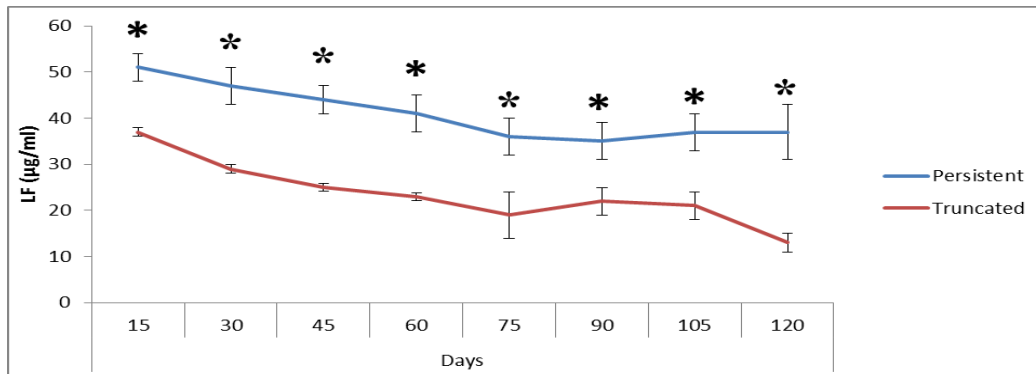


Fig.6 Milk α -LA profile in experimental Sahiwal cows



* indicates the significant difference ($p < 0.05$) within same day between the groups

Fig.7 Milk LTF profile in Experimental Sahiwal Cows



* indicates the significant difference ($p < 0.05$) within same day between the groups

Plasma Oxytocin

The plasma oxytocin concentration during different periods varied from 15.57 ± 4.78 to 18.31 ± 4.09 pM/L and 15.93 ± 4.86 to 20.17 ± 4.58 pM/L in persistent and truncated cows respectively (Table-2). The result shows non-significant difference ($P < 0.05$) between persistent and truncated cows in oxytocin levels (Fig.-5). Exogenous oxytocin was associated with increased milk production of both dairy cows and sheep (Lollivier & Marnet, 2005 and Zamiri *et al.*, 2001). Oxytocin might act on the mammary epithelial cells (Lollivier *et al.*, 2002) to influence both the protein secretion process and the transfer of fat globules on the apical part of the epithelial cells. The present study

shows no direct significant role on persistency of Sahiwal cows since its role is mainly prevalent as milk ejecting hormone which is in agreement with Papich, (2015). However, milk ejection is very important for regulating persistency of lactation in directly.

α -Lactalbumin

The α -LA concentration during different period varied from 1.21 ± 0.006 to 1.48 ± 0.007 $\mu\text{g/mL}$ and 0.82 ± 0.02 to 0.96 ± 0.06 $\mu\text{g/mL}$ in persistent and truncated cows respectively (Table-3). The result shows significant difference ($P < 0.05$) between persistent and truncated cows in α -LA levels on 15th, 30th, 45th, 60th, 90th, 105th and 120th day postpartum (Fig. 6). Presence of α -LA is

indicator of responsiveness of mammary gland to the lactogenic hormonal complex (Husveth, 2011) which is responsible for the differentiation of the mammary secretory epithelium. The development in lactogenesis of the rough as well as smooth endoplasmic reticulum, and the Golgi apparatus results in mammary synthesis of proteins, fat and lactose, respectively. α -LA being the important whey protein is also dependent on prolactin. Prolactin directly stimulates the transcription of casein and other protein genes. Prolactin induces the secretion of α -lactalbumin, an enzyme moiety of lactose synthase which helps in maintaining the persistency of lactation. α -Lactalbumin has been found to increase in response to addition of bovine prolactin (Akers and Denbow, 2008). α -LA also has role in proper involution of mammary gland without which the new cell formation can't take place properly. Bovine α -LA reduced the viability of primary bovine mammary epithelial cells (BMEC) and induced caspase activity in mammospheres which leads to proper involution (Riley *et al.*, 2008). Hence, less α -LA in truncated cows may be due to decreased response of mammary gland to prolactin and reduced lactose synthase activity which leads to less lactose formation and seized milk production.

Lactoferrin

LTF concentration during different periods varied from 0.35 ± 0.04 to 0.51 ± 0.03 $\mu\text{g/mL}$ and 0.13 ± 0.02 to 0.37 ± 0.11 $\mu\text{g/mL}$ in persistent and truncated cows respectively (Table-3). The result shows significant difference ($P < 0.05$) between persistent and truncated cows in LTF levels on 30th, 45th, 60th, 90th, 105th and 120th day postpartum (Fig.-7). Lactoferrin has antibacterial activity which is affected through the sequestration of iron necessary for microbial growth. Lactoferrin may also serve as a modulator of immune and inflammatory responses

(Legrand, 2005). Lactoferrin enhanced cell proliferation and the stimulatory effect of lactoferrin on proliferation depended on the degree of iron saturation (Bi *et al.*, 1997). LTF is able to influence cutaneous immune and inflammatory processes secondary to regulation of the production of TNF- α and possibly other cytokines (Kimber *et al.*, 2002). The significant difference shown in the level of LTF in milk of truncated Sahiwal cows may point towards the impaired prolactin mechanism. Prolactin is essential hormone when it comes to lactation. Hence, the less LTF in truncated cows may be due to less PRL stimulated LF synthesis in BMECs.

In conclusion the plasma NEFA and cortisol levels were significantly higher in the truncated while plasma GH, milk α -lactalbumin and lactoferrin were higher in the persistent cows. These findings could be utilized to address the problems of truncated lactation in Sahiwal cows.

Acknowledgement

The authors are grateful to Director, NDRI Karnal for providing necessary facilities to carry out the present research work.

Data availability statement

All the data of this study were represented in the figures and table. However, the raw data of ELISA results will be shared on request basis.

Conflict of interest

The authors declare no competing or financial interests.

Animal Welfare Statement

"The authors confirm that the ethical policies of the journal, as noted on the journal's author

guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes."

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How to cite this article:

Mishra, S. M., A. K. Roy, Singh Mahendra and Gupta, I. D. 2020. Effect of Certain Plasma Hormones, Metabolites, Milk α -lactalbumin and Lactoferrin on the Persistency of Lactation in Sahiwal Cattle. *Int.J.Curr.Microbiol.App.Sci*. 9(08): 2279-2290.
doi: <https://doi.org/10.20546/ijcmas.2020.908.261>