RESIDUAL ANALYSIS OF DEXAMETHASONE AND ITS EFFECT ON GOAT MILK

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| **Abstract** |  |  |  |

The aim of this study was to analyse the effect of intramuscular administration of dexamethasone on clinical, residual and milk composition parameters in goat. The dose of DXM 0.5 mg/kg BW once daily for 3 consecutive days. Clinical parameters (pulse rate, respiratory rate and rectal temperature) and milk samples for residual analysis of DXM and its effect on goat milk. Milk Sample were collected at 2, 8, 16, 32, 48, 72, 96, 120, 144, 168 hr, A significant (P˂0.05) increase in pulse rate and respiratory rate were noticed at 2, 8, 16, 32, 48 and 72 and 96 hr. and the rectal temperature was increased (P<0.05) only at 02 hr post drug administration. The highest residual level of dexamethasone was found at 32 hr (2.70 ng/ml) and lowest at 168 hr (0.25 ng/ml). Milk Fat % significantly increased (P˂0.01) at 32, 48 and 72 hr and (P<0.05) at 2, 8, 16, 96,120 hr and then gradually returned to pre-treatment value by 144 hr. The mean of milk protein concentration was increased (P˂0.01) at 8, 16, 32, 48, 72 and 120 hr and (P<0.05) at 2 and 96 hr. Milk Solid Not Fat level was increased (P<0.05) at 16, 32, 48, 72 and 96 hr, however, at 120 hr this increase was significant at (P<0.01). Milk yields non-significantly decreased from 2 – 16 hr as compared to control then, decreased (P<0.05) at 32, 48, 72, 96, 120, 144 hr following DXM exposure subsequently. It was concluded that, the therapeutic dose of dexamethasone 0.5 mg/kg BW once daily for 3 consecutive days produced significant effects on clinical, residual level and milk composition parameters in goat.

**Keyword:** Dexamethasone, goat milk, Residual analysis

**Introduction**

Milk is the secretion of the mammary gland and it is the characteristics of all mammals. It is an important food for young ones because it provides nutrients like minerals and vitamins in an appropriate amount (Roadhouse and Henderson, 2015).The milk of all animals contains the same kind of ingredients but with varying degrees of its composition. Within a particular species, genetic factors and environmental conditions such as climate and stage of lactation may also affect the composition of milk. However, effect of DXM on milk composition is remain incomplete understood.

Corticosteroids are a big group of naturally occurring and synthetic chemical compounds used in veterinary and human medicine. There are two types of corticosteroids: mineralocorticoids characterized by their effects on fluid and electrolyte balance; and glucocorticoids together with immunosuppressive and anti-inflammatory effects and it is also important for the carbohydrate, lipid metabolism, regulation of blood pressure and maintenance of muscle tone and bone density (Kufe et al., 2013).

DXM has a long history in veterinary medicine to treat a range of metabolic diseases and inflammatory disorders in domestic animals. It is commonly used as a therapeutic agent to treat chronic inflammatory, allergic and autoimmune diseases i-e: leukemia’s, lymphomas and to prevent allograft rejection following transplantation (Ito *et al.,* 2016). It is regularly used in high doses in emergency allergic reactions, spinal cord trauma or shock and treatment of immune disorders mediated such as immune-mediated thrombocytopenia or hemolytic anemia, acetonaemia and some types of cancer and allergic diseases such as chronic obstructive pulmonary disease (COPD), asthma, hives, itching, skin disease and some neurological diseases. It may also help prevent hypersensitivity reactions associated with certain medications. It is beneficial to use with other medications which often causes nausea and vomiting (Sousa, 2015). DXM clinical success, its use may result in drug residues in dairy and other products of animal origin aimed for human consumption. There is an indication that long-term use of low concentrations of DXM may have adverse effects on public health (Becker, 2011; Reig *et al.,* 2016).

DXM residues has been reported in the various biological samples such as urine, faces, meat, liver in different animals (Chen *et al.,* 2011; Cherlet *et al.,* 2014). Importantly, their therapeutic use is also limited by establishing maximum residue limits (MRLs) in milk and edible tissues. The MRLs of DXM in milk samples are 0.3 μg/kg−1. DXM residues may also affect milk composition in various species (Macrina *et al.,* 2014; Thanasak *et al.,* 2015) .Hence, it is necessary to develop comprehensive control measures to monitor DXM residues in goat milk because goat milk is commonly consumed by children.

Number of studies have also been conducted to determine DXM residues and its effects on the milk in different animal species. To the best of our knowledge, such studies are limited in goats especially in our local breeds so far. Therefore, it was indispensable to find out the residual profile of DXM and its effects on the composition of milk in our local goat breeds.

**Materials and Methods**

**Experimental Protocol**

In this study, six lactating healthy goats of mix breeds were used. The goats were kept indoor at Livestock Experimental Station, Sindh Agriculture University Tandojam. The animals were acclimatized for three weeks. All animals were dewormed and vaccinated during the experimental period. All animals were clearly identified by the use of an ear tag assigned a number from G1 to G6. Dexamethasone (DXM) (Trade name: Dexafar, Farvet Pharmaceutical company) was administered intramuscularly to evaluate its residual profile and its effect on goat milk composition.

**Experimental Procedure**

Milk samples were collected before and after drug administration. DXM was administered at a therapeutic dose of 0.5 mg/kg body weight intramuscularly (I.M) for 03 consecutive days to six healthy lactating dairy goats (based on high yield lactating animals) with an interval of 24 hrs. Then, milk samples were collected at 2h, 8h, 16h, 32h, 48h, 72h, 96h, 120h, 144h and 168hour post drug administration. Milk samples were collected in two set of test tubes, one set containing 05 ml for residual analysis which was kept at -35 oC until analysis and another set of test tube having 100 ml for milk composition which was analyzed immediately.

The effect of DXM on milk composition and residual analysis were investigated at the Department of Animal Products Technology, Sindh Agriculture University, Tandojam.

**Following parameters were recorded**

**Clinical parameters**

Pulse rate (PR), Respiratory rate (RR) and rectal temperature (RT) recorded before administration of DXM and after 2, 8, 16, 32, 48, 72, 96, 120, 144 and 168 hrs post dosage regimen respectively.

1. **Analysis of Milk**

**Fat Content**

For the determination of fat content, Gerber method was used as described by Kleyn *et al.* (2001). Briefly, 11 ml milk sample was mixed with 10 ml of 90% Sulfuric Acid and 01 ml Amyl Alcohol in Butyrometer and then closed with a rubber cork. The Butyrometer was placed in a Gerber machine and centrifuged for 05 minutes at 1100 rpm. The percentage of fat was identified on the scale of butyrometer.

**Protein content**

The determination of protein content was carried out by following protocol described by Barbano et al. (1999). Briefly, a 05ml milk sample was used in Micro-Kjeldahl digester in the existence of catalyst 0.2 g CuSO4 and 02 g Sodium/Potassium Sulphate where 30 ml H2SO4 was used. The digested sample was diluted by adding 250 ml distilled water. Subsequently, 05 ml diluted sample was taken and distilled with 40% of Sodium hydroxide using Micro-Kjeldahl distillation unit where steam was distilled in 05 ml of 2% H3BO3 (Boric Acid) containing an indicator for 03 minutes. The Ammonia trapped in H3BO3and was determined by titrating with 0.1 HCl. The Nitrogen Percentage was analyzed using the formula written as under:

Where;

V1=Value of titrated milk sample

V2= Value of titrated blank sample

Protein content was evaluated by modifying nitrogen percentage to protein, believing that, all nitrogen was available in milk as a protein i.e., protein percentage = N% × conversion factor. Conversion factor = 100/N% in milk products (i.e., 15.66).

**Lactose content**

Milk Lactose determination was done through difference method using the following formula:

Lactose% = TS% - (Fat% + Protein% + Ash %)

**Solid Not Fat content (SNF)**

Determination of SNF content in milk was performed by difference method using following formula:

Solid Not Fat%= TS% - Fat%

**Residual detection:**

The residues of DXM in goat’s milk were determined using Direct Competitive ELISA (AgraQuant® COKDA0800) according to manufacturer’s instructions.

**Statistical Analyses**

Statistical analysis was performed using a computer program, Student Edition of Statistic (SXW), Version 8.1 (Copyright 2005, Analytical software-USA). The data was more analyzed by linear models, where analysis of variance with three- way ANOVA was done in the case of significant difference existed; the means were additional computed applying least significant difference (LSD) test at 5% probability level.

**Results**

This study was aimed to observe various effects associated with the short-term administration of dexamethasone (DXM) in goat species.

**Clinical parameters**

**Heat beat, Respiratory rate, Rectal Temperature**

The pulse rate was significantly increased (P<0.05) at various time-points from 8 - 96 hr as compared to control. However, pulse rate significantly decreased up to 168 hr as compared to control.The respiratory rate was significantly increased (P˂0.05) at 2 hr and at 8, 16, 32, 48, 72 and 96 hr (P<0.01) post-DXM administration. The maximum increase of respiratory rate was found at 16 hr after treatment. Afterward, the respiratory rate gradually returned to control value by 168 hr. The rectal temperature was significantly increased (P<0.05) at 02 hr post-treatment, however, this increase was non-significant in subsequent hrs compared with control value. All parameters are showed in (Figure-01).

**Figure-1. Mean values of Clinical Parameters of goats (n=6) obtained after I/M administration of dexamethasone.**

**Dexamethasone Residues in Milk**

Administration of DEX increased (P<0.01) its residual level at 02, 08, 16, 32, 48, 72, 96, 120 and 144 hr. However, the statistical analysis showed non-significant increased value at 168 hr. The highest and lowest mean values of DEX residues in goat milk were found at 32 and 168 hr following DEX administration respectively (Figure-2).

**Figure-2. Residue analysis of intramuscularly administered dexamethasone at the therapeutic dose of 0.5mg/kg for 3 consecutive days in goat (n=6) milk.**

**Effect of dexamethasone on milk**

1. **Milk Fat, Milk protein, Milk Sugar (Lactose), SNF**

Figure no 3 shows the result of milk fat, milk protein, milk sugar, milk SNF, figure indicated the, the means of pre-treatment value of milk fat was 3.68%. Administration of DXM for three days increased (P˂0.05) in milk fat content at 2, 8, 16, 96 and 120 hrs as compared to control value, whereas, highly significant increased (P<0.01) was found at 32, 48 and 72 hr post drug administration. The highest and lowest mean values of milk fat were found at 48 and 144 hr post drug administration respectively. The milk fat values at 144 and 168 hr were found to be statistically non-significant as compared to control. **Milk protein** DXM administration for 03 days caused a significant increase (P<0.05) at 2 and 96 hr, whereas, at 8, 16, 32, 48, 72 and 120 hr increased (P<0.01) in the concentration of milk protein. The highest and lowest mean values of DEX were found at 48 and 144 hr post drug administration respectively. At 144 and 168 hr, the values showed a non-significant increase in comparison with the control value. **Milk Sugar (Lactose),**The therapeutic dose of DXM for 03 days in goat showed the non-significant difference in the level of milk lactose at all designated time points of observations as compared to control. **SNF,** The administration of DXM for 03 days milk SNF level was increased (P<0.05) at 16, 32, 48, 72 and 96 hr, however, at 120 hr this increase was significant at (P<0.01). Then, the values at 144 and 168 hr gradually returned to pre-treatment level and were found non-significant as compared to control value. The highest and lowest mean values of DEX were found at 120 and 168 hr post drug administration respectively.

**Figure-03. Mean values of Milk analysis of goats (n=6) obtained after administration of I/M dexamethasone.**

1. **Milk Yield**

DXM-induced effects caused non-significant decrease in milk yield 2–16 hr (203.83±9.27), as compared to pretreatment observations, thereafter, a significantly decreased (P<0.05). However, milk yield returned to control value by 168 hr (206.67±9.60) in lactating goats (Figure-03).

**Figure-03. Mean values milk yield of goats (n=6) obtained after administration of I/M dexamethasone.**

**Discussion**

In the current study, short-term administration of DXM results observed a significant cardiac response in goat. DXM at therapeutic dose i.e. 0.5 mg/kg b.w. caused a significant increase in pulse rate. The pulse rate was increased (P<0.05) at 8 and 72 hr and (P<0.01) on 2, 16, 32 and 48 after administration of 3 consecutive days of the dosage regimen of DXM but this increased response gradually returned to normal up to 120 hr. It is reported that corticosteroids characterized their effects on CVS as a result of its effect on plasma volume, and balance of electrolyte, synthesis of adrenaline and angiotensin levels, all of which leading in maintaining normal blood pressure and cardiac output. Corticosteroids have effects on the heart muscle responses, permeability fluid and electrolyte balance concerned with proper carbohydrate, lipid metabolism, regulation of blood pressure, and bone density (Becker, 2011).The increase in pulse rate (cardiac output) as well as the blood pressure has also been reported by others with DXM treatment. Increase in pulse rate and blood pressure have been observed in infants (Fauser *et al.,* 2003). In this study, the respiratory rate was significantly increased (P<0.05) at 2 hr and (P<0.01) on 08, 16, 32, 48, 72 and 96 hr following dosage regimen of DXM. The maximum increase of respiration rate occurred at 16 hrs. Then, the respiration rate gradually returned to normal by 120 hr. This study is in the agreement with the findings reported by Durand *et al.* (2012); Ohlsson *et al.* (2012) in humans and animals. It is stated that glucocorticoids have a positive inotropic effect on the cardiopulmonary system. It stimulates heart muscle contraction and increases heart rate, this increase in heart rate and cardiac output increases the volume of blood flow (Washburn *et al.,* 2003). This increased blood flow with CO2 crosses blood brain and blood CSF barriers. CO2 combines with H2O to form H2CO3 that dissociates into HCO3 Ions and H+ ions. This H+ stimulates chemo-sensitive area (Carotid bodies at the bifurcation of common carotid arteries). It is reported in fetal sheep that the increased respiration and blood pressure in part is related to the glucocorticoid-induced increased pulmonary angiotensin conversion enzyme (ACE) (Zimmermann *et al.,* 2013). The rectal temperature was significantly increased (P<0.05) at 2 hr post-DXM treatment then, this increase was detected non-significant until 168 hr. Coelho *et al.* (2006); Yared *et al.* (2006) reported that DEX treatment, either before or after endotoxin injection markedly inhibits temperature as a result of increased plasma interleukin and prostaglandin. In contrast to this, the non-significant effects on present study probably show the effect of DXM Independent to temperature. It is possible that the mild increase in temperature in this study is presumably due to the inhibition or the release of many biologically active substances by DXM. In this study, it has been observed that DEX causes an excessive urination, as well as defecation in all experiment animals with this condition animals, showed polydipsia. The effects were again drug-related the animals recovered from these conditions with 72 hrs after drug administration. The result is inconsistent with the results reported in the dog (Bughio *et al.,* 2015) and in elephants (Mikota and Plumb, 2013)

There was a decrease in body weight in all experimental animals. The result of this study is in the line of findings reported by (Ohlsson *et al.,* 2010). In this study, the body weight temporary decreased and animals regained their body weight within 8 days following the DEX administration. Change in the level of glucocorticoid or mineralocorticoid can lead to muscle abnormalities and bone loss but the condition usually associated with chronic hypercorticism. It is reported that increased level of glucocorticoid causes wasting of muscle and it is due to their catabolically affected on protein metabolism. Insufficient corticosteroid shows in low work capability of striated muscle, weakness, and fatigue (Kufe *et al.,* 2013).

**Residual Analysis**

DXM residues before treatment were 0 in all six goats which were kept as control, whereas marked alterations were recorded after treatment in milk samples of goats after DXM administration for three days. A highly significant increase was observed in the residual mass of DXM in goat milk at 2, 8, 16, 32, 48, 72, 96, 120 and 144 hr (Figure -02) after dosage regimen compared with the pretreatment value. Others have also reported similar findings in different animals. Chen *et al.* (2011), Cherlet *et al.* (2014), Draisci et al. (2005) reported residues of DEX in different biological samples i.e. urine, feces, meat, liver or milk in various animals. Besides the clinical usage of DXM in relieving pain and inflammation, its use may result in drug residues in dairy and other products of animal source aimed for human consumption. Several other authors have been reported serious threats for long-term usage of low concentrations of DXM, which might have adverse effects on public health (Becker, 2011; Reig *et al.,* 2016). Additionally, the therapeutic use also limits due to the launching of MRLs in milk and edible tissues. DXM is more than 50 times the strength of the steroid cortisol(Becker, 2011). Since Fairclough *et al.* (2009) reported that I.M administration of DXM most probably due to the formation of phosphate and acetate esters which can lead to the sustained release of DXM into the systemic circulation. Coelho et al. (2006) reported that no significant difference in relative tissue residual levels also reported that with the administration of phosphate and acetate esters plasma levels of DEX is also elevated. So accumulation in plasma or muscles as from I.M injections, one can surely expect residues in milk. DXM are administered to animals either by injections (parenterally), orally in feed/water, topically on the skin or by intramammary and intrauterine infusions and may lead to residues of drugs in foods of animal origin such as milk, meat, and eggs (Turnipseed *et al.,* 2011).Calves fed milk and/or colostrum’s from cows receiving drugs are also included in the cause list of residues (Guest and Paige, 2001).

The result of the current study is also consistent with those observed by (Falahatpisheh *et al.,* 2011) who investigated residues of DXM in cow milk via ELISA. Somewhat similar observations were also noticed by Caloni *et al*., 2000 who found residues of DEX in lactating cows after administration of a therapeutic dose of DXM once daily, they further stated that with recommended doses the maximum tolerated residue limit of DXM may exceed and suggest a withdrawal period of 3-3.5 days.

**Compositional Analysis**

DEX at therapeutic dose i.e 0.5mg/kg BW caused a significant increase in Fat content at 48 hr and non-significant at 144 and 168 hr post drug administration respectively. The concentration then started to decrease toward the pre-treatment level, so they remained higher than in the control. The fat is slowly increased and reached a peak level at 48 hr then the content gradually returns to decrease at 168 hr. The lack of negative effect of DEX on fat effect on fluid secretion explains the increase in the concentration of fat in the treated cows(Varner and Johnson, 2003).

The dose of 0.5mg/kg BW of DEX caused a significant increase in protein content at 48 hr (Figure-03,) and non-significant at 168 hr after drug administration respectively. Consequently, the concentration of protein increased then decreased and remained indirectly proportional to the changes in the milk yield. The concentration of protein was higher as compared to control. The secretion of protein was reduced after 48 hr. The decline of unwanted effect of DEX on protein on fluid secretion clarifies the increase in the concentration of protein in the treated animals (Varner and Johnson, 2003). The present finding for milk protein content was found in contrast to those observed by Shamay *et al.* (2000). They reported decreased level of protein in milk after induction of DXM in cows. This contrast may be attributed to interspecies differences between cow and goat. Similar findings were also reported by Varner and Johnson (2003) also reported similar finding, he reported that there is decreased level of protein content in milk composition. These variant findings may be due to climatic or nutritional factors which ultimately have caused increased protein level.

The dose of 0.5mg/kg BW of DEX showed a non-significant decrease in lactose content after drug administration. The present findings of lactose content were non-significant which were in agreement to those found by Silanikove et al. (2006). They reported decreased level of lactose content in milk after induction of DXM to the cow. The basic cause in this decreased level of lactose in milk might be due to the relation between the activation of hypothalamic pituitary adrenal axis and reduction in the output of osmotic components from the alveoli into the gland lumen through the production of active biological substance from β-casein by the plasmin system in milk. This decreased the level of lactose also may involve another factor such as decrease secretion of milk from glandular cells which resulted in reversion of lactose level to pre-treatment values in a directly proportional manner (Silanikove *et al.,* 2006).

The dose of 0.5mg/kg BW of DEX showed a non-significant increase in SNF on initial observation. However, with the passage of time SNF showed a significant increase till 120 hr and then gradually returned to the non-significant level at 144 and 168 hr post treatment. DXM Residues may affect milk composition in various species (Macrina *et al.,* 2014; Shamay *et al.,* 2000; Thanasak *et al.*, 2004) The present findings of milk SNF content were significant which were in agreement to those found by Walsh *et al.* (2001). They reported increased level of SNF content in milk after induction of DXM to the cow. The basic cause in this increased level of SNF in milk might be due to the genetic potential of individual animals, age, stage of lactation, infections of udder and the type of feeding.

**Milk Yield**

In the present study milk yield non-significantly decreased by therapeutic administration of DXM in lactating goats till 16 hr. Afterward, a significant decrease was observed in milk yield up to 144 hr and after that completely returned to pretreatment levels up to 168 hr (Figure-03). Similar observations were also reported by (Shamay *et al.,* 2000) who recorded decrease in milk yield with the administration of a therapeutic dose of DXM in lactating cows. It has been reported that administration of ACTH and DXM to lactating cows caused a proportional decrease in milk yield (Hartmann and Kronfeld, 2003). This decrease in milk yield might be due to the disruption of the cellular integrity of mammary epithelial cells of tight junctions which have caused lower milk yield in goats(Stelwagen *et al.,* 2015). Another possible factor to which decrease milk yield may be attributed to increasing in milk sodium and chlorine due to their leakage from blood and decrease in potassium concentration which is leaked from milk to blood(Stelwagen *et al.,* 2014). It has also been suggested that decreased the availability of glucose or decrease glucose uptake by mammary glands also resulted in lower milk yield (Shamay et al., 2000).

**Conclusions**

It is concluded that, dexamethasone residues were significantly found up to 32 hrs which gradually decreased on subsequent observations up to 168 hr. Hence its withdrawal period in goat milk remained 168 hrs (07 days). Milk fat and protein increased significantly and showed their highest level in 48 hr however these parameters showed decrease till 168 hr. Milk lactose showed a non-significant increase which completely returned to pre-medication value by 48 hr, whereas SNF of milk was affected and showed a significant increase upto 120 hr and decreased till 168 hr. Dexamethasone produced some significant clinical effects on various systems of the body in goat, but these effects did not remain for long duration.

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