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Clinico-therapeutic efficacy of oral formulation in the treatment of clinical ketosis

KEYWORDS: Ketone bodies, ketosis, herbal gel, rothera's test, triglycerides.

Sudhir Kumar Trimbakrao Borikar
K.N.P college of Veterinary Science, Shirval, Maharashtra

V.R. Patodkar
K.N.P college of Veterinary Science, Shirval, Maharashtra

S.B. Swami
K.N.P college of Veterinary Science, Shirval, Maharashtra

B.N. Ambore
K.N.P college of Veterinary Science, Shirval, Maharashtra

Anurag Borthakur
R&D Deptt. Ayurvet, Baddi, Himachal Pradesh

K. Ravikanth
R&D Deptt. Ayurvet, Baddi, Himachal Pradesh

Shivi Maini
R&D Deptt. Ayurvet, Baddi, Himachal Pradesh

ABSTRACT Present study was carried out to investigate the role of herbal gel in effective control of ketosis. A total of 24 cows were selected for the study, 16 of them diagnosed with ketosis and 8 healthy cows. Group T0 (n=8) was kept as negative control consisting of healthy cows. Group T1 (n=8) was treated with AV/KPC/10 (M/S Ayurvet limited) at the rate of 1 tube/day/animal for 3-5 days. Group T2 (n=8) was treated with CoFeCu at the rate of 2 tablets once daily for 3-5 days. Results revealed that serum ketone bodies were significantly reduced in the AV/KPC/10 treated group T1 as compared to group T2. The serum glucose, calcium and inorganic phosphorus levels were also significantly higher in group T1 when compared to group T2. The serum triglyceride level was also significantly reduced in group T1. Milk yield was significantly increased in the herbal gel treated group T1. Negative Rothera's Test and Diastrix strip tests also indicated significant improvement in ketosis in group T1.

Introduction

In a dairy farming, among many diseases, metabolic/calving diseases are of great concern to dairy producers worldwide **(Senthilkumar et al., 2013)**. Ketosis is a common metabolic disorder in high yielding dairy cows **(Reddy et al., 2014)**. Ketosis, or acetonemia, is an increase of "ketone bodies" (acetone, acetoacetate, and /3-hydroxybutyrate, subsequently referred to as ketones) in blood until they eventually begin to spill over into urine and (or) milk **(Littledike et al., 1981)**. Ketosis in dairy cattle occurs due to a period of negative energy balance (NEB) that occurs almost universally at the beginning of lactation **(Herdt, 2000)**. In a heavy milking animal 60% to 80% of the blood glucose is utilized by the mammary glands in the production of milk **(Annison and Linzell, 1963)** causing the animal to slip into negative energy balance. The causes of ketosis include under nutrition / feed restriction relative to high yields, inadequate energy intake, high protein feeds with low fiber content, inadequate forage feeding space, feeding from compacted self-feed silage and inadequate concentrate inputs **(Shridhar, 2009)**. Several herbal medicines have been tried and tested in the treatment of bovine ketosis and their results have encouraged the scientific community to investigate further in that direction. Antioxidants have been successful in alleviating signs of ketosis by reducing the oxidative stress **(Sahoo et al., 2009)**. *Phyllanthus niruri* has been known to possess anti- oxidant property **(Harish and Shivanandappa, 2006)** and has been employed in the treatment of ketosis. Future research should be directed toward understanding mechanisms conferring priority on milk production and regulating appetite. The current study is designed to evaluate the effect of herbal oral formulation (*M/S Ayurvet Limited, India*) for effective control of ketosis.

MATERIALS AND METHODS

Experimental design

The study was carried out in the KNP college of veterinary science Shirval, Dist. Satara, Maharashtra to evaluate the effect of herbal oral gel formulation AV/KPC/10 and Liquid Anabolite (Pfizer) in ameliorating bovine ketosis. A total of 24 lactating cows were selected for the purpose of study. Among the 24 cows, 16 cows were diagnosed to be suffering from ketosis and 8 were healthy. The cows were allocated into three different groups T0, T1 and T2 with 8 numbers in each groups. Cows diagnosed with ketosis were allotted into group T1 (n=8) and T2 (n=8) and healthy animals were allotted into group T0 (n=8). Group T0 was kept as negative control and no treatment was given. In group T1, the cows were treated with AV/KPC/10 @ 1 tube/day/animal for 3-5 days. In group T2, the cows were treated with anabolite liquid (Pflizer) @ 200 ml bid for the first day and 100 ml bid for the rest of 3-5 days. Biochemical tests viz. serum tests, urine tests, and also milk yield measurement were done on 0, 3rd, 7th and 30th day.

Statistical analysis

All the results were analyzed statistically by analysis of variance to determine the means and standard error as per the methods described by **Snedecor and Cochran (1994)**.

Results and Discussion

Milk yield

The milk yield in the control group (healthy animals) T0 was recorded to be 7.75 litres on day 0 and 8.31 litres on day 30th (table 1). In the AV/KPC/10 treated group T1, the milk yield recorded on day 0 was 5.72 litres on day 0 which was increased to 7.64 litres on day 30th, an increase of 33.56 % milk yield was observed following treatment with AV/KPC/10. In the anabolite treated group T2, the milk yield recorded on day 0 was 5.82 litres which was also increased to 7.09 litres on day 30th, an increase of 21.82% milk yield was observed. The milk yield recorded in group T1 was significantly higher in comparison to group T2. The increase in milk yield may be attributed to the ant- oxidant and free radical scavenging property of *Phyllanthus niruri* **(Thakur and Bigoniya, 2014)** which is a constituent ingredient of AV/KPC/10 which can help offset some of the deleterious effects of lipid metabolism **(Rajeshwar et al., 2008)**. The important physiological effects of ketosis are an increased mobilization of depot fat and fatty infiltration of the liver and an increased mobilization of amino acids from body protein to the liver,

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in which some are converted to glucose and glycogen **(Dye and Dougherty, 1956)**. Fatty liver exacerbates the outcome of metabolic diseases, in particular displaced abomasum and ketosis, because fatty liver decreases glucose availability for peripheral tissues **(Veenhuizen *et al.*, 1991)**. Both fatty liver and ketosis are part of a spectrum of conditions associated with intense fat mobilization in cattle. The increased milk yield following recuperation from ketosis may be attributed to the hepatoprotective effect of *Phyllanthus niruri* **(Manjekar et al., 2008)** which can significantly ameliorate the progression of the fatty infiltration in liver. The increased milk yield may also be ascribed to the presence of another herb *Glycyrrhiza glabra*, a constituent ingredient in AV/KPC/12, which possesses protective effect against lipid peroxidation of lisosomal membrane **(Rackova et al., 2007)**.

Table 1. Milk yield in cows in the control and treated groups.

Group	Milk yield (lit/day)			
	Day 0	Day 3rd	Day 7th	Day 30th
T0	7.75^{Aa} + 0.05	7.89^{Aa} + 0.07	8.08^{Aa} + 0.13	8.31^{Ab} + 0.05
T1	$5,72^{Bb}$ + 0.06	6.23^{Bc} + 0.07	6.91^{Bb} + 0.07	7.64^{Ba} + 0.05
T2	5.82^{Bb} + 0.06	6.31^{Bc} + 0.09	6.76^{Bb} + 0.08	7.09^{Ca} + 0.06

Ketone bodies

The ketone bodies in the healthy control group T0 was recorded to be 4.52 mg/dl on day 0, 4.33 mg/dl on day 3rd ,4.12 mg/dl on day 7th and 4.14 mg/dl on day 30th (table 2). The ketone bodies (mg/dl) recorded in group T1 on day 0 was 61.88 mg/dl which was significantly reduced to 3.87 mg/dl on day 30th following the treatment with AV/KPC/10, a significant decrease of 93.89 % in ketone bodies was recorded. The ketone bodies were also significantly reduced in group T2 from 56.80 mg/dl on day 0 to 6.46 mg/dl on day 30th. However, the decrease in ketone bodies was significantly greater (93.89% decrease) in AV/KPC/10 treated group T1 as compared to Anabolite treated group T2 (88.62% decrease).This may be attributed to the alleviation of oxidative stress **(Baskaran et al.,2010)** and a reduction of blood cholesterol and triglycerides **(Patel et al., 2012)**, protective effects conferred by *Phyllanthus niruri*. The nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) is an important gene regulator in glucose and lipid metabolism. Amorfrutin A1, an active ingredient of *Glycyrrhiza glabra* is a natural glucose-lowering compound that selectively modulates PPARγ **(Weidner et al., 2013)**. This may be how *Glycyrrhiza glabra*, a constituent ingredient of AV/KPC/10 is effective in treatment of ketosis.

Table 2. Serum ketone bodies in the control and treated groups.

Group	Ketone Bodies (mg/dl)			
	Day 0	Day 3rd	Day 7th	Day 30th
T0	4.52^{Ca} ±0.79	4.33^{Ca}±0.63	4.12^{Ba}±0.43	4.14^{Ba}± 0.70
T1	61.88^{Aa}±1.46	18.83^{Bb}±0.40	8.28^{Ac}±0.27	3.87^{Bd}±0.25
T2	56.80^{Ba} ±1.15	37.39^{Ab}±1.09	8.23^{Ac} ±1.05	6.46^{Ac}±0.14

Glucose

The serum glucose level in the healthy control group was recorded to be 58.75 mg/dl on day 0, 57.99 mg/dl on day 3rd, 56.07 mg/dl on day 7th and 54.65 mg/dl on day 30th. There was significant increase in the blood glucose level in the AV/KPC/10 treated group T1 from 32.47 mg/dl on day 0 to 58.55 mg/dl on day 30th, an increase of 80.32 % in the glucose level was recorded. The glucose level was also elevated in the Anabolite treated group T2 from 32.35 mg/dl on day 0 to 55.87 mg/dl on day 30th, an increase of 72.70% in the glucose level was recorded. The increase in glucose level was significantly higher in the AV/KPC/10 treated group T1 when compared to Anabolite treated group T2. Many of the active constituents to which the biological activity of P. niruri has been attributed include lignans, tannins, coumarins, terpenes, flavonoids, alkaloids, saponins and phenylpropanoids, which have been found in the leaves, stem and roots of this plant. Common lipids, sterols and flavonols also occur in the plant **(Dhar et al., 1968)**. These phytochemicals have been reported to exhibit diverse pharmacological and biochemical actions when ingested by animals **(Amadi et al., 2006)** as well as exhibiting physiological activity **(Sofowora, 1993)**. The increase in glucose level in the blood may be on account of the influence of tannic acid, on the digestibility of starch by alpha-amylase **(Gin et al., 1999)**.

Table 3. Serum glucose in the control and the treated groups.

Group	Glucose (mg/dl)			
	Day 0	Day 3rd	Day 7th	Day 30th
T0	58.75^{Aa} ± 0.55	57.99^{Aa} ± 0.59	56.07^{Ab} ± 0.57	54.65^{Cb} ± 0.51
T1	32.47^{Bd}±0.58	49.69^{Bc} ±0.57	55.09^{Ab} ±0.29	58.55^{Aa}±0.28
T2	32.35^{Bd}±0.49	46.92^{Cc}±0.60	53.05^{Bb}±0..75	55.87Ba± 0.38

Calcium

The serum calcium level (mg/dl) in the healthy control group was recorded to be 8.31 mg/dl on day 0, 8.59 mg/dl on day 3rd, 8.54 mg/dl on 7th day and 8.31 mg/dl on day 30th. A significant increase in the calcium level was recorded in AV/KPC/10 treated group T1 from 7.33 mg/dl on day 0 to 8.65 mg/dl on day 30th, an increase of 18% serum calcium level was recorded after treatment with AV/KPC/10. Significant increase in the calcium level was also observed in the Anabolite treated T2 group in which the calcium level recorded on day 0 was 7.27 mg/dl and was increased to 8.11 mg/dl on 30th day. However, the increase in the calcium level was significantly higher in the AV/KPC/10 treated group T1 (18% increase) when compared to the Anabolite treated group T2 (11.55%). Several reports confirmed presence of hypocalcemia in cows with clinical and subclinical ketosis **(Radostits et al., 2007; Yameogo et al., 2008)**. The increase in calcum level is probably due to improvement of ketosis following the administration of AV/APC/10.

Table 4. Serum calcium in the control and treated groups.

Group	Calcium (mg/dl)			
	Day 0	Day 3rd	Day 7th	Day 30th
T0	8.31^{Aa}±0.05	8.59^{Aa}±0.14	8.54^{Aa} ±0.14	8.31^{Ba} ±0.15
T1	7.33^{Bd}±0.07	8.06^{Bc}±0.03	8.37^{Ab}±0.03	8.65^{Aa}±0.04
T2	7.27^{Bd}±0.05	7.71^{Cc}±0.02	7.88^{Bb}±0.04	8.11^{Ba}±0.03

Inorganic Phosphorus

The serum inorganic phosphorus (mg/dl) in the healthy control group T0 was recorded to be 5.69 mg/dl on day 0, 5.58 mg/dl on day 3rd, 5.56 mg/dl on day 7th and 5.32 mg/dl on day 30th. Significant increase in the inorganic phosphorus level was observed in the AV/KPC/10 treated group T1 from 5.22 mg/dl to 5.67 mg/dl, an increase of 8.12 % inorganic phosphorus was recorded. Elevation in inorganic phosphorus was also observed in the Anabolite treated group T2. However, the increase in the inorganic phosphorus in the AV/KPC/10 treated group T1 was significantly higher as compared to Anabolite treated group T2. Insufficient phosphorus supply in the diet, prolonged anorexia, and increase durinary phosphorus excretion due to hyperparathyroidism could explain presence of hypophosphatemia in Ketosis **(Youseff et al., 2010)**. The decrease of phosphorus and magnesium level coincided with that obtained by **Stockham and Scott 2002** and **Žiogas et al., 2007**. The convalesce from ketosis following the treatment with AV/KPC/10 may be the reason for elevation in the phosphorus level.

Table 5. Inorganic phosphorus in control and treated groups.

Group	Inorganic Phosphorus (mg/dl)			
	Day 0	Day 3rd	Day 7tth	Day 30th
T0	5.69^{Aa} ± 0.07	5.58^{Aa} ± 0.08	5.56^{Aa} ± 0.06	5.32^{Ba} ± 0.07
T1	5.22^{Bb} ±0.02	5.37^{Bc} ± 0.02	5.53^{Ab} ± 0.02	5.67^{Aa} ± 0.03
T2	5.34^{Ba} ± 0.02	5.30^{Ba} ± 0.02	5.34^{Ba} ± 0.02	5.40^{Ba} ±0.03

Triglyceride

The serum triglyceride level (mg/dl) in the healthy control group was recorded to be 21.06 mg/dl on day 0, 24.32 mg/dl on day 3rd, 21.39 mg/dl on day 7th and 33.55 mg/dl on 30th. The triglyceride level was significantly decreased in the AV/KPC/10 treated group T1 from 42.37 mg/dl on day 0 to 28.82 mg/dl on day 30th. Significant reduction in the triglyceride level was also observed in the Anabolite treated

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group T2. There are reports which suggest that *Phyllanthus niruri* has hypolipidemic property inherent in it. **(Latha et al., 2010, Chandra et al., 2000)**. The reduction in triglyceride level may be attributed to the presence of *Phyllanthus niruri* as a constituent ingredient in AV/KPC/10.

Table 6. Serum triglyceride in control and treated groups

Group	Serum triglycerides (mg/dl)			
	Day 0	Day 3rd	Day 7tth	Day 30th
T0	$21.06^{Bc} \pm 0.34$	$24.32^{Cb} \pm 0.30$	$21.39^{Cc} \pm 0.36$	$33.55^{Aa} \pm 0.25$
T1	$42.37^{Aa} \pm 0.18$	$35.41^{Ab} \pm 0.15$	$30.40^{Ac} \pm 0.07$	$28.82^{Bd} \pm 0.37$
T2	$43.14^{Aa} \pm 0.28$	$33.30^{Bb} \pm 0.22$	$29.06^{Bc} \pm 0.22$	$26.98^{Cd} \pm 0.26$

Rothera's Test

Rothera's test is used to detect whether acetone or ketone bodies are present in the given sample of urine. The Rothera's test was negative on day 7th and day 30th in group AV/KPC/10 treated group T1 indicating absence of ketone bodies in the urine.

Keto Diastix Strip Test

The diastix strip test reading was recorded as trace (5 mg/dl of aceto-acetic acid) on day 7th and negative on day 30th in group T1 indicating significant improvement in ketosis after treatment.

CONCLUSION

Results indicate that there is a pattern of decline in the ketone bodies and triglycerides following the treatment with herbal gel AV/KPC/10. It was also observed that the serum glucose, calcium and inorganic phosphorus were also increased post treatment with the herbal gel. Thus, it is conspicuous from the results that AV/KPC/12 is highly efficacious in mitigating signs of ketosis.

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