EFFECT OF RICE STRAW ON SOME BLOOD BIOCHEMICAL PARAMETERS OF IRAQI BUFFALO(*Bubalus Bubalis*)

Ali J. I.
Researcher
Direct. Diyala Agric, Min. of Agric.
ali.jaleel1101a@coagri.uobaghdad.edu.iq

W. H. Al-Samaraae
Prof.
Dept. of Anim. Prod. /Coll. of Agricu. Engin. Sci./ University. of Baghdad
Wafaa.h@coagri.uobaghdad.edu.iq

ABSTRACT

This study was aimed to show the changes of some blood parameters by the effect of biological and chemical treatment of rice straw ,It was conducted at The Ruminant Research Station in Abu Ghraib / Directorate of / Agricultural Researches / Ministry of Agriculture, for the period from June 1, 2021 until August 27, 2021 (preceded by the preliminary period 14 days), The study included 12 Iraqi buffalo calves aged 5 - 6 months. the calves were randomly divided into three equal groups (4 calves / group), kept in individual pens and distributed among group.T1 (the control group) in which the calves were fed on untreated rice straw T2 the calves were fed on rice straw treated with urea at a rate of 3% T3 the calves were fed on rice straw treated with Trichoderma harzianum at a rate of 0.1%. The triglycerides concentration were decreased significantly (p≤0.05) in the T2 (11.28 mg/dL) and T3 (11.32 mg/dL) compared with T1 (20.79 mg/dL). similarly, the level of very low-density lipoprotein (vLDL) was decreased 2.25, 2.26, 4.16 mg/dL for T2, T3, and T1, respectively. The creatinine concentration in T2(87.19 mmol/L) was significantly decrease than that of T1(177.8 mmol/L) and T3(176.75 mmol/L). Also, ALP concentration increased in T2 (238.93 IU/liter) compared with T1. (151.82 IU/liter), while it did not differ significantly with T3(205.83 IU/liter) Also T3 did not differ significantly from T1. The differences in the concentration of lipid profile, protein fractions and enzymes activity among groups lacked significance , feeding of rice straw treated biologically and chemically had no negative effect on buffalo calves health.

Key words: ruminant, urea, fungi, serum profile, biological treatment, chemical treatment

*** Part of the Ph.D. dissertation of the 1st author.**

تأثير تبن الرز في بعض معايير الدم في عجول الجاموس العراقي

علي جليل ابراهيم
باحث
مديرية الزراعة في محافظة ديالى/ وزارة الزراعة

وفاء حميد السامرائي
استاذ
قسم الانتاج الحيواني/كلية علوم الهندسة الزراعية /جامعة بغداد

المستخلص

هدفت الدراسة الى بيان تغييرات بعض معايير الدم بتأثير المعاملة الكيميائية والبايلوجية لتبن الرز وأُجريت في محطة أبحاث المجترات في أبي غريب / قسم بحوث الثروة الحيوانية / دائرة البحوث الزراعية / وزارة الزراعة ، للمدة من 1 حزيران 2021 ولغاية 27 آب 2021 (سبقتها الفترة التمهيدية 14 يوم) ، استعمل في هذه التجربة 12عجل من الجاموس العراقي بعمر5 – 6 (شهر) ، قسمت هذه العجول عشوائيا إلى ثلاث مجاميع متساوية (4 عجل/مجموعة) في حضائر منفردة. غذيت العجول بصورة فردية على عليقة مركزة وبنسبة 3 % من وزن الجسم الحي وقدم العلف الخشن (تبن الرز) بصورة حرة وقد شملت المعاملات التجريبية على ما يلي T1 (مجموعة السيطرة غذيت على تبن غير معامل) T2(غذيت على تبن معامل باليوريا بنسبة 3 %) T3 (غذيت على تبن معامل بالفطر Trichoderma harzianum بنسبة 0.1%). انخفض معنويا (p≤0,05) مستوى الدهون الثلاثية في المعاملة الثانية (11.28) والثالثة (11.32) مقارنة مع المعاملة الاولى (20.79). وفي نفس الاتجاه كانت تسير مستوى الدهون واطئة الكثافة جدا (vLDL) والتي بلغت 2.25 ، 2.26 ، 4.16 ملغم/ديسلتر للمعاملة الثانية والثالثة والاولى على التوالي .كما لوحظ انخفاض معنوي في تركيز الكرياتينين في المعاملة الثانية 87.19 عن المعاملتين الاولى 177.8 والثالثة 176.75 بينما لم تختلف معنويا المعاملة الثالثة عن المعاملة الاولى. كما ارتفعت فعالية تركيز ALP في المعاملة الثانية 238.93 مقارنة مع المعاملة الاولى 151.82 بينما لم تختلف معنويا مع المعاملة الثالثة 205.83 في حين لم تختلف معنويا المعاملة الثالثة عن المعاملة الاولى. انعدمت الفروق المعنوية في تركيز كل من مستوى الدهون وانواع البروتينات وفعالية بعض الانزيمات بين المجاميع المختلفة . لم تؤثر التغذية على تبن الرز المعامل كيميائيا وبايلوجيا في صحة عجول الجاموس.

الكلمات المفتاحية: المجترات، اليوريا، الفطر، مصل الدم, المعاملة البايلوجية، المعاملة الكيميائية

***البحث مستل من اطروحة دكتوراه للباحث الاول.**

Received: 14/1/2022, Accepted:27/4/2022

INTRODUCTION

Buffalo is a dual-purpose animal belonging to the bovid family (Bovidae) of the genus Bubalis. Its breeding spreads in Iraq from north to south and plays a major role in the animal production sector (2). One of the most important challenges facing Iraqi agriculture is protecting and improving national agricultural resources, including buffalo, to achieve sustainable food security (5). Roughage is an important part of the buffalo's diet, as it represents the filling ration due to the capacity of the alimentary canal of these animals, and then they need large quantities of feed to meet their needs, Several studies have been conducted to improve the nutritional value of low-quality rough forages such as the use of feed additives or physical treatments such as crushing and grinding, slicing and marinating (14, 33). moreover chemical treatments such as adding bases such as urea, NaOH, and other bases or organic solvents (15, 27).while biological treatments are using microorganisms that analyze the bonds between lignin, cellulose and hemicellulose to analyze the plant cell wall and make better use of its components To get a high digestibility coefficient and a high nutritional value for this forages (30). Biomarkers, such as various proteins and other biochemical attributes, lead an essential role in the biological systems of males and females (1 ,6). In order to develop a database for some biochemical parameters and lipid parameters in the blood of Iraqi buffalo calves, this study aimed to know the extent to which these parameters are affected by the changes that occur to rice straw during its biological and chemical treatment to improve its nutritional value, In Iraq, there is no studies about buffalo fed in rice straw treated by Trichoderma harzianum ,Therefore, this study is the first of its kind.

MATERIALS AND METHODS

The study was conducted at the ruminant Research Station in Abu Ghraib / Directorate Agricultural Researches / Ministry of Agriculture, for the period from June 1, 2021 until August 27, 2021. Twelve calves were selected post weaning, weighing 140-160 kg Individual feeding was conducted for 84 days of experiment preceded by 14 days as adaptation period .The concentrate diet was introduced by 3% of body weight consisted of 67% barley, 10% yellow corn, 12% soybean meal, 8% cottonseed meal, 2% lime, 1% salt. treatments then sampled for analysis (Table1). The calves were weighed bi - weekly and the concentrate diet was adjusted on the basis of weight. The roughages offered *ad libitum*, The remained roughages and concentrate were collected daily at the morning pre- feeding for calculating the amount of daily feed intake. The animals were weighed every two weeks pre- morning feeding during the experiment of period . All animals were provided clean water, vaccines and kept continuous veterinary supervision all experimental, First group (control) the calves were fed on rice straw only, while , the second group(T1) on urea treated rice straw and the third group (T2) were fed on rice straw treated with Trichoderma harzianum.

Table1. The chemical composition of rice straw (T1), treated rice straw (T2) and concentrate diet (% of dry matter).

Chemicals composition of Offered feed (%DM)	Treatment diet			
	Concentrate	T1	T2	T3
DM	93.09	93.89	90.75	90.60
OM	92.59	92.86	89.98	89.68
CP	14.75	3.94	8.75	10.94
CF	11.30	32.28	26.56	25.60
NFE	62.19	52.93	53.24	52.32
EE	4.35	1.25	1.43	1.30
Ash	7.41	9.60	10.02	9.84
*ME	12.39	9.89	10.28	10.32

***Metabolic energy (MJ/kg DM) = 0.012 × crude protein + 0.031 × ether extract + 0.005 × crude fiber + 0.014 × nitrogen free extract (18). Chemical analysis of feed applied to found dry matter (DM), organic matter (OM), Ash, crude protein (CP), crude fiber (CF), ether extract (EE). (9).**

Preparation of forage components

The rice straw was divided into three parts , each one was treated as follows :

1- The first part not treated control group was and represented as

2 - The second part was treated with urea, as 3% of the weight of the rice straw, (3 kg of urea were weighed and dissolved in 100 liters

of water), continuous stirring, and the treated rice straw was covered with a large piece of nylon. The weights were placed over it tightly to prevent the leakage of ammonia resulting from the decomposition of urea during the incubation period . On a clean floor under the sun, the rice straw was daily stirring to dry it, then a sample of rice straw was taken for the purpose of chemical analysis. It was filled and stored in bags until the experiment proceeded (29).

3-The third part was treated with the fungus Trichoderma . The rice straw was prepared in a dark and airtight room, as the straw was placed on a nylon (polyethylene) on the floor of the room, and the straw was moistened with water until a humidity of 60% was obtained. The solution containing the spores and growths of the fungus Trichoderma harzianum was sprayed at a rate of 0.1% (1 kg / ton of rice straw) on the rice straw by a spray device (holder) with a capacity of 100 liters with continuous stirring to ensure the liquid reaches all parts. The rice straw was covered by nylon (polyethylene) and left for two weeks to complete the process of fungus growth on the rice straw and at a room temperature between 25-30 C°. The plastic cover was removed from the rice straw, The treated straw was displayed on a clean floor under the sun to dry it and then put in bags for the purpose of preservation and used in the current experiment (7, 13).

blood sampling

Blood samples were collected from the jugular vein of calves on the last day of the experiment, before the morning feeding. The tests included measuring serum glucose, according to the method of Mendel et al (20). Cholesterol concentration was measured according to Richmond's method (23). Triglyceride and HDL (High-density Lipoproteins) and (LDL) Low-density Lipoproteins were estimated, and the concentration of total protein in blood plasma was determined according to Wotton (32). Also, albumin , alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities were estimated on Caird et al. (11). The globulin level in the blood serum, was estimated according to the method of (9) using the following equation:

Globulin (g/100ml) = total protein – albumin

Very Low Density Lipoproteins (vLDL) were calculated according to the following equation:

$$vLDL = \frac{\text{Triglyceride}}{5}$$ (28).

Statistical analysis

The experimental data analyzed as a complete randomized design (CRD) were and compared the significant differences among the averages by Duncan multiple range test (12) using statistical program SAS (24) the statical model was as follows:

$$\mathbf{Yij = \mu + Ti + eij}$$

As:

Yij = View value j Per transaction i

μ = The overall mean of the trait

ti = Treatment effect

eij = Random error that is normally distributed with mean equal to zero and variance of its magnitude σ 2 e.

RESULTS ANA DISCUSSION

The results showed that there were non-significant effects of the treatments on the concentrations of total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol. The treatments had a significant effects ($p \leq 0.05$) on triglycerides and very low density lipoproteins (vLDL), as the level of triglycerides decreased significantly in the second treatment being 11.28 mg/dL and in the third treatment being 11.32 mg/dL compared with the control group namely 20.79 mg/Dl. The second treatment did not differ from the third group significantly. Similarly, the vLDL concentrations were amounted to 4.159, 2.25, 2.26 mg/dL for the first, second and third treatments, respectively (Table2). The reason behind the decrease in triglycerides in second and third treatment may be attributed to high ALP enzyme activity in calves of the two treatments (Table 2) as high ALP activity promotes lipolysis by increasing the levels of free fatty acids (16).

Table 2 . Effect of treating rice straw with urea and Trichoderma harzianum on some blood biochemical parameters of Iraqi buffalo (mean ± SE):

Treatment	Cholesterol(mg/dL)	HDL (mg/dL)	LDL(mg/dL)	vLDL(mg/dL)	Triglycerides(mg/dL)
T1	61.29±9.55	790.10±59.41	35.04±6.12	a 4.16± 0.77	a 20.79±3.83
T2	45.75±2.41	713.08±28.40	32.14±5.33	b 2.25± 0.38	b 11.28±1.89
T3	56.76±11.0	784.74±17.54	27.92±6.04	b 2.26± 0.29	b 11.32±1.47
Significance level	N.S.	N.S.	N.S.	*	*

***Means with different superscripts within each column differ significantly (P <0.05). NS= non-significant differences; T1untreated rice straw , T2, urea treated rice straw T3 , Trichoderma harzianum treated rice straw**

Also, there was non-significant effect of all treatments on total protein, albumin and globulin (Table 3). Albumin and globulin values were measured to assess the long-term protein status, as well as to ascertain the presence of chronic inflammatory disease (31).

Table 3. Effect of treating rice straw with urea and Trichoderma harzianum on serum total protein and its fractions (Means ± SE)

Treatment	Total protein g/L	albumin g/L	globulin g/L
T1	63.04±1.98	35.61±1.36	63.04±1.98
T2	63.90±1.45	36.21±36.21	63.90±1.45
T3	65.47±0.68	37.75±0.58	65.47±0.68
Significance level	N.S.	N.S.	N.S.

N.S = non-significant

Table (4) shows that there were non-significant effects of the three treatments on the levels of serum glucose, urea and uric acid in the blood. These results agreed with Ahmad et al. (4) who fed buffalo calves on chemically-treated rice husks. also Syahrir et al. (26) reported that bull fed with 50% rice straw and 50% concentrate feed, their blood glucose level was 70-76 mg/dL, while a significant decrease in creatinine concentration was observed in the second treatment compared to the first and third treatments, namely 87.19, 177.80 and 176.75 mmol / liter for the second, first and third treatment, respectively. The second group whose weight was increased was the increase in adipose tissue, as Heymsfield et al. (17) and Proctor et al. (21) indicated that muscle mass can be predicted by creatinine concentration.

Table 4. Effect of treating rice straw with urea and Trichoderma harzianum on glucose, urea, creatinine and uric acid of Iraqi buffalo (mean ± SE):

Treatment	Glucose (mg/dL)	Urea (mmol/L)	Uric acid (mmol/L)	Creatinine (mmol/L)
T1	76.62±11.17	6.61±0.93	28.042±1.91	a 177.8± 19.68
T2	68.56±3.43	6.61±0.88	30.09±1.94	b 87.19± 16.77
T3	77.88±2.49	6.24±1.41	35.61±3.16	a 176.75± 14.64
Significance level	N.S.	N.S.	N.S.	**

**** Means with different superscripts within each column differ significantly (P<0.01); NS= non-significant differences.T1untreated rice straw , T2, urea treated rice straw T3 , Trichoderma harzianum treated rice straw**

Table 5 shows a significant effect of the treatments on the level of serum ALP, as the ALP concentration increased in the second and third treatments compared with the first treatment, while the second treatment did not differ significantly with the third treatment, being 238.93, 205.83, 151.82 U/L. for the second, third and first treatment respectively (Table 5) The ALP enzyme contains many substrates and participates in various metabolic and biosynthesis pathways (10) and since creatinine in this study gave an indication of the rise in adipose tissue in calves of the second treatment. The high activity of ALP enzyme may be attributed to the increase in adipose tissue, as Son et al. (25) indicated that the level of ALP in the blood is higher in obese subjects. It may also be attributed to the

increased activity of glucose synthesis (Gluconeogenesis), as ALP has a role in the process of uptake and transport of fats (22). It has a role in removing the phosphate group from various molecules such as proteins (35, 34). Table (5) shows that there were non-significant effects of the treatments on the activity of ALT and AST enzymes. The activity of the ALT enzyme in the blood serum was within the normal levels of the three treatments. The normal levels for ALT and AST in buffalo blood are 46-189 and 83-219 IU/L for AST and ALT, respectively(3). As these levels are an indicator of the good health of all experimental animals, as well as the absence of negative effects of the treatments on the feed intake or the metabolism of the experimental animals, because the high level of ALT enzyme in the blood serum is a sure indicator of liver cell damage in farm animals (19).

Table 5. Effect of treating rice straw with urea and Trichoderma harzianum on the activity of ALP, ALT and AST enzymes on the blood serum of Iraqi buffalo (means ± SE):

Treatment	ALP (U/L)	ALT (U/L)	AST(U/L)
T1	b 151.82±14.29	41.29±3.815	210.82±3.82
T2	a 238.93± 14.04	37.70±5.29	176.66±23.51
T3	ab 205.83± 26.34	38.79±1.27	222.14±31.78
Significance level	*	N.S.	N.S.

*** Means with different superscripts within each column differ significantly (P<0.05).N.S= non-significant differences, T1:untreated rice straw , T2: urea treated rice straw T3: Trichoderma harzianum treated rice straw.**

REFRENCES

1. Abdulkareem, T. A., S. M. Eidan, F. K. Alsaidy and N. K. Al-Hassani. 2023. Effect of pre-and post-mating vitamin AD3E treatment on reproductive performance of Awassi ewes. Iraqi Journal of Agricultural Sciences, 54(2), 431-437. https://doi.org/10.36103/ijas.v54i2.1717

2. Abdulkareem, T.A., S.A. Al-Sharifi, S.M. Eidan, and R.G.Sasser.2012. Reproductive and productive performance of Iraqi buffaloes as influenced of pre-mating and pre-calving concentrate supplementation. Pak. Vet. J, 32(3):345-348

3. Abdulkareem, T.A., S.M. Eidan, A.M. Shubber , F.F.Ibrahim, M.D. Ali, and O.A. Mohammed.2020. Reference Physiological Values in Different Animal Species.pp:18-21

4. Ahmad, F., A. Asghar, , F.Wadood, , A.Yaseen, , M.Akmal, M. Mubeeen, and N. Mujahid, .2019. Response of alkali treated rice husk in buffalo calves on growth, dry matter intake, nutrient digestibility and blood metabolites. Pak. J. Sci. 71(2).

5. Alkhazraji H. T., H J H, Banana. and M-BM R, Fakhrildin, 2023. Effect of cryoprotectants and cryopreservation methods on the quality of Buffalo Oocytes. Iraqi Journal of Agricultural Sciences, 54(5),1322-1328. https://doi.org/10.36103/ijas.v54i5.1832

6. Al-Saedi, A. J. A. and T. A. Abdulkareem. 2022. Comparison of semen quality for three lines of Holstein bulls. 1. Some immediate and microscopic characteristics. Iraqi Journal of Agricultural Sciences, 53 (4),752-759. https://doi.org/10.36103/ijas.v53i4.1585

7. ALWAELI, S. N. A. 2016. Effect of barley straw treatment with Trichoderma harzianum fungi, iraqi Probiotic and Baker's yeast on Awassi Lambs Performance. M.Sc., College of Agriculture/ University of Baghdad.pp:46-62. DOI: 10.13140/RG.2.2.26395.08481

8. AOAC. 2012. Official Methods of Analysis, 19th ed.; Association of Official Analytical Chemists: Gaithersburg, MD, USA

9. Bishop, M.L.; J.L. Dube-Engelkirk and E.P. Fody . 2000 . Clinical Chemistry: Principles, Correlation's , Procedures. 4th. ed., J.B. Lippincott Williams and Wilkins. Philadelphia pp.405-416

10. Buchet, R., J. L. Millán and D. Magne .2013. Multisystemic Functions of Alkaline Phosphatases. Phosphatase modulators,pp: 27-51. DOI: 10.1007/978-1-62703-562-0_3

11. Caird, F. and T. Judge , Modern . 1980. Topicsin Paediatric Dermatology. Edited by JULIAN Verbov. pp: 220, Illustrated. William Heinemann Medical Books, London, 1979.£ 11.50 . Postgraduate Medical Journal, 56:216-220.

12. Duncan, D. B. 1955. Multiple range and multiple Ftests. Biometrics, 11(1), 1-42. https://doi.org/10.2307/3001478
13. El-Naggar, S., S. Gad, Z. Mahasneh, M. Tawilla and G Abou-Ward. 2023. Impact of adding Ajwain seeds to the mail lambs rations as growth promoters. Iraqi Journal of Agricultural Sciences. 54(6):1557–1564. https://doi.org/10.36103/ijas.v54i6.1856
14. Ibrahim, A.J., Al-Samaraae, W.H. and Ajil, H.M., 2022. Effect of chemically and biologically treated rice straw on some productive characteristics of Iraqi buffalo (Bubalus bubalis) calves. *Journal of* Kerbala for Agricultural Sciences, *9*(3), pp.26-35. https://doi.org/10.59658/jkas.v9i3.993
15. Hassan, S. A., and S. M. N. Muhamad .2009. Responses of Karadi lambs to urea-treated and non-treated barley straw with two levels of rumen undegradable nitrogen. Jordan. J. Agric. Sci, 5: 98-110
16. Hernández-Mosqueira, C., C. Velez-delValle, and W. Kuri-Harcuch, .2015. Tissue alkaline phosphatase is involved in lipid metabolism and gene expression and secretion of adipokines in adipocytes. Biochimica et Biophysica Acta (BBA)-General Subjects, 1850(12), 2485-2496. https://doi.org/10.1016/j.bbagen.2015.09.014
17. Heymsfield, S. B., C.Arteaga, C. McManus , J. Smith , and S. Moffitt, .1983. Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. The American Journal of Clinical nutrition, 37(3), 478-494. https://doi.org/10.1093/ajcn/37.3.48
18. MAFF .1975. Energy allowances and feeding systems for ruminants. Ministry of Agriculture, Feed and Fisheries Technical Bulletin No. 33. (H.M.S.O.: London).
19. Mazur, N. P., V. V. Fedorovych, E. I. Fedorovych , O. V. Fedorovych, P. V. Bodnar, B. V. Gutyj , and , N. I. Pakholkiv 2020. Effect of morphological and biochemical blood composition on milk yield in Simmental breed cows of different production types. Ukrainian Journal of Ecology, 10(2): 61-67.
20. Mendel, B., A. Kemp, and D.K. Myers, 1954. A colorimetric micro-method for the determination of glucose. Biochemical Journal,56(4):639. https://doi.org/10.1042%2Fbj0560639
21. Proctor,D.N.,P.C.O'brien,E.J.Atkinson and K. S. Nair, .1999. Comparison of techniques to estimate total body skeletal muscle mass in people of different age groups. American Journal of Physiology-Endocrinology And Metabolism ,277(3):E489-E495. DOI:10.1152/ajpendo.1999.277..E489
22. Reust, C.E., and L.Hall .2001.What is the differential diagnosis of an elevated alkaline phosphatase (AP) level in an otherwise asymptomatic patient?. Journal of Family Practice, 50(6): 496-496
23. Richmond, W. 1973. Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clinical chemistry, 19(12),1350-1356. https://doi.org/10.1093/clinchem/19.12.1350
24. SAS. 2012. Statistical analysis system, users guide. Statistical. Version 9.1th ed.SAS .Inst.Inc.Cary .N.C.USA
25. Son, D. H., H. S. Ha and Y. J. Lee .2021. Association of serum alkaline phosphatase with the TG/HDL ratio and tyg index in korean adults. Biomolecules, 11(6), 882. https://doi.org/10.3390/biom11060882
26. Syahrir, S., K. G. Wiryawan, A. Parakkasi and M. Winugroho .2010. Blood profile of beef cattle offered mulberry leave meal to substitute feed concentrate. Jurnal Ilmu dan Teknologi Peternakan, 1(1): 12-18. https://doi.org/10.20956/jitp.v1i1.659
27. Thaer A. M. Al-Mamouri, J. A. Tawfeeq Al-Ani .2024. Effect of Replacing treated and untreated Corn impurities with Urea instead of Wheat Bran on productive performance of Awassi lambs. Iraqi Journal of Agricultural Sciences –2024:55(1):587-594 https://doi.org/10.36103/zr148430
28. Tietz, N. W. 1987 . Fundamentals of Clinical Chemistry, 3rd edn, W.B. Saunders, Philadelphia, PA
29. Tiwari, R. K., A. K. Garg, and P. Singh . 2013. Changes in the chemical composition of sugarcane crop residues treated with different levels of urea and moisture. Veterinary World, 6(12): 1004. https://doi.org/10.14202/vetworld.2013.1004-1007

30. Weiland, P. .1988. Principles of Solid-State Fermentation. Treatment of Lignocellulosic with White Rot Fungi . (ed) by Zadrazil, F. and Reiniger, P. Elseverier Appl. Sci., London and New York, pp : 64 – 76.

31. Whitaker, D. A., W. J. Goodger, M. Garcia , B. M. A. O. Perera and F. Wittwer .1999. Use of metabolic profiles in dairy cattle in tropical and subtropical countries on smallholder dairy farms. Preventive Veterinary Medicine, 38(2-3), 119-131. https://doi.org/10.1016/S01675877(98)00118-4

32. Wotton, I.D.P 1964. Micro - Analysis in Medical Biochemistry. 4th edn. Churchill Livingsstone, London.pp:509

33. Wanapat, M. and Wachirapakorn, C., 1990. Utilization of roughage and concentrate by feedlot swamp buffaloes (*Bubalus Bubalis*). Asian-Australasian Journal of Animal Sciences, *3*(3), pp.195-203. https://doi.org/10.5713/ajas.1990.195

34. Zakrzewska, H., J.Udala, , and B. Blaszczyk, 2002. In vitro influence of sodium fluoride on ram semen quality and enzyme activities. Fluoride, 35(3), 153-160.