RELATIONSHIP BETWEEN DIETARY PROTEIN SOURCE AND SOME REPRODUCTIVE PERFORMANCE OF GROWING FRIESIAN MALE CALVES.

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Abstract

The present study aimed to define the effect of rumen un-degradable protein (RUP) as different protein sources on the reproductive performance of growing Friesian male calves from March to September, 2010. Fifteen healthy male calves, (8:10 months of age, with average live body weight of 180±40 kg) were used and allocated to three groups (five animals each). Dietary treatments were: "control" (protein source: cotton seed meal (CSM) and maize gluten feed (MGF); (CP 13.15 %, 33% RUP of CP and TDN 66%), (SBM) ration (protein source was: soybean meal (CP 16.72, 34% RUP of CP and TDN 67%) and (MGM) ration (protein source was: maize gluten meal (CP 15.75, 45% RUP of CP and TDN 66%) . Feed intake, body weight and testicular size were measured monthly. Blood samples were taken monthly and semen was collected twice a week by artificial vagina from the 14th month of age in order to estimate semen characteristics.

Average daily dry matter intake (DMI) was 7.9, 7.12 and 7.29 kg for control, SBM and MGM groups, respectively. No significant differences were observed in average body weight gain (253.7, 256.0 and 261.7 kg), average daily gain (1.09, 1.15 and 1.12 kg), scrotal circumference (28.0, 29.4 and 29.0 cm), semen ejaculate volume (4.14, 4.25 and 4.10 ml), sperm-cell concentration (1.52, 1.49 and 1.53 X10^9/ml), sperm wave motion (80, 77 and 83 %) , dead spermatozoa (20, 23, and 17) and abnormalities sperm (6, 7 and 7) for control, SBM and MGM groups, respectively. There was a significant correlation between scrotal circumference and body weight ($P<0.001$, $r = 0.76$). No differences in blood measurements (total protein, albumen, aspartate-aminotransferase; (AST), alanine-aminotransferase; (ALT), alkaline phosphatase (ALP) acid phosphatase (ACP) and creatinine*)* were observed among treatments. The same result was recorded with blood testosterone. However, triiodothyronin (T3) hormone concentration was ($P<0.05$) different. Consequently, feeding male calves on rations contained different protein sources don't improve their reproductive performance.

Key words: Male calves, protein source, blood, semen, enzymes.

Introduction

Reproductive performance of livestock is determined by four factors: genetic, physical environment, nutrition and management. Also, most researchers suggested

that nutritional factors are perhaps the most crucial in terms of their direct effects on reproductive performance (**Smith and Akinbamijo, 2000).** Moreover, adequate nutrition is a vital part for bull's management, since; it allows young bulls to enhance the genetical expression for growth, that effects the predicted potential performance of their offspring, It is helping puberty to begin on time and allowing breeding starts moderately on 15 to 17 months of age. Furthermore, severing of hypo-nourishment may cause irreversible testicular damage in young bulls and decrease sperm production in mature bulls, there is no doubt that protein deficiency can reduce semen quality and sexual activity **(Okolski *et al.,* 1971 and Brown, 1994)**. Carbohydrate, protein and nucleic acid metabolism or their deficiency may impair fertility through spermatogenesis and libido in males. Eighty to 90% of the protein content of some feedstuffs with high-quality protein may be degraded in the rumen **(Beever, 1984)** which can result in protein deficiency for maximum live weight gain in growing ruminants **(Grigsby *et al.,* 1989).** In situations of nutritional stress, protein supplementation decreased age at puberty of bulls and improved semen quality **(Tegegne *et al.,* 1992)**. Likewise, nutrient availability supplementation with rumen un-degradable protein (RUP) reduced the decline in testicular size and improved epididymal sperm reserves (**Entwistle, (1983) and Ndama *et al*., (1983)**.

The objectives of the present study aimed to establish the effect of feeding a source high in RUP (gluten) and moderate (soy bean meal) and low (cotton seed meal+ gluten feed) on growth and reproductive performance of growing male Friesian calves.

Materials and Methods

The present study carried out at El-Karda Experimental Station, Kafer El-Sheikh Governorate, through (6 months) from March to September 2010. Fifteen healthy male calves, about 8 to 10 months of age, with average live body weight about 180±40 kg were allocated randomly into three equal groups according to their ages and their live body weights (5 animals each). In the first group, calves were fed ration contains both of cotton seed meal (CSM) and maize gluten-feed (MGF), as main sources of protein. While in the second and third group, calves were fed ration contains soybean meal (SBM) and maize gluten meal (MGM), respectively, as the main source of protein in their rations. Chemical analysis of the experimental rations was performed according to the methods of **A.O.A.C. (2002)** as shown in Table (1).

The nitrogen free extract (NFE) and organic matter (OM) were calculated by the difference. Also, rumen undegradable protein was determined subsequent ingredient according to NRC (1989). Daily feed residuals, were recorded and daily feed as dray matter (DMI), total nutrient digestibility (TDN) and crud protein (CP) / head / day consumptions were determined subsequent ingredient allowances according to NRC (1989) were adjusted monthly on the basis of the change of body weight.

Animals were weighed monthly. The testicular measurements including (scrotal circumference; (SC), testicular length; (TL) and testicular width; (TW)) of all calves were taken every month during the experimental period. Paired testicular volume (PTV) was calculated by the following formula [volume = 0.5236 x length x width2] according to **Bailey *et al.* (1998).**

Table 1. Chemical composition of the experimental rations (on dry matter basis).

Item	DM%	OM%	ASH%	CP%	EE%	CF%	NFE%	RUP%
Control ration	90.2	88.81	11.19	13.15	2.31	7.12	66.23	4.37
Soy bean ration	92.26	90.03	9.97	16.72	2.77	5.00	65.54	5.70
Gluten ration	88.35	91.96	8.04	15.75	3.95	6.20	66.06	7.11

Dray matter (DM), organic matter (OM), crud protein (CP), ether extract (EE), crud fiber (CF), nitrogen free extract (NFE) , organic matter (OM), rumen undegradable protein (RUP)

Semen collection was done twice a week from each bull in the morning between 7.00: 9.00 am. semen was transferred to a waterbath maintained at 37^{o}C. and direct microscopic examination was done. Semen-ejaculate volume (ml) was measured immediately after collection by the graduated semen collection tube, **(World Health Organization, (WHO, 1999)**. Progressive motility was observed by putting drop of semen sample on a warm glass-slide under bright field microscopy at 40X magnification and the percentage of sperm waves motion is determined **(WHO ,1999)**.

Sperm-cell concentration (X10^{9}/ml) was evaluated by the spectrophotometer. Spectrophotometer-clean cuvette containing 4.95 ml of 2.9% (wt/vol) -Na-citrate solution was inserted into the sample chamber of the spectrophotometer and percent transmission in wave length 550 (%T) was adjusted to 100 (**Muhammad *et al.*, 2009).** Live spermatozoa were estimated by differential staining technique using Eosin-Nigrosin stain (**Campbell *et al.*, 1953).** The morphological abnormalities of

spermatozoa were determined in the same slide smears, which prepared for live-dead ratio under light microscope at 100 X magnification.

Blood parameters:

Plasma total protein was measured colorimetrically according to **Gornal *et al.* (1949).** Plasma albumin was determined colorimetrically and Plasma creatinine Kineticly methods according to **Doumas *et al.* (1971)..** Also, plasma activity of aspartate aminotransferase (AST) and activity of alanine aminotransferase (ALT) enzymes were determined colorimetrically according to the methods described by **Young (1990)**. Blood plasma alkaline phosphatase (ALP) was determined by Kinetic method according to **Young (1990).** Plasma acid phosphatase (ACP) was determined colorimetrically according to **Kind and King (1954).**

Hormonal measurements:

Blood plasma testosterone was determined according to the method described by **Wilson and Foster (1992)**. Blood plasma triiodothyronin (T3) was determined by Coat-A- Total T3 Radioimmunoassay kit (USA) according to the method described by **Tietz (1995).**

Statistical analysis:

The experimental results were statistically analyzed using the General Linear Model Program **(SAS, 1999)** as data were subjected to analysis of variance and correlation. The differences among means were tested using Duncan's multiple range test **(Duncan, 1955)**.

Results and Discussion

Feed intake

Table 2. Effect of protein source on feed intake (kg).

Item	Control	SBM	MGM
Berseem hay	1.85	1.7	1.7
Silage	1.25	1.25	1.25
Experimental ration	4.8	4.17	4.34
DMI/head(kg)	7.9	7.12	7.29
TDN/ head(kg)	4.57	4.52	4.57
CP /head(kg)	0.95	0.99	0.98

Dray mater (DMI), total nutrient digestibility (TDN) and crud protein (CP), SBM= soy bean meal, MGM= maize gluten meal

The daily feed intake expressed as DM, TDN and CP per head per day for the different experimental rations are presented in Table (2). Data showed that, there were insignificant (P>0.05) differences among different groups in DMI/head (kg), TDN/head (kg) and in CP/head (kg).

Body weight and testicular measurements:

Mean values of body weight (BW) and average daily gain (ADG) are shown in Table (3). Both BW and ADG were insignificantly affected by diet, also mean ADG values being higher for SBM than MGM and CSM+MGF (control). As well as, body weight gain during 147-d experimental period did not differ between treatments. Furthermore, high undegradable protein source (UDP) produced body weight and average daily gain near that of the basal diet. This result is in agreement with **Steen (1988), Comerford *et al.* (1992) and Shafqat *et al.* (2002),** who fed beef cattle, beef steers and buffalo bull on ruminal escape protein source and found that source of supplementary protein did not influence body weights. However, **Grigsby *et al.* (1989), Karges *et al.* (1992), Zinn and Owens (1993) and Rocha *et al.* (1995)** found an increase in growth rate for calves steers and bull fed high ruminally degradable protein (RDP) diets. These results may indicate that bulls received sufficient amounts of an adequate diet can meet specific requirements **(Brown, 1994).**

Table 3. Effect of protein source on body weight changes (kg).

Item	Control	SBM	MGM	± SE
Initial weight	173.50	169.70	174.00	11.53
Final weight	330.40	335.00	336.80	15.70
Average weight	253.72	256.02	261.77	13.28
Average weight gain	157.00	165.40	163.00	10.33
Average Daily gain	1.09	1.15	1.12	0.05

Control: cotton seed meal + maize gluten feed was the main source of protein in diet. SBM: Soy bean meal was the main source of protein in diet. MGM: maize gluten meal was the main source of protein in diet. SE: (stander error).

Effect of protein source on testicular measurements is illustrated in Table (4). There were no significant difference between diets in SC, TW, TL and TV. Also, SC and TV values were insignificantly lower in control as compared to SBM and MGM groups. These results are in agreement with those of **Nolan *et al.* (1990) and Rocha *et al.* (1995)** who found no significant effect of different protein sources (fish meal and soy bean meal) on testicular dimensions in bulls. Furthermore, **Masters and Fels (1984)** showed that testicular volume is controlled by nutrition, nutrition appears to mediate its effect through increasing the frequency of pulses of leutinizing hormone

Table 4. Effect of protein source on testicular measurements at the experiment.

Item	Control	SBM	MGM	± SE
Scrotal circumference (cm)	28.05	29.40	29.00	0.752
Testicular width (cm)	5.44	5.95	5.98	0.173
Testicular length (cm)	13.06	12.68	13.25	0.401
Testicular volume (cm^3)	208.05	238.71	253.12	19.50

Control: cotton seed meal + maize gluten feed. SBM: Soy bean meal. MGM: maize gluten meal. SE: (stander error).

(LH) and probably follicles stimulating hormone (FSH) (**Sutherland and Martin, 1980, Lindsay *et al.*, 1984, Boukhliq *et al.*, 1997 and Hotzel *et al.*, 2003).**

Relationship between protein source to body weight and scrotal circumference in bulls is presented in Table (5). Correlation coefficient (r= 0.12) between protein source and ADG was found. In general, correlation coefficient was not statistically significant. Nevertheless, there was highly correlation between body weight, testicular parameter. Values were (r=0.93, r=0.79 and 0.65) between testicular volume with testicular width, scrotal circumference and testicular length, respectively.

Table 5. Correlation coefficients between body weights (BW), scrotal circumference (SC), paired testiculur volume (PTV), and treatment.

Item	Treatment	Body weight	Average daily gain	Testicular volume	Testicular length	Testicular width
Body Weight	0.08					
Average daily gain	0.12	0.55				
Testicular volume	0.29	0.70**	0.33			
Testicular length	0.06	0.40*	0.14	0.65*		
Testicular width	0.34*	0.70**	0.34*	0.93**	0.35*	
Scrotal circumference	0.1	0.76**	0.42*	0.79**	0.45*	0.79**

*=Significant correlation (P<0.05)

Semen characteristics:

The average semen-ejaculate volume produced by the bulls in control and treatment groups was 4.14, 4.25 and 4.10 (ml)groups, respectively (Table,6). Semen-ejaculate volume tended to be nearly similar between treatments. As well as, sperm-cell concentration ($X10^9$/ml), sperm motility and sperm abnormalities percentages were not affected by type of diet.

Also, the difference wasn't significant between treatments with live and dead spermatozoa (%). These results are similar to those obtained with **Wolfe *et al.* (1965)** and **Rocha *et al.* (1995)** who reported that there is no effect of feeding different protein sources on bull semen characteristics. These results could be expected due to the absence of an effect on testicular development or on testosterone concentration. In

addition, there is no effect on sperm-ell onentration in bulls fed adequate amounts of protein and energy that provided essential amino acids and nutrient according to those recommended by **NRC (1988)** which modulates reproductive function **(Donovan and O'Keefe, 1966 and McCann *et al*., 1972)**

A negative relationship between semen characteristics and type of diets was observed in Table (7). Likewise, no significant correlation between semen characteristics was observed but correlation between sperm abnormalities and dead spermatozoa was ($P<0.05$) significant.

Table 6. Effect of protein source on the bull semen characteristics.

Item	Control	SBM	MGM
Semen ejaculate volume (ml)	4.14± 0.45	4.25 ± 0.50	4.10±0.45
Sperm cell concentration ($x10^9$/ml)	1.52 ± 0.06	1.49± 0.07	1.53± 0.06
Live spermatozoa (%)	80.00± 2.02	77.00± 2.51	83.00± 2.39
Dead spermatozoa (%)	20.00± 2.37	23.00 ± 2.87	17.00 ±2.58
Sperm abnormalities (%)	6.00± 0.50	7.00± 0.55	7.00 ± 0.53
Wave motion (%)	80.00± 2.8	77.00± 2.00	78.00 ±2.15

Table 7. Correlation coefficients between semen parameter and treatment.

Item	Treatment	Semen ejaculate volume	Sperm motility	Sperm-cell concentration	Sperm abnormalities	Dead spermatozoa
Semen ejaculate volume	-0.23					
Wave motion	-0.20	0.14				
Sperm cell concentration	0.05	0.21	-0.12			
Sperm abnormalities	-0.14	-0.01	-0.02	-0.02		
Dead spermatozoa	-0.17	0.08	0.06	-0.25	0.29	
live spermatozoa	0.17	-0.08	-0.06	0.25	-0.29	-1**

Blood parameters:

No differences in blood plasma measurements were found between treatments Table, (8). in diet. SE: (stande

Most plasma parameters level was higher ($P>0.05$) in MGM treatment group more than SBM and control. The lowest ($P>0.05$) average values of acid and alkaline phosphatase activity were determined in control group. The same result was recorded in the concentration of plasma ALT. The mean values of total protein, albumen and creatinine weren't affected by diet.

Differences in blood plasma testosterone concentrations between treatments weren't significantly. However, values for SBM group were higher than for other groups. Concentration of triiodothyronin (T3) was significantly different by protein type. The higher value ($P < 0.05$) was recorded in control followed by MGM and

Table 8. Effect of protein source on blood plasma parameters.

Item	control	SBM	MGM	±SE
Total protein (gm /dl)	5.09	5.05	6.00	0.87
Albumen(gm/dl)	3.45	3.84	4.12	0.36
alkaline phosphatase (U/L)	40.97	69.99	76.35	25.99
Acid phosphatase (U/L)	11.71	12.71	17.30	3.35
Alanine aminotransferase (U/L)	11.92	13.39	13.60	1.43
Aspartate aminotransferase (U/L)	2.51	1.98	2.37	0.39
Creatinine (mg/dl)	0.83	0.88	0.77	0.07
Testosterone (ng/ml)	5.90	8.40	7.70	3.78
Triiodothyronin (ng/ml)	136.3^{a}	110.2^{b}	129.5^{ab}	7.15

Control: cotton seed meal + maize gluten feed SBM: Soy bean meal. MGM: maize gluten meal

SBM groups. The lack of difference in testosterone concentrations between bulls in the two treatments may be due to the lack of differences in testicular development **(Rocha *et al.*, 1995).**

The relationship between blood constituent and the type of protein is shown in (Table, 9) There was no significant (P > 0, 05) correlation between plasma testosterone, T3 concentration, and protein type. (r = 0.16 and 0.19, respectively). These results are in agreement with those of **Louis *et al.* (1994)** who found that testosterone concentration was not affected by protein intake (P >0 .0 5) in boar and indicating similar pituitary responsiveness to gonad trophic releasing hormone (GnRH) and (or) similar stores of LH in the pituitary.

Table 9. Correlation coefficients between blood plasma parameters and treatment ($p<0.05$).

Item	Trt	TP	ALB	ALP	ACP	AST	ALT	Cri	T
TP	-0.12								
ALB	-0.33	0.03							
ALP	-0.19	0.27	0.06						
ACP	0.15	-0.44	0.03	0.15					
AST	-0.28	-0.02	-0.04	-0.17	-0.12				
ALT	0.23	-0.51	0.06	-0.20	0.31	-0.31			
Cri	-0.05	-0.39	0.43	0.26	0.41	-0.24	0.25		
T	0.16	-0.14	0.04	0.12	-0.11	0.02	0.22	0.23	
T3	0.19	-0.14	0.14	-0.10	0.45	-0.13	0.12	0.29	0.04

Trt= treatments, TP =Total protein, ALB= Albumen, ALP= alkaline phosphatase, ACP= Acid phosphatase, AST=aspartate aminotransferase, ALT= alanine aminotransferase, Cri= Critinene, T= Testosterone, T3= Triiodothyronin

Conclusion

In conclusion, feeding male calves on rations (cotton seed meal with gluten-feed or soy bean meal or gluten meal) don't improve their reproductive performance. On the other hand, there was highly correlation between body weight and testicular parameter.

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