Comparison of time frame critical for feed supplement on reproductive hormones of Azawak cattle in semi-arid zone of Niger Republic.

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Abstract

This experiment was conducted to evaluate the effects of feed supplementation on laying reproductive hormones level. In total, 36 Azawak cattle breed (18 bulls and 18 heifers) with an average initial body weight (BW) of 184 ± 40 kg aged 3 to 4 years owned by CERRA, Maradi ranch were used for this experiment. A time frame critical for utilization of supplement (NS: No supplementation, SMD: Supplementation early at 8:00 am in the morning daily before moving out for grazing, SED: Supplementation in evening at 5:00 pm daily after the afternoon grazing, SMED: Supplementation in the morning at 8:00 am and in the evening at 5:00 pm daily, S2DM: Supplementation once every two days in the morning at 8:00 am before morning out for grazing and S2DE: Supplementation once every two days in the evening at 5:00 pm). The feeding trial lasted for 90 days. The data of chemical composition of feed supplement were analyzed by the following statistical procedures: one-way ANOVA. The data from reproductive hormone levels after feeding supplementation were analyzed by using 2×6 factorial laid in Completely Randomized Design (CRD). The factors evaluated were two sexes (bull and heifer) by the six time and frame (treatments) of supplementation. Differences between sex and treatment were determined by the T-test mean $\pm$ SD separation and Least Significant Difference (LSD). For all analyses, a P value < 0.05 was considered statistically significant and a P value > 0.05 was considered not significant. The results showed that effects of inorganic minerals on reproductive hormones per treatment were obtained. The higher (2.78 ± 5.68 ng/mL) and lower (0.34 ± 0.37 ng/mL) values of testosterone content was obtained from SMD and NS followed by (1.79 ± 0.60 ng/mL) and (1.17 ± 0.25 ng/mL) from progesterone in NS and S2DE while values of estradiol E_2 were (281.67 ± 70.48 pg/mL) and (217.17 ± 52.06 pg/mL) in NS and SMD respectively. However, a higher (5.20 ± 0.67 mIU/mL) and lower (4.25 ± 0.88 mIU/mL) values of follicle-stimulating hormone was obtained from S2DM and SMD. Accordingly, result obtained of heifer indicated higher (0.47 ± 0.49 ng/ml) and lower (0.18 ± 0.02 ng/ml) values of testosterone content was obtained from SMD and NS followed by (1.60 ± 0.65 ng/mL) and (1.02 ± 0.21 ng/mL) from progesterone in NS and SMD while values of estradiol E_2 were (237.33 ± 44.28 pg/mL) and (198.33 ± 32.08 pg/mL) in NS and SMD, respectively. However, a higher (5.23 ± 0.84 mIU/mL) and lower (4.10 ± 0.98 mIU/mL) values of follicle-stimulating hormone was obtained from S2DM and SMED, respectively. According to result obtained of bull, the higher (5.09 ± 8.02 ng/mL) and lower (0.49 ± 0.52 ng/mL) values of testosterone content was obtained from SMD and NS followed by (1.97 ± 0.60 ng/mL) and (1.16 ± 0.07 ng/mL) from progesterone in NS and S2DE while values of estradiol E_2 were (326.00 ± 67.55 pg/mL) and (234.00 ± 32.14 pg/mL) in NS and SMED respectively. However, a higher (5.17 ± 0.64 mIU/mL) and lower (4.13 ± 0.50 mIU/mL) values of follicle-stimulating hormone was obtained from S2DM and SED. Conclusively, inorganic minerals in feed

supplementation have beneficial effect on reproductive hormones of Azawak cattle breed.

Key words: Reproductive Hormones, Feed Supplement, Azawak Cattle Breed, Semi-arid Zone.

Introduction

Hormones are chemicals released by a gland at critical times and transfers messages from one part of the body to the target cells. Normally, types of cells which release specific hormones are gathered into a gland such as thyroid, testes or pituitary (Yazdan, 2013). Hormones can be released from one area in the body and be distributed by the bloodstream and cause physiological changes in other parts of the body (Griffin, 2000). Generally, hormonal functions are classified in 3 main parts which includes manipulation of growth, effect on productions and effect on reproduction (Squires, 2003). Growth is the increase in body size and weight which is happening by increase in size and weight of cells. Growth is also due to increase in deposition of protein, as well as lipid. Hormones affect the extent of the deposition of muscle and fat which affect the lean yield of carcass. Hormone can also affect feed intake; feed conversion efficiency; growth performance; meat quality, tenderness, juiciness, flavour and water holding capacity (Yazdan, 2013). The aim of this study was comparison of time frame critical for feed supplementation on reproductive hormones of azawak cattle breed in semi-arid zone of Niger Republic.

Materials and methods

Experimental animals and management

A total number of thirty six (36) Azawak cattle (18 males and 18 females) with an average initial body weight (BW) of 184±40 kg aged 3 to 4 years owned by CERRA, Maradi ranch, Niger Republic were used for the experiment. The animals were quarantined for 3 weeks and ear tagged for identification. The trial was

conducted at CERRA, Maradi. The cattle were grouped into six animals per experimental diet. They were arranged in six different treatments of feed. The experimental animals were allowed to move out for normal grazing within the rangeland twice daily (8:30am–1:30pm and 2:30pm–4:30pm) and supplement were given daily. The houses were disinfected with Izal® solutions and were allowed to dry for one week before the commencement of the experiment. The pens were cleaned fortnightly. The animals were housed in a cage (3 m long, 3 m wide and 2 m high). The cages were enriched with iron platforms and parallel iron bars. The house is located at the border of a gallery forest, in an area of environmental protection. Before the commencement of the experiment, animals were given prophylactic treatment of Ivermectin (0.2 mg/kg sub-cutaneous), Oxytetracycline (5 mg/kg intra-muscular) and multivitamin (10 ml/kg intra-muscular) injections were administered. The animals were supplemented to provide necessary minerals for 90 days. Water was supplied *ad-libitum* through the watering place.

Feed composition of the experimental diet

The composition of experimental diet contained 35, 15, 10, 15 and 1% of millet stalk, cotton seed cake, *Faidhebia albida* pod, wheat bran and salt. Also, added to it were cowpea haulms (13%), urea (1%), phosphorus (5%) and calcium (5%).

Chemical analysis

The samples of feed supplement used in the experiment was grounded in a Wiley mill through a 1mm screen and were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash according to the standard methods of AOAC (2000). Nitrogen content was

determined by the micro-Kjeldahl method, while crude protein (CP) was calculated by multiplying the nitrogen content with a factor of 6.25. However, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the methods of Van Soest et al. (1991). Calcium (Ca) and Total Phosphorus (TP) were estimated using an atomic absorption spectrophotometer (AAS).

Experimental design

For this study, samples of blood were taken via jugular vein from Azawak cattle, a time frame critical for utilization of supplement: i) No supplementation (NS), ii) Supplementation early in the morning (8:00am) daily before moving out for grazing (SMD), iii) Supplementation in the evening (5:00pm) daily after the grazing (SED), iv) Supplementation in the morning (8:00am) and in the evening (5:00pm) daily (SMED), v) Supplementation once every two days in the morning (8:00am) before moving out for grazing (S2DM) and vi) Supplementation once every two days in the evening (5:00pm) (S2DE) and two (2) sexes (male and female) were used. Bloodletting was performed from apparently health cattle. Blood samples were collected in test tubes containing Ethylene Diamine Tetraacetic Acid (EDTA) as an anticoagulant. These tubes were placed in an icebox and carried to the laboratory within 4 h of collection. In the laboratory, these samples were centrifuged at 3000 rpm for 10 minutes; the plasma was separated and stored at -4°C for further analysis. The separated serum was stored at -20°C in sterilized glass vials for estimation of testosterone. Testosterone concentration in blood serum samples was determined using RIA kits supplied by Immunotech, France. The unknown samples and standard samples were incubated with ¹²⁵I serum-labeled testosterone in antibody

coated tubes. After incubation, the liquid content of the tubes was aspirated and the bound radioactivity was determined in a gamma counter (Packard, USA). A standard curve was prepared with 6 standards and testosterone concentration in unknown samples was obtained from the curve by interpolation. The serum was separated from the blood and was transferred into sterile EDTA tubes, labeled and stored in deep freeze (-20°C) until hormonal assay for estradiol E₂ was carried out. Estradiol E₂ hormone levels were estimated by Enzyme-Linked Immuno-Sorbent Assay (ELISA) method. Plasma progesterone concentration was assayed as described by Webb *et al.* (1977) and plasma follicle stimulating hormone (FSH) was assayed as described by Webb *et al.* (1980). The assay reliability criteria did not differ significantly from those previously published.

Statistical analysis

The data from this study were coded and later analyzed using statistical package for social science (SPSS) version 20. The data of chemical composition were analyzed by the following statistical procedures: one-way ANOVA. Significant differences were detected with $P < 0.05$ and means were separated by Duncan's Multiple Range Test (DMRT). The data from reproductive hormone levels after feeding supplementation were analyzed by using 2 x 6 factorial laid in Completely Randomized Design (CRD). The factors evaluated were two sexes (bull and heifer) by the six time and frame (treatments) of supplementation. Differences between sex and treatment were determined by the T-test mean $\pm$ SD separation and Least Significant Difference (LSD). For all analyses, a P value < 0.05 was considered statistically significant and a P value > 0.05 was considered not significant.

Time frame critical for feed supplement on reproductive hormones

Results

Chemical composition of feed supplementation

The results of the proximate composition of feed supplementation added inorganic minerals sample were presented in Table 1. From the results obtained, the value of ash content was obtained ($9.18 \pm 0.25\%$) followed by $8.92 \pm 0.59\%$ from moisture while value of protein were $22.11 \pm 1.22\%$. However, the value of crude fiber was

obtained from $45.64 \pm 0.54\%$. Also, the value of ether extract was obtained from 9.85 ± 0.35 while nitrogen free extract had $13.24 \pm 2.37\%$. Value of metabolizable energy was 2086.57 ± 10.16 kcal and value of dry matter was $91.09 \pm 0.59\%$. Acid detergent fiber had 40.65 ± 0.35 value while the value of nitrogen detergent fiber was obtained from $50.28 \pm 0.55\%$. Calcium value was 148.53 ± 5.35 mg/kg while total phosphorous was 45.86 ± 1.36 mg/kg.

Table 1: Chemical composition of experimental diet

Feed materials	Percentage
Millet stalk	35
Cowpea haulms	13
<i>Faidhebia albida</i> pods	10
Wheat bran	15
Cotton seed cake	15
Common salt	1
Urea	1
Phosphorus	5
Calcium	5
Total	100
Constituents	Mean$\pm$SD
DM(%)	91.09 ± 0.59
Ash(%)	9.18 ± 0.25
CP(%)	22.11 ± 1.22
CF(%)	45.64 ± 0.54
EE(%)	9.85 ± 0.35
NFE(%)	13.24 ± 2.37
ME (kcal/gDM)	2.09 ± 0.01
ADF(%)	40.65 ± 0.35
NDF(%)	50.28 ± 0.55
Ca (%)	14.85 ± 0.54
TP (%)	4.59 ± 0.14

DM = Dry Matter, CP = Crude Protein, CF = Crude Fiber, EE = Ether Extract, NFE = Nitrogen Free Extract, ADF= Acid Detergent Fiber, NDF = Nitrogen Detergent Fiber, ME = Metabolizable Energy, Ca = Calcium, TP = Total Phosphorus, SD = Standard Deviation.

Effect of time and frame of feed supplementation on reproductive hormones profiles (bull and heifer)

Effect of time frame of feed supplementation on reproductive hormones profiles (male and female combined) were presented in Table 2. From the results obtained, the higher (2.78 ± 5.68 ng/mL) and lower (0.34 ± 0.37 ng/mL) values of testosterone content was obtained from

SMD and NS followed by (1.79 ± 0.60 ng/mL) and (1.17 ± 0.25 ng/mL) from progesterone in NS and S2DE while values of estradiol E2 were (281.67 ± 70.48 pg/mL) and (217.17 ± 52.06 pg/mL) in NS and SMD, respectively. However, a higher (5.20 ± 0.67 mIU/mL) and lower (4.25 ± 0.88 mIU/mL) values of follicle-stimulating hormone was obtained from S2DE and SMD.

Table 2: Effect of time frame of feed supplementation on reproductive hormones profiles (bull and heifer)

Reproductive Hormones	Treatment						Pr>F
	NS	SMD	SED	SMED	S2DM	S2DE	
Testosterone (ng/mL)	0.34±0.37	2.78±5.68	0.76±0.68	0.94±1.25	1.42±2.68	0.43±0.34	0.009*
Pogesterone (ng/mL)	1.79±0.60	1.28±0.75	1.27±0.28	1.31±0.39	1.35±0.48	1.17±0.25	0.182ns
Estradiol E ₂ (pg/mL)	281.67±70.48	217.17±52.06	241.17±27.15	226.00±29.42	244.17±45.18	237.50±39.14	0.608ns
FSH (mIU/mL)	4.45±1.91	4.25±0.88	4.25±1.03	4.58±1.44	5.20±0.67	5.03±1.86	0.040*

NS = No Supplementation, SMD = Supplementation early in the morning (8:00am) daily before moving out for grazing, SED = Supplementation in the evening (5:00pm) daily after the grazing, SMED = Supplementation in the morning (8:00am) and in the evening (5:00pm) daily, S2DM = Supplementation once every two days in the morning (8:00am) before moving out for grazing, S2DE = Supplementation once every two days in the evening (5:00pm), FSH = Follicle-Stimulating Hormone, SD = Standard Deviation, Pr = Probability, **= highly significant, *= significant, ns = no significant

Effect of time frame of feed supplementation on reproductive hormones profiles of heifer

Table 3 presented the results of effect of time frame of feed supplementation on reproductive hormones profiles from female. From the results obtained, the higher (0.47±0.49 ng/mL) and lower (0.18±0.02 ng/mL) values of testosterone content was obtained from SMD and NS

followed by (1.60±0.65 ng/mL) and (1.02±0.21 ng/mL) from progesterone in NS and SMD while values of estradiol E₂ were (237.33±44.28 pg/ mL) and (198.33±32.08 pg/ml) in NS and SMD, respectively. However, a higher (5.23±0.84 mIU/mL) and lower (4.10±0.98 mIU/mL) values of follicle-stimulating hormone was obtained from S2DM and SMED, respectively.

Table 3: Effect of time frame of feed supplementation on reproductive hormones profiles of heifer

Reproductive Hormones	Treatment						Pr>F
	NS	SMD	SED	SMED	S2DM	S2DE	
Testosterone (ng/mL)	0.18±0.02	0.47±0.49	0.23±0.01	0.21±0.04	0.20±0.04	0.19±0.05	0.000**
Progesterone (ng/mL)	1.60±0.65	1.02±0.21	1.29±0.31	1.35±0.50	1.40±0.76	1.19±0.38	0.137ns
Estradiol E ₂ (pg/mL)	237.33±44.28	198.33±32.08	235.67±7.77	218.00±30.64	224.33±57.74	231.00±59.51	0.238ns
FSH (mIU/mL)	4.53±2.50	4.17±1.37	4.37±1.53	4.10±0.98	5.23±0.84	5.17±2.68	0.100ns

NS = No Supplementation, SMD = Supplementation early in the morning (8:00am) daily before moving out for grazing, SED = Supplementation in the evening (5:00pm) daily after the grazing, SMED = Supplementation in the morning (8:00am) and in the evening (5:00pm) daily, S2DM = Supplementation once every two days in the morning (8:00am) before moving out for grazing, S2DE = Supplementation once every two days in the evening (5:00pm), FSH = Follicle-Stimulating Hormone, SD = Standard Deviation, Pr = Probability, **= highly significant, *= significant, ns = no significant

Effect of time frame of feed supplementation on reproductive hormones profiles of bull

Table 4 presented the results of effect of time frame of feed supplementation on

reproductive hormones profiles from male. From the results obtained, the higher (5.09±8.02 ng/mL) and lower (0.49±0.52 ng/mL) values of testosterone content was obtained from SMD and NS followed by

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(1.97±0.60 ng/mL) and (1.16±0.07 ng/mL) from progesterone in NS and S2DE while values of estradiol E₂ were (326.00±67.55 pg/mL) and (234.00±32.14 pg/mL) in NS and SMED, respectively. However, a

higher (5.17±0.64 mIU/mL) and lower (4.13±0.50 mIU/mL) values of follicle-stimulating hormone was obtained from S2DM and SED.

Table 4: Effect of time frame of feed supplementation on reproductive hormones profiles of bull

Reproductive Hormones	Treatment						Pr>F
	NS	SMD	SED	SMED	S2DM	S2DE	
Testosterone (ng/mL)	0.49±0.52	5.09±8.02	1.30±0.54	1.67±1.53	2.64±3.67	0.67±0.34	0.000**
Progesterone (ng/mL)	1.97±0.60	1.53±1.07	1.26±0.31	1.28±0.34	1.30±0.02	1.16±0.07	0.002**
Estradiol E ₂ (pg/mL)	326.00±67.55	236.00±68.42	246.67±41.14	234.00±32.14	264.00±24.25	244.00±12.77	0.034*
FSH (mIU/mL)	4.37±1.67	4.33±0.25	4.13±0.50	5.07±1.88	5.17±0.64	4.90±1.18	0.018*

NS = No Supplementation, SMD = Supplementation early in the morning (8:00am) daily before moving out for grazing, SED = Supplementation in the evening (5:00pm) daily after the grazing, SMED = Supplementation in the morning (8:00am) and in the evening (5:00pm) daily, S2DM = Supplementation once every two days in the morning (8:00am) before moving out for grazing, S2DE = Supplementation on ce every two days in the evening (5:00pm), FSH = Follicle-Stimulating Hormone, SD = Standard Deviation, Pr = Probability, **= highly significant, *= significant, ns = no significant

Discussion

The nutritive characteristics of feed supplementation ingredients are shown in Table 1. This dry matter content indicates all constituents excluding water of the ingredients used in the formulation. Higher DM content was recorded in feed supplementation. The DM in this study is slightly above the values reported by Mubi *et al.* (2013) but similar to the range reported by (Zarah *et al.*, 2014). The crude protein content of the feed from this study was slightly lower than what was reported by Addass *et al.* (2011) in an experiment using cattle. This variation was attributed to the environmental factor, diet formulation and analytical procedure. However, the crude protein content of the diet is sufficient for ruminants which will provide ammonia required by rumen microorganism to support optimum microbial activity (Norton 2003). This differences and variation in crude protein percentage among diets may be due to the type of protein source and its level of inclusions in

the rations. This is slightly different from what was reported by Onwuka (1999); Mohammed *et al.* (2007) and Huiling *et al.* (2009), respectively. The differences observed could also be associated with soil nitrogen condition, level of maturity of the crop residue and varietal differences. The values presented from Crude fibre was also agrees with the report by Addass *et al.* (2011) and also in line with the report by Malgwi *et al.* (2015). This feed supplementation recorded highest crude fibre level. Such high crude fibre content of the diet could be due to the quality and fibrous nature of ingredients used which reduces digestibility rate of the diet as well. Ether extract was obtained was higher compared to the work of Kinfemi *et al.* (2009) The mean ash content of feed supplementation is a little higher than the values reported by Dibal (1991) for semi-arid browse plants. The ME content in MJ/kg DM also followed the same trend as reported by various authors. However, The NDF content from feed was higher than that

was reported by Pereira *et al.* (1998), Batajoo and Shaver (1998). The ADF content from diet was higher than the report by Pereira *et al.*, (1998) and NRC (2001). Many factors affect chemical composition such as oil extraction process (Mara *et al.*, 1999) stage of growth (Promkot and Wanapat, 2004) maturity and species or variety (von Keyserlingk *et al.*, 1996; Agbagla-Dohnani *et al.*, 2001). Values of Ca and TP was in agreement with the reports by (Ikram *et al.*, 2010) who reported that the observed differences in mineral composition in these products may be due to genetic factor added in different level. Decrease in minerals, energy and protein contents also contribute to lower intake, reduced digestibility and consequently losses in weight by grazing animals. The report of Chinast and Mayer (1977) was in line with the findings of this study who reported that the crude protein content of tropical forages decreases during the advanced periods of the dry season. Feeding plays an important role in the performance of the animals. An experiment is designed to provide higher plane of nutrition (Chauhan *et al.*, 2000) and vitamin E and mineral supplementation for augmenting the improvement in reproductive performance. Effect of feed supplementation on testosterone and FSH concentration in test group increased significantly as compared to control. In test group, the end of treatment from testosterone and FSH concentrations in serum were significantly ($P < 0.05$) higher as compared to initial day. Kohrle *et al.* (2007) reported that sufficient amount of inorganic minerals is required for thyroid hormone homeostasis because deiodinases are seleno-enzymes which catalyze activation of testosterone. Similar reports were recorded by Kumar *et al.* (2006) and Imam *et al.* (2009) in crossbred bulls. In buffalo bulls, after supplementation with

inorganic minerals, Om *et al.* (1996) reported decreased conversion of testosterone to dihydrotestosterone in liver, increased formation of estradiol from testosterone and decreased luteinizing hormone, testosterone and estradiol level in cattle fed with Zn deficient diets. The effect of inorganic minerals on progesterone and estradiol E_2 were not differed significantly ($P > 0.05$) after treatment. Estradiol can also inhibit pulsatile LH secretion in cows (Walters *et al.*, 1982).

Conclusion

It could be concluded that inorganic minerals in feed supplementation has beneficial effect on reproductive hormones of Azawak cattle breed. Further studies may need to evaluate the effects of semen quality parameter and libido of bulls.

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