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Influence of Different Cutting Dates on Cornell Net Carbohydrate and Protein System (CNCPS) Parameters and the Fatty Acid Compositons of Caramba Hay *(Lolium multiflorum cv. caramba)*

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ABSTRACT

The aim of the study was to determine the influence of different cutting dates on Cornell Net Carbohydrate and Protein System (CNCPS) parameters and the fatty acid (FAs) compositons of caramba hay (*Lolium multiflorum cv caramba*). The samples were taken from the five randomized plots at the three different cuts (first cut:before blossom, second cut:blossom 50%, and third cut:after blossom). The samples were analyzed including the crude protein (CP), ether extract (EE), CP fractions (A= NPN, B_1= fast, B_2= intermediate, B_3= slow and C= not fermented and available for the animal), degradable intake protein (DIP), undegradable intake protein (UIP) and the FAs compositions (C14:0, C16:0, C18:0, C18:1, C18:2 and C18:3). The CP, EE and A_1 fraction were negatively affected by the different cutting dates ($P<0.05$), while the B_2, B_3 and C fractions were increased by growing stage ($P<0.05$). Different cutting dates affected total major FAs, and decreased the concentration of C18:3 ($P<0.05$) and increased those C16:0 ($P<0.05$) and C18:2 ($P>0.05$). A positive strong linear relation was found between the C18:3 and CP contents in caramba hay (R^2= 0.769, $P<0.001$). The study showed that CP, soluble protein ($A+B_1$ fraction) and C18:3 were significantly decreased, the other crude protein fractions (B_2, B_3 and C) and other major FAs (C16:0 and C18:2) were increased by growing stage.

Keywords: CNCPS parameters; Fatty acids; Cutting date; Caramba

1. Introduction

Caramba (*Lolium multiflorum, cv. caramba)* which is a perennial forage grass, is rich especially protein, minerals and water-soluble carbohydrate content. Further its stem does not mature quickly until the time of harvest (Dewhurst et al 2001). In recent years it is stated that caramba is quite well adapted to Turkey climate and soil condition, so caramba has been recognized as potential forage for ruminant animals (Özelçam et al 2015). The livestock breeding in Turkey is largely based on pasture. However, because of agricultural mechanization, total range or pasture areas of Turkey have been drastically reduced (Kusvuran & Tansı 2011).

It is very important to know the crude protein (CP) fractions and total fatty acids (FAs) of the dietary feedstuffs to determine diet composition for target milk or meat composition (Ferlay et al 2006). The CP and FAs of perennial forages are affected by numerous factors such as plant species and variety, climate, day length, rainfall, fertilization, stage of growth and storage methods (Amrane & Michalet-Doreau 1993; Aganga et al 2004; Kalač & Samková 2010). In perennial grasses, the highest level of CP and the C18:3 content of FAs in young plants at the first cut, and then it decreases during summer, particularly around blooming (Dewhurst et al 2001; Boufaïed et al 2003). Also, knowledge of the factors that influence the CP and FAs of forages could help farmers to optimize cultivation and harvest conditions and thereby improve the quality of their forages (Khan et al 2012). The efficiency of nitrogen use and decrease nitrogen excretion to the environment is the important sustainability parameters on ruminant farms (Haugen et al 2006). The organic forages are the most necessary key to prevent the protein deficiency in organic ruminant rations, so forage protein analysis comes on the top of the list in accurately formulating rations (Pacheco et al 2012). Some researchers stated that rumen CP degradability may be reliably predicted from Cornell Net Carbohydrate and Protein System (CNCPS) parameters (Shannak et al 2000; Branco et al 2012). The perennial grass has been used as an animal feed as a fresh grass with grazing or after the processes of silage making or haymaking. The important losses occur in the content of FAs in perennial ryegrass, because of the loss of precursor fatty acids during the processes of silage making or haymaking. Therefore, the major FAs of fresh caramba are higher than silage or hay forms (Aii et al 1988). Although hays contain relatively low level of FAs on lipids, they are cheapest and often the major source of unsaturated FAs in ruminant diets (Kalač & Samková 2010). Also, the data on chemical composition and the FAs of caramba hay have been scarce compared with fresh or silage forms (Glasser et al 2013; Özelçam et al 2015).

The objective of the study was to investigate the influence of three cutting dates on CNCPS parameters and the FAs compositons of caramba hay.

2. Material and Methods

In this study, caramba *(Lolium multiflorum cv. Caramba*) is used as the material from Küçük Menderes basin at the Aegean Region which has the characteristics of Mediterranean climate. The summer season is warm and dry, and winters are temperate and rainy. The caramba was planted the research plots of Ege University, Ödemiş Vocational School Experiment Farm at Izmir (38°13'03" North, 27°57'50" East) from November in 2010 to June in 2011. Caramba samples were taken from the five randomized plots (2 m x 5 m) of the experimental farm at the three cuts of 2011 year. The three cuts were the first cut (before blossom), second cut (50%, blossom) and third cut (after blossom). The dry matter (DM) contents of the fresh caramba were 180.1, 200.1 and 264.7 g kg^{-1} for the first cut, second cut and third cut, respectively.

2.1. The climate, soil, planting and harvesting conditions

The soil and climate conditions of the experimental farm did not show a restrictive effect on caramba planting in irrigated conditions. The average temperature is 15.3 °C and total rainfall precipitation is 510 mm from November in 2010 to June in 2011 (Anonymous 2014). The soil (0-20 cm) where the study conducted had pH 7.28, salt 0.030-0.095%, organic matter 1.13-1.58%, $CaCO_3$ 1.44-21.52%, N 0.11-0.16%, P 20.50-40.52 mg kg^{-1}, K 110-400 mg kg^{-1}. The soil was generally sandy-loam texture. In the experiment, the raw spacing was 20 cm and the amount of the seed per hectare was 25 kg. Before planting, the plots were received 50 kg N (nitrogen) and P_2O_5 (phosphorus) per hectare by taking 15.15.15 compose base fertilizer. In spring, each plot was received 50 kg N per hectare as base application. Harvesting were made at 4-5 cm height at the before blossom, blossom (50, %) and after blossom.

2.2. The chemical compositions and the Cornell net carbohydrate and protein system parameters

The DM, CP and ether extract (EE) values of the caramba hay (air-dried samples) were measured by AOAC-approved methods (AOAC 1995). The standardized method of Licitra et al (1996) was used to determine the CNCPS parameters. The caramba hay samples were grinded using 1 mm sieve. The A fraction (non-protein nitrogen/NPN) is traditionally the nitrogen passing into the filtrate after precipitation with tungstic acid. The B_1 fraction is true soluble protein in borate-phosphate buffer at rumen pH (6.7-6.8). The Whatman#54 filter paper was used for filtration without vacuum to determine the A and B_1 fractions. The A+B_1 fractions generate total soluble protein (SolP). The cell wall components of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by Van Soest (1994). After the *r*esidue paper of NDF and ADF were transferred into a Kjeldhal flask for protein determination of neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP). Degradable intake protein (DIP, g kg^{-1} DM) was calculated by using the following equations and undegradable intake protein (UIP) was calculated by CP-DIP. The equations: A fraction (NPN): RDPA, rumen soluble protein; B_1 fraction (fast soluble protein): $RDPB_1$, $(B_1x(Kd_{B1}/Kd_{B1}+Kp_{1x}))$; B_2 fraction (intermediate degradable protein): $RDPB_2$: $(B_2x(Kd_{B2}/Kd_{B2}+Kp_{1x}))$; B_3 fraction (slow degradable protein): $RDPB_3$, $(B_3x(Kd_{B3}/Kd_{B3}+Kp_{1x}))$; DIP (Degradable intake protein): RDP_{TOTAL}= RDPA+$RDPB_1$+$RDPB_2$+$RDPB_3$. In these calculations the values stated in Sniffen et al (1992) and Fox et al (2003) were used for the coefficients of outflow rate on the different levels of DM intake (Kp) and degradation rate of B fractions (Kd), respectively. (DIP_{1X}= at 1x maintenance level of intake, DIP_{2x}= at 2x maintenance level of intake, and DIP_{3x}= at 3x maintenance level of intake)

2.3. The fatty acids (FAs)

The lipid fraction (approximately 0.5 g) from caramba hay was extracted with chloroform-methanol at a ratio of 2:1; the lipid was then isolated in the chloroform phase after adjustment of the solvent ratio to 2:2:1 (chloroformmethanol-water, v/v). The chloroform phase was removed and evaporated to dry under vacuum heater below 40 °C (Folch, Lees & Sloane-Stanley). The lipid is refluxed with a 1M solution of potassium hydroxide in 95% methanol. Then all lipid samples were analyzed using a gas-liquid chromatograph to determine the FAs (Agilent Technologies 6890 N Network GC System, Anaheim, CA, USA, Thermo Scientific TRACE TR-FAME GC Column; 60 mL, 0.25 mm ID, 0.25 um thick) at the University of Ege, Central Analytical Laboratory. Detector temperature: 250 °C, injection block temperature: 250 °C, Owen temperature: gradually from 2 °C to 240 °C, Split flow 119.9 mL min^{-1}, helium as the carrier gas. The FAs were identified by comparing their retention time and fragmentation pattern with an established standard (SUPELCO 37 Comp. Fame mix 10 mg mL^{-1} in $CH_{12}Cl_2$). The FAs; saturated (myristic C14:0, palmitic C16:0 and stearic C18:0), monounsaturated (oleic C18:1 and linoleic C 18:2) and polyunsaturated (γ-linoleic C18:3) were expressed as the percentages of total lipids.

2.4. Statistical analyses

All data were subjected to one-way ANOVA by using the statistical package of SPSS (15.0®) (SPSS 2006). Significant differences among the means were determined by the Duncan's multiple range tests. The relationship between the values the unsaturated FAs and the CP in caramba hay were determined by stepwise simple linear regressions.

3. Results and Discussion

3.1. The chemical compositions and crude protein fractions

The results of chemical compositions and CP fractions of caramba hay are shown in Table 1. When the growth period is increased by the different cuts (before blossom, 50% blossom and after blossom), the results of the CP and EE were decreased as an expected (Table 1). The differences between these contents were largely due to the different cutting

dates. The first cut had the highest CP content of caramba hay compared to the other cuts (P<0.05). The CP results in this experiment were agreed with Amrane & Michalet-Doreau (1993) and Aganga et al (2004) that CP of caramba hay is decreased depending on different cutting dates. Similar to these references, first cut had the highest CP content. The CP and EE contents of second cut of caramba hay in the present experiment were close to the CNCPS ver. 5 feedbank data (Fox et al 2003) (respectively, 86.0 g kg^{-1} DM and 22.0 g kg^{-1} DM). Also, similar to the this study with third cut, Özelçam et al (2015) stated that the CP was 63.5 g kg^{-1} DM and EE was 18.4 g kg^{-1} DM in the for caramba hay.

The CNCPS parameters of chemical compositions were compared with the values of the CNCPS ver. 5 feedbank and those determined by Fortina et al (2003). The CNCPS parameters of caramba hay on the CNCPS ver. 5 feedbank were similar to the second cut of the our results (bloosom) except NPN (SolP%). The average NPN (96 of SolP%) on the feedbank was higher than in our results for the all cutting times (Table 1). The differences between the NPN could be attributed to the different reagents (tungistic acid vs tricloratic acid) and filtration methods (Fortina et al 2003). However, we used tungistic acid in our study. On the other hand, when we compared our results with Fortina et al (2003), for caramba hay, although CP content (176 g kg^{-1} DM) is higher than our results, SolP (37.2 CP, %) was very close for the second cut. SolP, NPN (Solp%) and A fraction of the study were decreased by increasing vegetation stage and this decrease was important for Solp and A fraction (P<0.05). Similarly, Villiers & Ryssen (2001) stated that **t**he soluble N fraction, rate of degradation of the potentially degradable fraction and effective N degradability of herbage decreases with advancing stage of growth. Also, the results of B_1 fraction for the all cuts were low as explained in Sniffen et al (1992) that B_1 fraction of forages is lower than other fractions. The crude protein fractions of the Fortina et al (2003) study were close to the results for A, B_1 and B_2 fractions, were higher for B_3 fractions and lower for C fractions. The variations for the B_3 and C fractions could be due to the conventional or filter bag methods to determine NDIP and ADIP (Bovera et al 2003). Like Polat et al (2014), DIP values decreased and UIP values increased in accordance with the increased feeding level of DM intake (1x,

Table 1- Chemical compositions and crude protein fractions of caramba hay

Parameters	*1st cut (Before blossom)*	*2nd cut (Blossom, 50%)*	*3th cut (After blossom)*	*P value*
DM, g kg^{-1}	923.50±1.70 b	932.20±1.50 a	935.50±0.90 a	0.003
CP, g kg^{-1} DM	106.10±4.30 a	92.40±3.10 b	62.70±3.10c	0.000
EE, g kg^{-1} DM	28.40±1.30 a	24.40±2.10 ab	20.30±1.20 b	0.013
SolP, % of CP	50.08±2.01 a	36.73±1.59 b	17.63±1.58 c	0.000
NPN, % of SolP	84.30±3.32	82.80±3.99	78.41±4.58	0.568
NDIP, % of CP	24.93±0.79 c	32.17±1.05 b	36.95±1.64 a	0.000
Crude protein fractions, % of CP				
A= NPN	42.44±2.51 a	29.97±1.16 b	13.56±1.37 c	0.000
B_1	7.64±1.30	6.87±1.74	5.82±0.98	0.650
B_2	18.76±1.64 c	25.96±2.04 b	35.81±1.62 a	0.000
B_3	18.21±1.27 b	23.69±1.62 a	25.35±1.44 a	0.005
C (ADIP)	6.72±0.91 b	8.48±1.15 ab	11.60±1.21 a	0.013

DM, dry matter; CP, crude protein; EE, crude fat; SolP, soluble protein; NPN, nonprotein nitrogen (% of SolP); NDIP, neutral detergent insoluble protein; A fraction (NPN), non-protein nitrogen; B_1, fast true soluble protein; B_2, intermediate degradable protein; B_3, slow degradable protein; C (ADIP), acid detergent insoluble protein; Different letters (a, b, c) in the same row are statistically different (P<0.05)

2x and 3x) (Figure 1). Because of high protein solubility at the first cut, all DIP values are higher than second and third cuts. Similar to the feeding levels, DIP values were decreased by growing period from the first cut to third cut, all UIP values were significantly increased (P<0.05). We found UIP_{1X} values of caramba hay according to the first cut, second cut and third cut respectively, 32.19, 33.39 and 34.26 g kg^{-1} DM. Similarly, Fox et al (2003) reported the amount of the UIP_{1x} values were 30.53 g kg^{-1} DM for caramba hay.

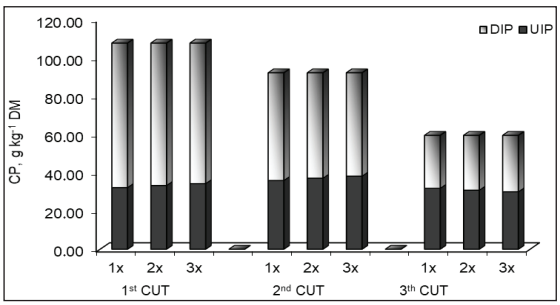

Figure 1- Rumen degradable intake protein (DIP) and Rumen undegradable intake protein (UIP) values of caramba hay according to the DM intakes based on CNCPS. CP, crude protein; DIP, degradable intake protein; UIP, undegradable intake protein (fed at 1x maintenance level, at 2x maintenance level of intake, and at 3x maintenance level of intake)

3.2. The fatty acids and relationship between major fatty acids and crude protein

The FAs of caramba hay with the different cuts are presented in Table 2. The C18:3 was the main FAs present in caramba hay ranging from first cut (43.06%) to third cut (20.71%). The other major FAs were C18:2 and C16:0, which both represented on an average from first cut to third cut 27.93% and 36.73%, respectively (Table 2). The C14:0, C18:0 and C18:1 represented the lower percent of total FAs. This is in agreement with the finding of Boufaïed et al (2003), Elgersma et al (2005) and Ferlay et al (2006). The C14:0, C16:0, C18:0, C18:1, C18:2 and C18:3 content of caramba (fresh) were reported as 1.04, 16.7, 1.73, 2.42, 12.3 and 61.0%, respectively (Aii et al 1988). Ferlay et al (2006) confirmed that C14:0, C16:0, C18:0, C18:1, C18:2 and C18:3 content of caramba hay (contain 133 g kg^{-1} DM of CP) were 0.6, 15.8, 1.8, 2.0, 14.0 and 55.9%, respectively. Ferlay et al (2006) study was close to our study than Glasser et al (2013). Because, as reported in Glasser et al (2013) that haymaking induced a slight decrease in total fat and FAs, among the FA a decrease in C18:3, mainly compensated for by an increase in C16:1. Even this decrease in C18:3 were higher when the drying conditions were not good, because of lipolysis and oxidation of polyunsaturated FAs (Aii et al 1988).

Similar to the study, Dewhurst et al (2001) concluded that the C18:3 of major FAs content (20.4 g kg^{-1} DM) were highest in early season and cutting date were found significantly important for *Lolium multiflorum* (n= 4, P<0.001). This high concentration of C18:3 in first cut (early summer), were explained by Elgersma et al (2005) for perennial ryegrass that the leaf-stem ratio of the herbage probably has effect on FAs content. After first cut, total FAs contained

Table 2- The major fatty acids of caramba hay

The fatty acids (g 100 g^{-1} lipid extract)	*1st cut (Before blossom)*	*2nd cut (Blossom, 50%)*	*3th cut (After blossom)*	*P value*
C14:0	0.71±0.01	0.74±0.07	0.96± 0.03	0.065
C16:0	15.40±0.60 b	17.87±1.13 b	20.61±0.20 a	0.003
C18:0	3.25±0.37 a	2.09±0.14 b	3.20±0.25 a	0.016
C18:1	7.99±1.03 a	4.88±0.61 b	6.29±0.61 ab	0.047
C18:2	12.53±1.03	14.27±1.17	16.12±1.38	0.149
C18:3	43.06±0.96 a	37.96±3.65 a	20.71±1.33 b	0.000
Others	17.10±1.90 b	22.20±3.00 b	31.90±2.90 a	0.006

Different letters (a, b, c) in the same row are statistically different (P<0.05)

lower concentrations of FAs for caramba hay (Table 2) with the consistent with Dewhurst et al (2001). Glasser et al (2013) reported that predominant factor was the vegetation stage and when the forage grew older, CP decreased, along with the EE, total FAs and the content of C18:3. These variations are first due to the decrease in the proportions of leaves that are richer than stems and seeds in membrane lipids. Also, Witkowska et al (2008) stated that because of lower temperature (the lowest daily total radiation), C18:3 content of FAs in early summer was higher than mid-summer in perennial ryegrass. In line with findings of Elgersma et al (2005), Boufaïed et al (2003) and Witkowska et al (2008), the positive linear relation was found between the content of major FAs (C16:0, C18:2 and C18:3) and CP in caramba hay (Figure 2). The simple regression relationship between C18:3 and CP (n= 15, R^2= 0.769, P<0.001) was the highest and significantly important compared to C16:0 and C18:2 (Figure 2). This relationship must be a result of indirect associations. Photosynthetic tissue is formed during growth, which is simulated by nitrogen. Furthermore, with the consistent in our study, the relation between the C18:3 and crude protein (R^2= 0.84, P<0.001) was the highest in Boufaïed et al (2003) study compared to the C16:0 and C18:2. However, in our result was disagreement with Boufaïed et al (2003) that the relationship between the C16:0 and C18:2 with crude protein were insignificant.

In conclusion, the crude protein, the soluble protein ($A+B_1$ fraction) and C18:3 were significantly decreased by growing stage from first cut to third cut. Because of photosynthetic activity, the plant has a higher CP contents at the first cut and this decreases as the crop ages. On the other

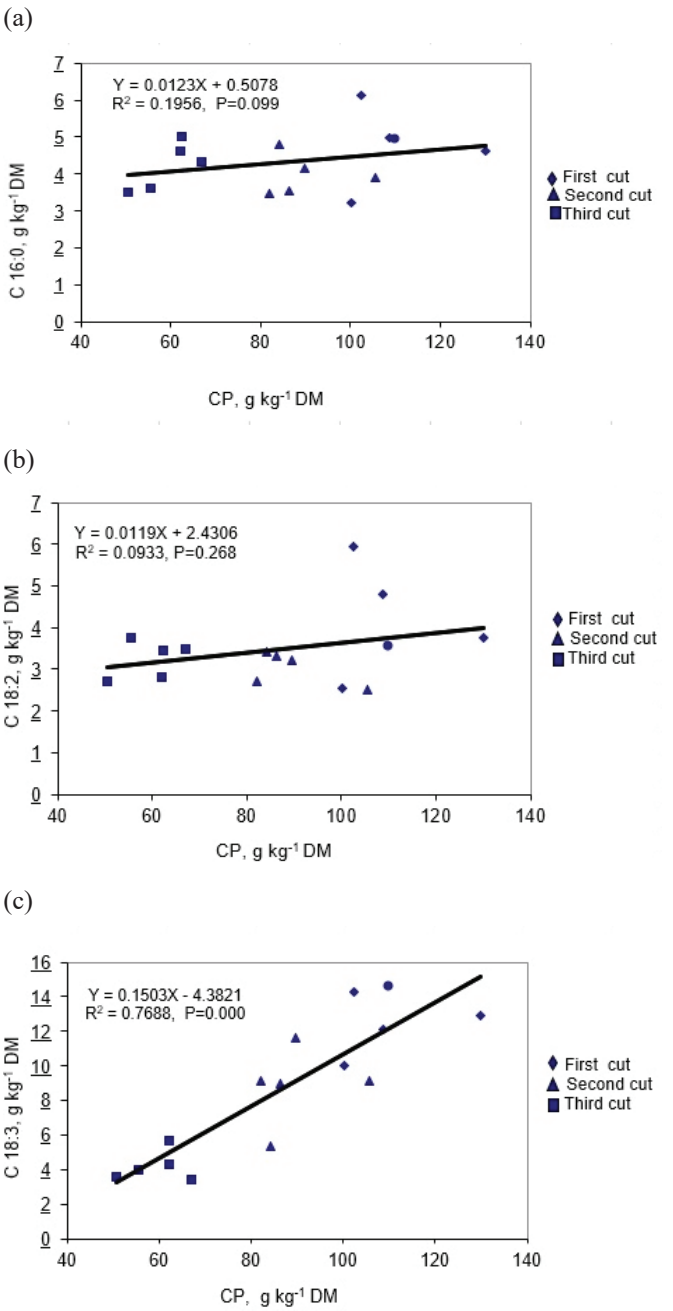

Figure 2- The relationship between the major FAs: C16:0 (a), C18:2 (b), C18:3 (c) and the crude protein in caramba hay harvested at three cutting dates

side, other CP fractions (B_2, B_3 and C) and major FAs (C16:0 and C18:2) values of in caramba hay were increased from first cut to third cut. Further studies are needed to determine the effects of the growth stage and storage methods on relationship between major fatty acids and CP in perennial ryegrass.

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